RELATIVE EFFICACY OF POTASSIUM IODIDE AND AMMONIUM PERCHLORATE AS ANTIDOTES TO RADIOIODIDE EXPOSURE IN THE ADULT RAT AND ITS IMPLICATIONS ON DISASTER PREPAREDNESS

by

CURTIS ANDREW HARRIS

(Under the Direction of Cham E. Dallas)

ABSTRACT

In consideration of the therapeutic efficacy of pharmaceutical intervention for blocking uptake of radioiodide ($^{131}I^-$) into the thyroid gland from nuclear fallout or terrorist attack, there is a paucity of data for treatment post $^{131}I^-$ contamination for differing pharmaceutical approaches to the treatment of $^{131}I^-$ poisoning. Currently the only method of treating $^{131}I^-$ exposure that is approved by the Food and Drug Administration is potassium iodide (KI), and though effective, it has significant limitations, as evidence by over 10,000 thyroid cancers following KI treatment of Chernobyl victims. Experiments were conducted to compare KI to perchlorate (ClO_4^-), a known iodide uptake inhibitor with a higher affinity for the sodium-iodide symporter and thyroid receptor sites, to determine if advantages could be manifested by perchlorate administration rather than KI for $^{131}I^-$ poisoning. In initial experiments, it was determined that both KI and perchlorate dosed rats had a relatively equal efficiency in blocking the uptake of $^{131}I^-$ into the thyroid when administered following the $^{131}I^-$ dose. However, when serum and urine endpoints were considered, we discovered that animals dosed with perchlorate contained markedly lower serum concentrations of $^{131}I^-$ and markedly increased cumulative urine amounts of $^{131}I^-$.

Following these results the focus was primarily on urinary excretion as the underlying determinant of which prophylactic approach was more beneficial. Urine time-course data revealed that over three days of urine collection no significance between cumulative urinary $^{131}I^-$ excretions existed between KI and perchlorate dosed animals. However, during the first day of collection it was determined that animals on perchlorate treatment excreted $^{131}I^-$ more than did animals administered KI. Thyroidal $^{131}I^-$ discrepancies were noted with significantly reduced concentrations of $^{131}I^-$ in animals administered KI. Thyroxine was then administered in conjunction with KI and perchlorate therapy with similar urinary excretion profiles and no significance in thyroidal concentrations of $^{131}I^-$. We concluded from the current work that perchlorate has an equivalent efficacy in blocking uptake of thyroidal $^{131}I^-$ accumulation, while excreting $^{131}I^-$ with a higher intensity than KI. It is therefore our recommendation that perchlorate be more thoroughly investigated as a pharmaceutical intervention for acute $^{131}I^-$ poisoning.

INDEX WORDS: Perchlorate, Iodide, $^{131}I^-$, Thyroid, Urine, Kinetics

RELATIVE EFFICACY OF POTASSIUM IODIDE AND AMMONIUM PERCHLORATE AS ANTIDOTES TO RADIOIODIDE EXPOSURE IN THE ADULT RAT AND ITS IMPLICATIONS ON DISASTER PREPAREDNESS

by

CURTIS ANDREW HARRIS

BS, Georgia College and State University, 2003

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2008

RELATIVE EFFICACY OF POTASSIUM IODIDE AND AMMONIUM PERCHLORATE AS ANTIDOTES TO RADIOIODIDE EXPOSURE IN THE ADULT RAT AND ITS IMPLICATIONS ON DISASTER PREPAREDNESS

by

CURTIS ANDREW HARRIS

Major Professor: Cham E. Dallas

Committee: Jeffery W. Fisher
Catherine A. White
Michael G. Bartlett
Anthony C. Capomacchia

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2008

DEDICATION

This work is dedicated to my family and wife. The support I received from each of them over the past years has given me the drive and strength I needed in order to complete my degree. They will never know how much it meant to me and how this could not have been done without them.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my major professor, Dr Cham Dallas, for his encouragement, patience, and financial support. I would also like to thank Dr Jeff Fisher for his dedication to the project and for all his help and support along the way. Without question, I would like to thank Ed Rollor III for all the time and energy that he put into the project. The project could not have been completed without him. To my remaining committee members Dr. Catherine White, Dr Michael Bartlett, and Dr Tony Capomacchia, I would like to thank you for all of your guidance and direction throughout all of my years at The University of Georgia. Finally, I would like to thank the CDC who funded all of my research and also contributed a lot of time and effort running analytical analysis of my many samples.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND REVIEW OF THE LITERATURE

The development and efficacy of new agents to protect against and diminish radiation toxicity is becoming vitally important to our society. Whether by terrorist attacks, reactor meltdowns, or nuclear warfare, radiation can be extremely harmful or even deadly if not properly treated. A particular radioactive isotope that has become a cause for concern over the last couple of decades is radioactive iodine-131 ($^{131}I^-$). $^{131}I^-$ is a beta and gamma emitting byproduct of nuclear fission reactions of uranium atoms produced during the operation of nuclear reactors and by fission reactions of plutonium (or uranium) involved in the detonation of nuclear weapons (http://www.epa.gov/radiation/radionuclides/iodine.htm). It has a short half-life, eight days, and once ingested is physiologically indistinguishable in the body from stable iodide ($^{127}I^-$) (Ramsden 1967; Book, McNeill et al. 1980; Sternthal 1980). This property of $^{131}I^-$ can be both advantageous and detrimental to human health. Modern medicine has found a way to utilize the radioactive and physical/chemical properties of $^{131}I^-$ when a patient is diagnosed with hyperthyroidism, papillary carcinoma, or follicular carcinoma (Zidan, Hefer et al. 2004). Since $^{131}I^-$ has the ability to shut down the entire thyroid or selected sections of the thyroid, it has been used therapeutically to ablate the thyroid in order to slow down hormone production and prevent thyroid cancer from metastasizing (Zidan, Hefer et al. 2004). While in the hospital setting, doctors are able to specifically target sections of the thyroid that need to be ablated while leaving

normal, functioning sections of the thyroid completely unaffected by the radiation. Doctors are also able to give specific dosings of $^{131}I^-$ so that a patient receives adequate concentrations of radiation to ablate the thyroid while posing a minimal risk of thyroid cancer.

A nuclear incident poses a different set of complications. In the case of nuclear fallout, specific targeting of the isotope to abnormal functioning sections of the thyroid is not possible. The potential dangers worsen once large concentrations of $^{131}I^-$ enter the thyroid and begin incorporation into thyroid hormones. Once incorporated into thyroid hormones the thyroid gland begins slowly releasing the organified form of $^{131}I^-$ as thyroid hormones 3,5,3',5'-tetraiodothyronine (T_4) and 3,5,3'-triiodothyronine (T_3), which results in a significantly higher absorbed dose of radiation (Zanzonico and Becker 2000). These problems can lead to numerous thyroid related conditions such as autoimmune thyroiditis and thyroid cancer. In some cases the absorbed radiation may result in death. Each of these outcomes was documented by the thousands after the Chernobyl disaster of 1986, especially in children and adolescence.

The Chernobyl disaster emitted more than 100 times the amount of radiation of the Hiroshima and Nagasaki atomic bombs combined and resulted in the release of approximately 50 MCi of $^{131}I^-$, about half of the iodine in the reactor, when Chernobyl-4 caught fire and burned for ten days on April 25, 1986 (Becker and Zanzonico 1997). The medical community witnessed an increase in thyroid related cancer in children from 18 cases between 1986 and 1989 to 510 cases between 1990 and 1995 (Bleuer, Averkin et al. 1997). The rapid onset of cancer in this population was a phenomenon that had never been seen before. The data from the population affected by the Nagasaki and Hiroshima atomic detonations indicated that a cancer spike in patients due to radiation poisoning is generally not developed for approximately 15 years. The

onset of cancer in patients affected by the Chernobyl meltdown began in just four years (Nitta, Endo et al. 2001).

Children and adolescents are much more susceptible to $^{131}I^-$ poisoning than adults for two main reasons: smaller thyroid mass and greater consumption of milk (Mahoney, Lawvere et al. 2004). When $^{131}I^-$ exposure occurs by inhalation, both adults and children receive the same concentration of radioactive dose. However, when comparing the size of an adult's thyroid to that of a child's, the child actually receives a much higher concentration because he/she receives the same amount of energy but in a much smaller tissue mass. Studies indicate that for newborns, the thyroid dose is about 16 times higher than that for adults for the same ingested radioactivity (http://www.atsdr.cdc.gov/HEC/CSEM/iodine/whosat_risk.html). Similarly, the absorbed dose is about 8 times higher for children under 1 year old and 4 times higher for children 5 years old. Milk consumption was also a leading cause of the increased number of reported cases of children's thyroid cancer compared to adult thyroid cancer (Blum and Eisenbud 1967). On average children drink much more milk than adults, especially in infancy. $^{131}I^-$ has been shown to accumulate both in breast milk and in fresh milk from cows that grazed on contaminated fields (Zanzonico and Becker 2000). This was a significant problem for the children of Chernobyl. Not only did the children receive the inhalation dose of $^{131}I^-$ in a smaller tissue mass, but they also received concentrated doses of $^{131}I^-$ from the ingested contaminated milk. These two phenomena contributed to the large increase in the number of cases of thyroid cancer in children over the short four-year period.

As noted by studies of the Chernobyl disaster and the Hiroshima and Nagasaki atomic bombs, $^{131}I^-$ is an extremely harmful isotope that begins to negatively affect the thyroid almost immediately after ingestion. It is now critical that there be a pronounced pharmaceutical

intervention that can protect the thyroid from its uptake. However, before trying to block radio-iodide from entering the thyroid, it is first important to understand the mechanism by which iodide is concentrated in the thyroid.

The thyroid's main function is to concentrate inorganic iodide, oxidize the inorganic iodide into molecular iodine, iodinate tyrosyl residues of thyroglobulin (Tg), and couple the iodotyrosines into thyroid hormones T_4 and T_3 (Yu, Narayanan et al. 2002). The thyroid is able to produce its metabolic hormones via other hormones, proteins, and enzymes located both internally and externally to the thyroid. The process of making thyroid hormones starts as soon as iodide is taken into the body. For most of North America the recommended daily allowance of iodine comes primarily from the diet and is approximately 150 μg/day (Burman and Wartofsky 2000; Zanzonico and Becker 2000). Iodine is found in a wide variety of foods, but a North American's main intake comes from processed flour, iodized salt, and seafood (Burman and Wartofsky 2000). Iodide (I^-, the dissociation product of salts such as potassium iodide) that has been ingested in the body is rapidly absorbed through the gastrointestinal (GI) tract and moves into systemic circulation (Burman and Wartofsky 2000). Once in systemic circulation, the iodide is actively sequestered by the sodium-iodide symporter (NIS), a protein channel located in the basolateral membrane of thyroid follicular cells, and transported to the thyroid and other NIS non-thyroidal tissues such as the gastric mucosa, salivary glands, and mammary glands (Burman and Wartofsky 2000; Riedel, Dohan et al. 2001). With the help of NIS and sodium ions, iodide is then able to move into the thyroid follicular cell against an electrochemical gradient, where for every iodide ion that enters the thyroid follicular cell two sodium ions are pumped out (Yu, Narayanan et al. 2002). From there iodide undergoes passive diffusion through the apical membrane from the follicular cell into the colloid where the

inorganic iodide is converted to molecular iodine by thyroid peroxidase (TPO), an enzyme associated with the apical membrane, and hydrogen peroxide (H_2O_2) (Yu, Narayanan et al. 2002). Organification of the molecular iodine then takes place in the colloid where tyrosyl residues of Tg are iodinated and coupled together to form Tg-T_3 and Tg-T_4 complexes. These complexes are able to diffuse back across the apical membrane from the colloid into the follicular cell where proteolysis of Tg occurs and thyroid hormones T_3 and T_4 are liberated and secreted into the systemic circulation (Yu, Narayanan et al. 2002). The production of thyroid hormones is a delicate and circadian process and must be vigorously controlled so that there is not too much nor too little hormone in the body. This control process is a function of the hypothalamus-pituitary-thyroid (HPT) axis.

Two control loops make up the HPT axis, a positive feedback loop and a negative feedback loop. The negative feedback loop allows the hypothalamus to detect when T_3 and T_4 concentrations are low and then synthesize a tripeptide hormone, thyroid-releasing hormone (TRH) (Yu, Narayanan et al. 2002). TRH then starts the positive feedback loop when it stimulates the pituitary gland to produce thyroid-stimulating hormone (TSH). After production, TSH enters systemic circulation and binds to TSH receptor sites on the thyroid. The binding of TSH causes active up-regulation of iodide by increasing production of NIS (Yu, Narayanan et al. 2002). Once the HPT axis detects adequate amounts of thyroid hormones in circulation, TRH and THS production are halted until the need for more hormone arises. An understanding of thyroid physiology and the iodide concentrating mechanism allow for consideration of how $^{131}I^-$ can be inhibited from accumulating in the thyroid via pharmaceutical intervention.

Potassium iodide (KI) is one pharmaceutical intervention that has been studied by many researchers since Plummer introduced iodine therapy for the treatment of thyrotoxicosis in 1923

(Plummer 1923). KI's mechanism of action is to compete with $^{131}I^-$ for the receptor sites of NIS and the thyroid. The rationale for using KI as a protector against radioiodide accumulation in the thyroid is obvious. If all isotopes of iodide truly are indistinguishable in the body, then each isotope has to be using the same transport mechanism to enter the thyroid. So, if it were possible to saturate the transport mechanisms and receptor sites at the thyroid with stable iodide, the radioiodide would never have a chance to enter the thyroid and disperse harmful gamma and beta radiation. Essentially it becomes a situation of competitive inhibition: whether the stable or radioactive iodide reaches NIS or the thyroid first. This makes concentration and time of administration of KI vitally important.

Studies in the 1960's found that for acute exposures of $^{131}I^-$ a blocking dose of 100-200 mg will reduce the amount of irradiation exposure by greater than 98% (Adams and Bonnell 1962; Blum and Eisenbud 1967; Ramsden 1967). The decisive factor of whether or not there was successful blockage of iodide was determined by an estimation of the amount of serum inorganic iodide, where elevated concentrations of inorganic iodide were denoted as significant decrease in uptake (Blum and Eisenbud 1967). It was also determined that increasing the dose of KI from 100-200 mg to 1000 mg yielded similar blocking effects, while increasing the incidence of side-effects and allergic reactions (Blum and Eisenbud 1967). From these studies and other literature review studies, the US Food and Drug Administration (FDA) stepped in and set guidelines for a dosing regimen of KI for radioiodine poisoning. The FDA concluded that adults should take doses of 130 mg and children less than 1 year of age should take 65 mg of KI daily in order to effectively block thyroid irradiation (Meck, Chen et al. 1985). The FDA later made revisions to the dosing regimen: adults (ages 18 and up) take 130 mg, children and adolescence (ages 12-18, except those approaching adult weight of >70 kg who should take the 130 mg dose)

should take 65 mg, children over 3 through age 12 should take 65 mg, children 1 month to 3 years should take 32 mg, and infants from birth to 1 month should take 16 mg of KI daily until the threat of radiation exposure no longer exists (FDA 2001). The benefits of KI administration as a blocking agent for radioiodine are vast, but several problems have been identified from its use as a therapeutic intervention. Two of the major problems from KI administration include that it must be taken for as long as there is an exposure risk, which is generally not an acute dose, and that there is a narrow therapeutic window in which KI can be administered and still be effective (Lengemann and Thompson 1963; Blum and Eisenbud 1967; Meck, Chen et al. 1985; Zanzonico and Becker 2000).

Depending on what type of event has occurred, the risk of radiation exposure could last weeks or even months, as was the case with the Chernobyl disaster and the Hiroshima and Nagasaki atomic bombs. These types of long-term events require patients to receive a significant dose of KI every day. The reason for the continued administration is two-fold: first, that patients that are still in the fall out zones and continue to receive radiation on a daily basis; and second, because the half-life of artificially elevated blood iodide levels is approximately 6 hours (Wolff 1980). While iodide intake is important to maintain hormone homeostasis, the amount of iodide needed in order to block $^{131}I^-$ would exceed the recommended daily allowance one thousand fold (RDA = 150 μg, blocking dose = 130 mg). This pronounced increase could lead to several thyroid related disorders from hypothyroidism, hyperthyroidism, iodide induced goiter, thyroid nodules, and death (Adams and Bonnell 1962; Meck, Chen et al. 1985; Zanzonico and Becker 2000). It has been estimated that for a 130 mg dose of KI the risk of death increases 10 fold from a single to a 10 day dosing regimen (Meck, Chen et al. 1985). Though death is not a likely scenario from taking a single or ten day dosing regimen, 0.1 in a million for a single dose and 1

in a million from a 10 day dose, other thyroid related disorders are more likely to occur depending on dose and duration of therapy (Meck, Chen et al. 1985). Studies have shown that for low doses (less than 25 mg/day), high doses (50-500 mg/day), and very high doses (greater than 1000 mg/day) of iodide adverse reactions have included iodide-induced thyrotoxicosis, iodide goiter and/or hypothyroidism, and thyroiditis, respectively (Becker, Braverman et al. 1984). Patients who have been previously diagnosed and suffer from goiter or autoimmune thyroid disease have various levels of iodide sensitivities and should be especially cautious about using KI as a prophylactic. However, patient knowledge of their medical history of pre-existing thyroid related conditions can help physicians in greatly reducing the chance of one of these severe adverse reactions taking place.

The major obstacle with iodine therapy relates to time of administration (Zanzonico and Becker 2000). Early administration establishes whether or not KI will be a useful intervention because the bulk of a single dose of radioiodide is incorporated into the thyroid within the first few hours of exposure (Wolff 1980). Human volunteer studies have shown that three hours following radioiodide contamination, half of the single exposure has accumulated in the thyroid with greater than 90% having been organified (Wolff 1980). When KI therapy was administered prior to or in conjunction with radioiodine, effective thyroid blocking was observed with greater than 90% blocking when KI was administered less than 10 hours prior to radioiodide. KI administration up to 40 hours prior to radioiodide resulted in 20-40% blocking. And finally, when KI was administered simultaneously with radioiodide, 100% blocking occurred (Wolff 1980). Time of administration and blocking efficiency was again considered by Zanzonico *et al.* (2000) with differing results. They demonstrated that administering KI to humans volunteers 96, 72, and 24 hours prior to radioiodide had blocking effects of 5%, 32%, and 90%, respectively

(Zanzonico and Becker 2000). Preventing radioiodide accumulation into the thyroid has been clearly demonstrated when KI was administered prior to radioiodide contamination; however, this option is not always an option.

It has been determined that KI effectiveness falls off rapidly as a function of time (Wolff 1980). Few data sets exist that have demonstrated the effect of KI treatment when administered post radioiodide dose. KI administered to humans 2, 8, and 24 hours post radioiodide dose was able to prevent accumulation into the thyroid by 80%, 40%, and 7%, respectively (Zanzonico and Becker 2000). Similar results were obtained when 100 mg of KI were administered to human volunteers 3 hours after radioiodide treatment, yielding a 60% blocking efficiency and taking 200 mg of KI four hours after radioiodide yielding a 34% blocking efficiency (Blum and Eisenbud 1967).

A compilation of these groundwork studies have suggested that if at all possible, KI should be taken prior to or coinciding with radioactive iodine and that KI has limited utility after four hours. The utility declines to zero after 12 hours. The narrow four-hour window of utility has dramatically limited the pharmaceutical efficacy of KI, especially in the case of mass exposure such as Chernobyl and the Nagasaki and Hiroshima atomic bombs. It is simply unrealistic to assume that in the time of a disaster it would be possible to distribute KI to hundreds of thousands, if not millions of people in four hours. This pharmaceutical reality has led to my hypothesis that perchlorate (ClO_4^-) will provide a more rapid thyroid radioprotection, in a later and more effective therapeutic window, than the current therapeutic use of KI.

Perchlorates are colorless, odorless, high melting point inorganic salts that are most commonly produced by the electrochemical oxidation of an aqueous solution of sodium chloride that proceeds via a series of one electron oxidations ($Cl^- \rightarrow ClO_2^- \rightarrow ClO_3^- \rightarrow ClO_4^-$) (Vogt H

1986). The five major perchlorate salts produced are magnesium perchlorate, potassium perchlorate, ammonium perchlorate (the majority of perchlorate produced), sodium perchlorate, and lithium perchlorate. The process of generating perchlorate salts generally begins with the production of the sodium perchlorate salt. However, other salt forms can be generated easily by selectively re-crystallizing the salts that are less soluble than sodium perchlorate, thus resulting in ion exchange to generate the desired product. The reaction proceeds as follows:

$Na^{+}_{(aq)} + ClO^{-}_{4\,(aq)} + M^{+}_{(aq)} + X^{-} \leftrightarrow MClO_{4(s)} + Na^{+}_{(aq)} + X^{-}_{(aq)}$, where M is Mg^{+}, K^{+}, NH_4^{+}, or Li^{+}; X is chloride, sulfate, or carbonate; and $MClO_4$ is the desired salt product (Wilbur 2005). Once produced, perchlorates exist in two forms, first as solids in the absence of water and secondly as solutions as it quickly dissolves in water. Once in an aqueous solution, perchlorate salts dissociate into the anion, perchlorate (ClO_4^-), and the cation; either magnesium (Mg^{+}), potassium (K^{+}), ammonium (NH_4^{+}), sodium (Na^{+}), or lithium (Li^{+}) (Wilbur 2005).

Perchlorate is similar in size and charge to iodide and has a short biological half-life of approximately 8 hours (Wolff 1998; Merrill, Clewell et al. 2003; Clewell, Merrill et al. 2004). Due to its similarity to iodide, perchlorate is able to mimic iodide's biological activity. Perchlorate's primary mechanism of action is to compete with circulating iodide for the receptor sites at NIS, and prevent uptake of iodide into the thyroid follicular cell (Anbar, Guttmann et al. 1959; Goldman and Stanbury 1973; Wolff 1998; Yu, Narayanan et al. 2002). Perchlorate does not appear to be metabolized in rats or humans and its main route of elimination is in the urine. In a metabolism study in humans it was reported that less than 0.1% of double-labeled perchlorate ($^{36}Cl^{18}O_4$) appeared in the urine as $^{36}Cl^{-}$ and $^{36}Cl^{18}O_3^{-}$ (Anbar, Guttmann et al. 1959). In a related study, rats were administered intraperitoneal (ip) injections of labeled perchlorate, $K^{36}ClO_4$, and sacrificed at various time intervals to check the biologic and metabolic activities of

perchlorate (Goldman and Stanbury 1973). From that study it was determined that the ion underwent no chemical change between injection and excretion and perchlorate's peak concentration in the thyroid occurred around the 4 hour time-point (concentration went to 5% of that value at 96 hours) (Goldman and Stanbury 1973). They concluded that there was no evidence of covalent binding of the radionuclide by the thyroid, there was no selective retention by other tissues tested, and there was an exponential disappearance from the body with a half life of approximately 20 hours (Goldman and Stanbury 1973). All of Goldman's (1973) conclusions were similar to that of iodide disposal in rats, except for the organification of iodide, as demonstrated in studies by Albert (1951). There appear to be no harmful metabolites from perchlorate, it has an unchanged elimination in the urine, and has an extreme iodide blocking effect and affinity for the thyroid. Researchers long ago began to explore perchlorate's pharmaceutical use for the treatment of hyperthyroidism, Grave's disease, and as a protectant for radioiodide exposure.

Perchlorate was extensively used in the 1950's and 1960's as an antithyroid agent due to its ability to competitively inhibit the process by which iodide circulating in the blood is actively transported into the thyroid (Crooks and Wayne 1960; Wolff and Maurey 1963; Fisher, Todd et al. 2000; Greer, Goodman et al. 2002; Merrill, Clewell et al. 2003; Clewell, Merrill et al. 2004). Studies have shown that effective control of hyperthyroidism could be maintained in 24 patients who were given 6 mg perchlorate/kg/day for up to 52 weeks (Godley and Stanbury 1954). Another study reported a 72 year old female who had a partial thyroidectomy in 1945 due to thyrotoxicosis having recurring symptoms of thyrotoxicosis in 1958 (Connell 1981). Instead of another surgery, she was treated with 1 g perchlorate/day for one month and maintained thereafter on 200 mg perchlorate/day. This therapy rendered the patient euthyroid for 22 years

with no complications or side effects. However, in 1980 the therapy was discontinued. Four weeks after discontinuation thyrotoxicosis symptoms began to develop and a diagnosis of Graves' disease was rendered. The study concluded that perchlorate was responsible for the maintenance of euthyroidism by continuous depletion of intrathyroidal iodine and that the withdrawal of perchlorate led to unblocked excessive iodide trapping, which resulted in excessive thyroid hormone production (Connell 1981). Subsequent to these findings and studies by Crooks and Wayne (1960), Morgans and Trotter (1960), and others, perchlorate became an accepted treatment for hyperthyroidism and was widely used for this purpose for several years (Crooks and Wayne 1960; Morgans 1960).

Thousands of patients were successfully treated in the 1950's and 1960's for thyrotoxicosis and Grave's disease with side effects equivalent to that of thionamides and a total incident of reaction to perchlorate of only 2-3% for doses of less than 1 g/day. However, seven reported cases of fatal aplastic anemia virtually stopped the use of perchlorate in a clinical setting (Trotter 1962; Wolff 1998). Aplastic anemia is characterized by drastic reductions in circulating granulocytes, erythrocytes, thrombocytes, and lack of erythropoietic and granulopoietic cells in the bone marrow (Hobson 1961; Johnson and Moore 1961; Krevans, Asper et al. 1962; Barzilai and Sheinfeld 1966). Of the seven reported cases of aplastic anemia, four of them were reported in Britain and one case each in the United States, Germany, and Israel (Wolff 1998). It was originally assumed that this condition was caused by excessive dosing of perchlorate, but this was not the case as the patients were receiving less than or equal to 1 g/day. The reason patients were developing this rare disease was in the duration of treatment. Patients who were administered thyrotoxicosis therapy were receiving daily doses of less than or equal to 1 g/day for 2-8 months, at which time the aplastic anemia started to proliferate (Wolff 1998). Another

theory that emerged to explain why patients were experiencing this fatal disease pointed to contaminated perchlorate. It was hypothesized that because four of the seven cases occurred in a single area in Britain, it is possible that the perchlorate could have been contaminated with other chemicals, though this theory was never further researched (Wolff 1998; Lawrence, Lamm et al. 1999). It should also be noted that all of the reported cases of death that resulted from aplastic anemia were women who ranged in age from 24-82 (Wilbur 2005). The reason women are more susceptible to aplastic anemia than men is because Grave's disease is much more common in women than in men. However, in a review paper published by Lawrence *et al.* (1999), it was concluded that the aplastic anemia observed in the 1950's and 1960's would be unlikely in the short-term use of perchlorate to prevent iodine-induce hyperthyroidism , because there have never been any reports of bone marrow suppression from any short-term dosing regimens (Lawrence, Lamm et al. 1999). Although aplastic anemia essentially stopped the prophylactic use of perchlorate for several decades, a newfound interest in perchlorate has developed that focuses on perchlorate's extensive oxidizing properties, public health concerns over contaminated water supplies, and occupational exposures to perchlorate from perchlorate production plants.

Perchlorates are utilized in a diverse set of industries, such as in rocket motors, fireworks, and explosives due to their ability under heat conditions to have intense oxidizing power while still remaining relatively stable at moderate temperatures (Vogt H 1986; Conkling 1996; Greer, Goodman et al. 2002). When perchlorate encounters increasing temperatures the anions begin to decompose into chlorine, chlorides, and oxygen. Self propagation, due to decomposition, increases heat production and allows the surrounding perchlorate anions to begin to oxidize, which in turn produces a large amount of energy in the presence of oxidizable materials (Wilbur

2005). The Department of Defense (DoD) and aerospace engineers (NASA), who in combination use an estimated 90% of perchlorates that are produced, have developed a method to use the explosive properties of perchlorates to their advantage to make weapons, such as rockets, and rocket propellants used in fighter jets and the space shuttles (Greer, Goodman et al. 2002; Wilbur 2005). Other applications of perchlorates include use in air bag inflation systems, oxygen generating systems, temporary adhesives for steel or other metallic plates, adjustments in ionic strength of electroplating baths, fertilizers, photography, engraving agents, and many others.(Vogt H 1986; Von Burg 1995; Smith, Theodorakis et al. 2001; Urbansky and Collette 2001). Perchlorate has extensive use in military, aeronautics, and many other industrial applications and therefore the production demand for perchlorate is quite high (50 million pounds produced in 1974) (Vogt H 1986). Leaks into the environment from its heavy industrial use have become a problem.

Perchlorates have been the topic of many recent publications because of their history as a contaminant, due to frequent leaching into the environment from weapons testing, large scale plant production, fertilizer use, military operations, aerospace programs, and recycling (Urbansky 2002). Perchlorates also have a limited shelf life and therefore require frequent replacement in rocket and missile fuels (Motzer 2001). Since the 1950's almost all perchlorate produced in the US has been disposed of in Nevada, California, and Utah, with minor amounts being disposed of in other states where rocket and missile fuels are produced (Motzer 2001). As of 1997, a sensitive new detection method for perchlorate was developed by the California Department of Health Services and analysis of soil and groundwater revealed widespread perchlorate contamination in Utah, California, Nevada, and Arizona (Greer, Goodman et al. 2002). This new method of analysis allowed the detection limit of perchlorate to drop by a factor

of 100, from 400 ppb to 4 ppb. Using the new detection method, it was determined that groundwater contamination in the Las Vegas, Nevada area ranged from 630,000 to 3,700,000 ppb with other states having concentrations of around 20 ppb (Motzer 2001). While perchlorates were found in high concentrations at the sites of known perchlorate producers, as would be expected, the more interesting data illustrated that perchlorates were also detected in high concentrations far from the source of production (Wilbur 2005). From this data it was determined that perchlorates are highly mobile in wet soil and are able to partition into surface and ground water. It was also determined that a large energetic barrier must be overcome in order for perchlorates to react, with dilute aqueous solutions of perchlorate having almost no oxidizing abilities and therefore remain relatively stable at moderate conditions (Wilbur 2005). Once in the environment, perchlorates tend to be quite persistent and their degradation properties have remained inconclusive at this point. However, it is known that there are a wide variety of microorganisms that are able to biodegrade perchlorate under anaerobic conditions (Wilbur 2005).

Due to perchlorate's ability to be highly mobile in aqueous systems and the new detection method developed, there have been at least eleven states with confirmed releases of perchlorate in ground or surface water (Urbansky 1999). This is a major concern for many toxicologists because of the relative paucity of information in the toxicological database available to more concretely determine the potential human health effects of low-level exposure (Siglin, Mattie et al. 2000). A 14 and 90 day drinking water toxicity study performed by Siglin *et al.* (2000) and two occupational studies performed by Gibbs *et al.* (1998) and Lamm *et al.* (1999) signify attempts to allay the concern from low-level environmental exposures. Siglin *et al.* (2000) administered perchlorate in drinking water to male and female rats at dosage levels of 0.0, 0.01,

0.05, 0.2, 1.0, and 10.0 mg/kg/day for 14 or 90 days. The study also included a non-treatment 30 day recovery period at the end of 90 days to assess recovery ability from the perchlorate effects at the 0.05, 1.0, and 10.0 mg/kg/day dose groups. Their study established that after 14 days of treatment, mean TSH levels were significantly increased in both males and females at 0.2 and 0.05 mg/kg/day and higher. Respectively, mean T_4 levels were significantly decreased in both males and females at the 10 mg/kg/day, and mean T_3 levels were significantly decreased in males starting at 0.01 mg/kg/day and higher. The decline of the percentage decrease of T_3 proceeded in a dose-dependent manner (Siglin, Mattie et al. 2000). T_3 levels in females were not significantly different from control animals in the 14 day study. These results verified the work of a previous 14 day study conducted by Caldwell *et al.* (1996) of thyroid hormone depletion and TSH elevation (Caldwell 1996). The 90-day study presented slightly contrasting data from the 14 day study. In the 90 day study, TSH levels were significantly increased in males at perchlorate doses of 0.2 mg/kg/day and higher and in females at doses of only 10 mg/kg/day, while T_3 and T_4 levels were significantly decreased in both sexes at levels of 0.01 mg/kg/day and higher, with percentages of decrease having a dose-dependent manner (Siglin, Mattie et al. 2000). After the 90-day study was completed, some animals were given a 30-day recovery period before another sacrifice and a third set of hormone concentration tests were performed. Following the recovery period, TSH was significantly increased in all three female recovery groups while no significant increase was found in their male counterparts. A direct contrast was observed in the T_4 concentrations as all three male groups showed a significant decrease in T_4 while the females showed no significant increase. There were also no significant decreases in T_3 concentrations in males or females except for the 10 mg/kg/day group in the female rats (Siglin, Mattie et al. 2000). The aim of this study was to develop a No Observable Effect Level (NOEL) for

ammonium perchlorate in drinking water. However, since increase in thyroid weight and histopathological changes could only be found in the 14 and 90 day study for the 10 mg/kg/day dose group for both males and females, and these changes were reversible after 30 days of nontreatment, the researchers were not able to establish a NOEL but rather a No Observable Adverse Effect Level (NOAEL). The NOAEL was set at 1.0 mg/kg/day based on the fact that this was the highest dose tested that produced THS depletion and thyroid hormone elevation with no increase in thyroid weight and no histopathological changes (Siglin, Mattie et al. 2000).

Occupational studies have also been conducted to assess the potential risk of thyroid related disorders, from constant exposures to varying concentrations of perchlorate, for shift workers at a production plant of ammonium perchlorate. In a study by Gibbs *et al.* (1998), employees at an ammonium perchlorate production plant in Nevada were monitored for airborne perchlorate exposure to assess if there were any measurable adverse effects on the thyroid. Three groups, including controls, were monitored for both acute and chronic exposure to perchlorate based on standard breathing rates. It was determined from the study that for acute exposures the concentration ranged from 0.2-436 μg/kg and that for chronic exposures the concentration ranged from 8,000-88,000 μg/kg (Gibbs, Ahmad et al. 1998). The study concluded that the amount of airborne exposure from perchlorate to workers at the plant was two to three orders of magnitude less than the historically prescribed dose for Graves' disease and two to three orders of magnitude greater than that obtained from drinking water from Lake Mead or the Colorado River for acute exposures (Gibbs, Ahmad et al. 1998). It was also concluded that for chronic exposure to perchlorate the amount of exposure to the workers could be up to ten times the cumulative dose that would result from the drinking water of Lake Mead or the Colorado River. Additionally, though these workers were receiving greater amounts of

perchlorate than what was in the drinking water, there were no exposure-related effects on thyroid related function (either acute or chronic) found. In fact, the only differences that were noted in hormone concentrations was that TSH levels were slightly elevated, but were consistent with published reports of circadian changes in serum TSH levels from the beginning to the end of a shift (Gibbs, Ahmad et al. 1998). A second occupational study by Lamm *et al.* (1999) confirmed the results demonstrated by Gibbs *et al.* (1998). The Lamm *et al.* (1999) study divided workers into four exposure scenarios with mean absorbed perchlorate doses of 1, 4, 11, and 34 mg/kg/day. This study also concluded that there were no differences in thyroid-function parameters (i.e. TSH, free T_4 index, T_4, T_3, thyroid hormone binding ratio, thyroid peroxidase antibodies, and clinical examination) found between the four various exposure levels (Lamm, Braverman et al. 1999). Because this study found no evidence of an adverse effect on thyroid function or blood cells, it established a NOAEL of 34 mg/day (or 0.49 mg/kg/day based on 70 kg man) for airborne occupational exposure to perchlorate (Lamm, Braverman et al. 1999).

Specific Aims

The ability of perchlorate and iodide to block the accumulation of iodide into the thyroid, small incidence of toxic side effects, and lack of data on relative perchlorate/iodide administration post-radioiodide dose have led to the scope of this dissertation and the following specific aims to be addressed:

Aim 1: Evaluate the relative prophylactic effects of potassium iodide and perchlorate to ameliorate $^{131}I^-$ exposure in the rat. High concentrations of KI and perchlorate were administered at contrasting time-points in rats following $^{131}I^-$ exposure. Results were compared based on percentage uptake into the thyroid versus saline dosed animals.

Aim2: Characterization of time-course urinary excretion profiles of $^{131}I^-$ in response to potassium iodide and perchlorate prophylaxis. As the main route of elimination of $^{131}I^-$ and therapeutic compounds are in the urine, results were based on percentage of the dose excreted in urine and comparisons were made between treatment groups.

Aim3: Evaluation of long term thyroid $^{131}I^-$ concentration and time-course urinary $^{131}I^-$ profiles in response to potassium iodide and perchlorate prophylaxis with hormone replacement therapy. Replacement T_4 doses were administered every 24 hours following $^{131}I^-$ exposure. Results were based on comparisons between the various treatment groups in Aim 3, as well as comparisons with similar treatment groups from Aim 2.

References

Adams, C. A. and J. A. Bonnell (1962). "Administration of stable iodide as a means of reducing thyroid irradiation resulting from inhalation of radioactive iodine." Hisp Med **7**: 127-49.

Anbar, M., S. Guttmann, et al. (1959). "The mode of action of perchlorate ions on the iodine uptake of the thyroid gland." Int J Appl Radiat Isot **7**: 87-96.

Barzilai, D. and M. Sheinfeld (1966). "Fatal complications following use of potassium perchlorate in thyrotoxicosis. Report of two cases and a review of the literature." Isr J Med Sci **2**(4): 453-6.

Becker, D. V., L. E. Braverman, et al. (1984). "The use of iodine as a thyroidal blocking agent in the event of a reactor accident. Report of the Environmental Hazards Committee of the American Thyroid Association." Jama **252**(5): 659-61.

Becker, D. V. and P. Zanzonico (1997). "Potassium iodide for thyroid blockade in a reactor accident: administrative policies that govern its use." Thyroid **7**(2): 193-7.

Bleuer, J. P., Y. I. Averkin, et al. (1997). "Chernobyl-related thyroid cancer: what evidence for role of short-lived iodines?" Environ Health Perspect **105 Suppl 6**: 1483-6.

Blum, M. and M. Eisenbud (1967). "Reduction of thyroid irradiation from 131-I by potassium iodide." JAMA **200**(12): 1036-40.

Book, S. A., D. A. McNeill, et al. (1980). "Comparative effects of iodine-132 and iodine-131 in rat thyroid glands." Radiat Res **81**(2): 246-53.

Braverman, L. E., Utiger, R. D. (1991). Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text: 6th ed., J. B. Lippincott, New York, NY.

Burman, K. D. and L. Wartofsky (2000). "Iodine effects on the thyroid gland: biochemical and clinical aspects." Rev Endocr Metab Disord **1**(1-2): 19-25.

Caldwell, D. J., Kinkead, E. R., Wolfe, R. E., King, J. H., Narayanan, L., Confer, P. D., and Mattie, D. R. (1996). "Changes in thyroid hormone levels after fourteen day oral dosing with ammonium perchlorate." The Toxicologist **30**(1): 67.

Clewell, R. A., E. A. Merrill, et al. (2004). "Evidence for competitive inhibition of iodide uptake by perchlorate and translocation of perchlorate into the thyroid." Int J Toxicol **23**(1): 17-23.

Clewell, R. A., E. A. Merrill, et al. (2001). "The use of physiologically based models to integrate diverse data sets and reduce uncertainty in the prediction of perchlorate and iodide kinetics across life stages and species." Toxicol Ind Health **17**(5-10): 210-22.

Conkling, J. A. (1996). "Pyrotechnics." In: Kroschwitz JI, Howe-Grant M, eds. Kirk-Othmer encyclopedia of chemical technology **20**: 680-697.

Connell, J. M. (1981). "Long-term use of potassium perchlorate." Postgrad Med J **57**(670): 516-7.

Crooks, J. and E. J. Wayne (1960). "A comparison of potassium perchlorate, methylthiouracil, and carbimazole in the treatment of thyrotoxicosis." Lancet **1**: 401-4.

Durand, J. (1938). "Recherches sur l'elimination des perchlorates, sur leur repartition dans les organes et sur leur toxicite [Research on the elimination of perchlorate, its distribution in organs and its toxicity]." Bull. Soc. Chim. Biol. **20**: 423-433.

Eichler, O. (1929). "Zur Pharmakologie der Perchloratwirkung [The pharmacology of the perchlorate effect]." Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. **144**: 251-260.

FDA (2001). Potassium Iodide as a Thyroid Blocking Agent in Radiation Emergencies. FDA. **66:** 1-15.

Fisher, J., P. Todd, et al. (2000). "Preliminary development of a physiological model for perchlorate in the adult male rat: a framework for further studies." Drug Chem Toxicol **23**(1): 243-58.

Gerlowski, L. E. and R. K. Jain (1983). "Physiologically based pharmacokinetic modeling: principles and applications." J Pharm Sci **72**(10): 1103-27.

Gibbs, J. P., R. Ahmad, et al. (1998). "Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function." J Occup Environ Med **40**(12): 1072-82.

Godley, A. F. and J. B. Stanbury (1954). "Preliminary experience in the treatment of hyperthyroidism with potassium perchlorate." J Clin Endocrinol Metab **14**(1): 70-8.

Goldman, S. J. and J. B. Stanbury (1973). "The metabolism of perchlorate in the rat." Endocrinology **92**(5): 1536-8.

Greer, M. A., G. Goodman, et al. (2002). "Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans." Environ Health Perspect **110**(9): 927-37.

Hobson, Q. T. (1961). "Aplastic anaemia due to treatment with potassium perchlorate." Br Med J **5236**: 1368-9.

http://www.atsdr.cdc.gov/HEC/CSEM/iodine/whosat_risk.html.

http://www.epa.gov/radiation/radionuclides/iodine.htm.

Johnson, R. S. and W. G. Moore (1961). "Fatal aplastic anaemia after treatment of thyrotoxicosis with potassium perchlorate." Br Med J **5236**: 1369-71.

Kamm, G., and Drescher, G. (1973). "Der Nachweis von Perchlorat im Urin [Demonstration of perchlorate in the urine]." Beitr. Gerichtl. Med. **30**(206-210).

Krevans, J. R., S. P. Asper, et al. (1962). "Fatal aplastic anemia following use of potassium perchlorate in thyrotoxicosis." JAMA **181**: 162-4.

Krishnan, K., Andersen, M. E. (1994). Physiologically based pharmacokinetic modeling in toxicology. In: Hayes AW, ed. Principles and methods of toxicology. 3rd ed. New York, NY, Raven Press, Ltd.**:** 149-188.

Lamm, S. H., L. E. Braverman, et al. (1999). "Thyroid health status of ammonium perchlorate workers: a cross-sectional occupational health study." J Occup Environ Med **41**(4): 248-60.

Lawrence, J. E., S. H. Lamm, et al. (1999). "The use of perchlorate for the prevention of thyrotoxicosis in patients given iodine rich contrast agents." J Endocrinol Invest **22**(5): 405-7.

Lengemann, F. W. and J. C. Thompson, Jr. (1963). "Prophylactic And Therapeutic Measures For Radioiodine Contamination--A Review." Health Phys **65**: 1391-7.

Mahoney, M. C., S. Lawvere, et al. (2004). "Thyroid cancer incidence trends in Belarus: examining the impact of Chernobyl." Int J Epidemiol **33**(5): 1025-33.

Meck, R. A., M. S. Chen, et al. (1985). "Criteria for the administration of KI for thyroid blocking of radioiodine." Health Phys **48**(2): 141-57.

Merrill, E. A., R. A. Clewell, et al. (2003). "PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat." Toxicol Sci **73**(2): 256-69.

Merrill, E. A., R. A. Clewell, et al. (2005). "PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans." Toxicol Sci **83**(1): 25-43.

Morgans, M. E., Trotter, W. R. (1960). "Potassium perchlorate in thyrotoxicosis." Br Med J **2**: 1086-1087.

Motzer, W. E. (2001). "Perchlorate: Problems, detection, and solutions." Environmental Forensics **2**: 301-311.

Nitta, Y., S. Endo, et al. (2001). "Age-dependent exposure to radioactive iodine (131I) in the thyroid and total body of newborn, pubertal and adult fischer 344 rats." J Radiat Res (Tokyo) **42**(2): 143-55.

Plummer, H. S. (1923). "Results of Administering Iodin to Patients Having Exophthalmic Goiter." JAMA **80**: 1955.

Ramsden, D. P., F. H.; Peabody, C. O.; Speight, R. G. (1967). "Radioiodine uptakes in the thyroid. Studies of the blocking and subsequent recovery of the gland following the administration of stable iodine." Health Phys **13**: 633-646.

Riedel, C., O. Dohan, et al. (2001). "Journey of the iodide transporter NIS: from its molecular identification to its clinical role in cancer." Trends Biochem Sci **26**(8): 490-6.

Siglin, J. C., D. R. Mattie, et al. (2000). "A 90-day drinking water toxicity study in rats of the environmental contaminant ammonium perchlorate." Toxicol Sci **57**(1): 61-74.

Smith, P. N., C. W. Theodorakis, et al. (2001). "Preliminary assessment of perchlorate in ecological receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas." Ecotoxicology **10**(5): 305-13.

Sternthal, E. L., L.; Stanley, B.; Abreau, C.; Fang, S. L.; Braverman, L. E.; (1980). "Suppression of thyroid radioiodine uptake by various doses of stable iodide " New England Journal Of Medicine **303**: 1083-1088.

Trotter, W. R. (1962). "The relative toxicity of antithyroid drugs." J New Drugs **2**: 333-43.

Urbansky, E. T. (2002). "Perchlorate as an environmental contaminant." Environ Sci Pollut Res Int **9**(3): 187-92.

Urbansky, E. T., and Schock, M. R. (1999). "Issues in managing risks associated with perchlorate in drinking water." Journal of Environmental Management **56**: 79-95.

Urbansky, E. T. and T. W. Collette (2001). "Comparison and evaluation of laboratory performance on a method for the determination of perchlorate in fertilizers." J Environ Monit **3**(5): 454-62.

Vogt H, B. J., Bennett JE, et al. (1986). "Chlorine oxides and chlorine oxygen acids." In: Gerhartz W, Yamamoto YS, Campbell FT, et al., eds. Ullmann's encyclopedia of industrial chemistry **A6**: 483-525.

Von Burg, R. (1995). "Perchlorates." J Appl Toxicol **15**(3): 237-41.

Wilbur, S. M. A., Diamond, Gary Ph.D., Llados, Fernando Ph.D., Odin, Marc M.S., D.A.B.T., Plewak, Daniel B.S., Tunkel, Jay Ph.D. (2005). Toxicological profile for perchlorates. P.

H. S. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry**:** 1-227.

Wolff, J. (1980). "Physiological aspects of iodide excess in relation to radiation protection." Journal of Molecular Medicine **4**: 151-165.

Wolff, J. (1998). "Perchlorate and the thyroid gland." Pharmacol Rev **50**(1): 89-105.

Wolff, J. and J. R. Maurey (1963). "Thyroidal iodide transport. IV. The role of ion size." Biochim Biophys Acta **69**: 58-67.

Yu, K. O., L. Narayanan, et al. (2002). "The pharmacokinetics of perchlorate and its effect on the hypothalamus-pituitary-thyroid axis in the male rat." Toxicol Appl Pharmacol **182**(2): 148-59.

Zanzonico, P. B. and D. V. Becker (2000). "Effects of time of administration and dietary iodine levels on potassium iodide (KI) blockade of thyroid irradiation by ^{131}I from radioactive fallout." Health Phys **78**(6): 660-7.

Zidan, J., E. Hefer, et al. (2004). "Efficacy of ^{131}I ablation therapy using different doses as determined by postoperative thyroid scan uptake in patients with differentiated thyroid cancer." Int J Radiat Oncol Biol Phys **59**(5): 1330-6.

CHAPTER 2

PROPHYLACTIC EVALUATION OF POTASSIUM IODIDE (KI) AND AMMONIUM PERCHLORATE (NH_4ClO_4) TO AMELIORATE $^{131}I^-$ EXPOSURE IN THE RAT[1]

[1] C. A. Harris, J. W. Fisher, E. A. Rollor III, D. C. Ferguson, B. C. Blount, L. Valentin-Blasini, M. A. Taylor, and C. E. Dallas.
Submitted to *Journal of Toxicology and Environmental Health* on September 29, 2008.

Abstract

The risk of radiation poisoning from radioactive iodide ($^{131}I^-$) has led to the need for an effective pharmaceutical intervention. Potassium iodide (KI) is the only intervention that is currently approved by the Food and Drug Administration for treating $^{131}I^-$ exposure. Though effective, stable iodide has significant limitations due to its short range of effective timing of administration. This phenomenon was experienced by thousands of significantly exposed people following the Chernobyl disaster, when the Soviet authorities did not administered KI until more than 72 hours after the start of the nuclear reactor fire, resulting in markedly reduced efficacy. Perchlorate (ClO_4^-) has been used therapeutically to displace non-organified iodine from the thyroid. The objective of this study was to test the relative efficiencies of large doses (30 mg/kg) of KI and ClO_4^- for displacing previously administered $^{131}I^-$. We found that both iodide- and perchlorate-dosed animals had a 65% and 75% blocking efficiency, respectively, compared to saline dosed animals for the 1.5 and 15 hour experiments. At the time of perchlorate and iodide dosing, 87-95% of the radioiodide that had accumulated in the thyroid had been organified. Animals administered perchlorate excreted 43% of the total administered $^{131}I^-$ and 56% of the total administered perchlorate. KI dosed animals excreted 30% of the $^{131}I^-$ and 47% of the therapeutic intervention by the termination of the 15 hour experiment. These experiments demonstrated that each prophylactic approach was capable of inhibiting the uptake of $^{131}I^-$ into the thyroid gland immediately after ingestion and maintain the inhibition over the course of the experiment. However, the perchlorate treatment groups excreted higher cumulative amounts of the $^{131}I^-$ as well as the therapeutic dose making perchlorate a more favorable treatment option compared to KI.

Introduction

Public health concerns over the events of Chernobyl and the Nagasaki and Hiroshima atomic bombs have led to a necessity for a pronounce pharmaceutical intervention for radiation poisoning (Morimoto, Yoshimoto *et al.* 1987; Robbins and Schneider 1998; Balonov 2007; Levin 2008). Of the many radioactive isotopes released during nuclear explosions, radioactive isotopes of iodine are among the most common (Lengemann and Thompson 1963). Iodide is used by the thyroid gland to make thyroid hormones, an important endocrine system in the body. The thyroid gland is unable to distinguish between the stable dietary iodide ($^{127}I^-$) and the radioactive forms of iodide such as $^{131}I^-$. Incorporation of radioactive iodide into the thyroid gland and the formation of radioactive thyroid hormones leads to various medical conditions such as autoimmune thyroiditis and thyroid cancer (Zanzonico and Becker 2000). Currently, the only recognized method of prophylaxis against $^{131}I^-$ poisoning is potassium iodide (KI) (FDA 2001).

Since the introduction of iodine for treatment of thyrotoxicosis in 1923 by Plummer, many researchers have evaluated the use of iodine for pharmaceutical intervention (Saxena, Chapman *et al.* 1962; Lengemann and Thompson 1963; Blum and Eisenbud 1967; Verger, Aurengo *et al.* 2001). Orally ingested iodide enters the systemic circulation and perfuses the thyroid gland. The sodium-iodide symporter (NIS) protein then actively transports iodide into the thyroid gland. Studies have shown excessively high doses (100-200 mg of KI) of iodide cause a temporary blocking effect on thyroidal uptake of iodine by greater than 98% of (Adams and Bonnell 1962; Blum and Eisenbud 1967; Ramsden, Passant *et al.* 1967). The Food and Drug Administration used these studies, as well as others, to outline a dosing regimen of KI for radioactive iodine poisoning based on age and weight. They concluded that an appropriate dose

of KI for adults is 130 mg daily until the threat of radiation exposure no longer exists (FDA 2001). A major shortcoming with the current public health recommendations is that the FDA did not consider the efficacy of a prophylactic dosing regimen for KI prior to the radioiodide exposure from radioactive fallout. Often it will not be possible for people to ingest KI before they are exposed to $^{131}I^-$ fallout. This public health scenario was experienced by thousands of people in the Chernobyl disaster when the Soviet Union administered KI starting 72 hours after the contamination had already occurred (Verger, Aurengo *et al.* 2001) and resulted in an unprecedented and very significant increase in thyroid cancer and thyroid related conditions among children and adolescence. These concerns have led to considerations of another pharmaceutical intervention, perchlorate, which may allow for an enhanced blocking effect for the thyroid gland and perhaps displacement potential of $^{131}I^-$ that has already filled the receptor sites of both NIS and the thyroid.

In 1952, Stanbury and Wyngaarden (Stanbury and Wyngaarden 1952) suggested that single doses of perchlorate strikingly depressed the accumulation rate of $^{131}I^-$ into the thyroid gland when administered prior to $^{131}I^-$. Decades ago Godley and Stanbury (Godley and Stanbury 1954) demonstrated that perchlorate was effective in treating hyperthyroidism in 24 patients by administration of 3-6 mg/kg/day of perchlorate for up to 52 weeks with minimal side effects that were alleviated by administering perchlorate with food. Perchlorate's similarity in size and charge to iodide allows it to compete with iodide for the receptor sites of sodium iodide symporter protein (NIS) (Stanbury and Wyngaarden 1952; Anbar, Guttmann *et al.* 1959; Goldman and Stanbury 1973; Wolff 1998; Merrill, Clewell *et al.* 2003)

The clinical use of both iodide and perchlorate to treat thyroid disorders has resulted in adverse responses. Perchlorate is reported to cause rash, fever, and lymphadenopathy in 2-3% of

patients treated with 3-6 mg/kg/day and 16-18% of patients treated with 17-28 mg/kg/day (Trotter 1962). The clinical use of perchlorate was halted, except for use of single doses for the perchlorate discharge test, in the late 1960's following an outbreak of seven cases of fatal aplastic anemia (Wolff 1998). Perchlorate has since reemerged as a treatment of thyrotoxicosis (Bartalena, Brogioni *et al.* 1996). Adverse effects from iodine administration include iodine-induced hyperthyroidism, iodine-induced hypothyroidism, and some non-thyroidal effects (Nauman and Wolff 1993). Cases of iodine-induce hyperthyroidism have been reported to vary between 2 and 12% of patients and increase in patients who were iodide deficient. Sudden acute or chronic exposure to iodine can cause the effects of hyperthyroidism in persons with normal thyroid activity, but is much more common in persons with multinodular thyroid glands and Grave's disease (Stanbury, Ermans *et al.* 1998). Atrial fibrillation has also been shown to occur in 15-20% of patients with hyperthyroidism, especially in elderly patients with coronary insufficiency (Dunn, Semigran *et al.* 1998). Iodine-induce hypothyroidism is much more common in newborns and preterm babies than in adults due to their low thyroid iodine levels (Brown, Bloomfield *et al.* 1997) and an underdeveloped thyroid uptake regulatory system. Long term effects from undiagnosed iodine-induced hypothyroidism in neonates can lead to impaired neurological and mental development (Nauman and Wolff 1993). Non-thyroidal allergic effects of iodine therapy include fever, swelling of the face and body, shortness of breath, and rashes (Crocker 1984; Pennington 1990).

Despite the drawbacks from the use of iodide or perchlorate, these drugs remain viable candidates for treatment of radioactive iodide exposure. The objective of the present study was to evaluate the prophylactic efficacy of a single dose of either KI or perchlorate after $^{131}I^-$ is administered to rats

Materials and Methods

Chemicals

Ammonium perchlorate (99.8%), trichloroacetic acid, bovine serum albumin, and propylthiouracil were purchased from Aldrich (Milwaukee, WI). Potassium iodide was purchased from J. T. Baker. Carrier-free iodine-131 was purchased from Amersham Biosciences (29.4 mCi/ug). Acepromazine maleate (10 mg/ml) and ketamine HCL (100 mg/ml) were purchased from Fort Dodge Animal Health (Fort Dodge, Iowa). Xylazine (20 mg/ml) was purchased from Ben Venue Laboratories (Bedford, Ohio).

Animals

Male Sprague-Dawley rats (330 ± 30 g) were used throughout the experiments and were obtained from Harlan Laboratories (Indianapolis, Indiana). The animals were individually housed in metabolism cages for urine collection and were given a 5 day acclimation period to the metabolism cage prior to the start of the experiment (a one week acclimation period in the animal facility was allowed before moving into the metabolism cages). The cages were stored in an environmentally controlled room (12 h light/12 h dark cycle, 22 ± 2°C room temperature, 50 ± 20% relative humidity, 10-15 air changes/hr). Animals were provided LabDiet Laboratory Rodent Diet 5001 rat chow and water *ad libitum*. Sera, urine, and tissue samples were stored at -80°C until analysis. The animals used in this study were handled in accordance with the procedures of The University of Georgia Institutional Animal Care and Use Committee (IACUC), AUP# A2005-10110-0.

Experimental Design

To test the objective, rats were orally administered $^{131}I^-$ followed by oral administration of saline, 30 mg/kg KI, or 30 mg/kg NH_4ClO_4 at designated time intervals post radioiodide dose. After sacrifice, thyroids, serum, and cumulative urine were analyzed for $^{131}I^-$ content and expressed as percentages of the saline dosings. Thirty-six animals were used for each experimental study. A summary of the experiments is shown in Figure 2.1. Animals were randomly assigned to individual metabolism cages and given a 5 day acclimation period prior to the start of the experiments. The night before the experiments food, but not water, was removed from the animals and was not returned. The following morning animals were weighed and gavaged with 1 ml of a 2.91 μCi (6 ng/kg) $^{131}I^-$ solution at time 0. Animals were then gavaged with approximately 1 ml solutions of 0.9% saline, 30 mg/kg KI dissolved in 0.9% saline, or 30 mg/kg perchlorate dissolved in 0.9% saline based on body weight at either time 0.5 or 3 hours post radioiodide exposure (Figure 2.1 A & B). The animals were anesthetized with a ketamine cocktail (0.1 ml per 100 g body weight) and moved from the experimental room to a surgical room where they were subsequently euthanized at time 1.5 and 15 hours, respectively, by CO_2 asphyxiation and thyroids, blood, and cumulative urine was collected (Figure 2.1 A & B). Blood was withdrawn via cardiac puncture and serum prepared by centrifugation at 1500 rpm at 4°C for 15 minutes; thyroid lobes were removed from the trachea, weighed, and placed in 400 μl of 1 mM PTU to prevent organification; and cumulative urine was collected from the metabolism cages and bladder.

Separate sets of control experiments were also performed in which no $^{127}I^-$ or perchlorate was given. Four animals for each control study were randomly assigned to metabolism cages and received a 5 day acclimation period prior to the start of the experiment. These experiments

were performed to characterize the percent organification of radioiodide in the thyroid at the time that the radioprotectant was administered and to see if there was any further accumulation of $^{131}I^-$ into the thyroids of animals that were therapeutically dosed. In the first experiment animals were given radioiodide at time 0 and then sacrificed at time 3 hours. In a second experiment animals were administered radioiodide at time 0 and sacrificed at time 0.5 hours. Blood serum, thyroids, and cumulative urine were collected as described previously for experimental treatment studies.

$^{131}I^-$ *Analysis*

After sacrifice, thyroid, urine, and serum samples were all placed on a 1470 Wallac Wizard Gamma Counter equipped with one detector to get raw $^{131}I^-$ counts/minute (cpm). Raw counts were recorded and the thyroids were homogenized with a mortar and pestle and centrifuged at 16,100 rpm for 1 hour at 4°C to pellet the homogenate. The supernatant was removed and filtered through a 0.45-µm filter to remove any leftover thyroid tissue. Supernatants were counted on the γ-counter. After counting the supernatants, procedures from Wolff Chaikoff (1948) and Goldman and Stanbury (1973) were modified for analysis of the bound and free fractions of $^{131}I^-$ in the thyroid. The precipitation was performed as follows: 50 µl of the supernatant were added to 500 µl of a 10% bovine serum albumin (BSA) solution and vortexed for 20 seconds, the resulting solution was allowed to incubate on ice for 30 minutes, then 500 µl of a 20% TCA solution was added and the resulting solution was vortexed for another 20 seconds. This mixture was allowed to incubate on ice for 15 minutes before being centrifuged at 16,100 rpm for 10 minutes at 4°C to form a TCA pellet and a TCA supernatant, the supernatant was then removed from the pellet and both the TCA pellet and supernatant were counted on the γ-counter.

$^{127}I^-$ and ClO_4^- Analysis

Non-radioactive analytes ($^{127}I^-$ and ClO_4^-) were quantified using ion chromatography coupled with mass spectrometry. Serum samples were spiked with internal standard ($^{129}I^-$ and $Cl^{18}O_4^-$), treated to remove proteins and analyzed by ion chromatography electrospray ionization mass spectrometry (Amitai, Winston *et al.* 2007). Urine samples were spiked with internal standard ($^{129}I^-$ and $Cl^{18}O_4^-$) and analyzed by ion chromatography electrospray ionization mass spectrometry (Valentin-Blasini, Blount *et al.* 2007).

Converting counts per minute to concentration

For solutions containing a specific activity of approximately 29.5 mCi $^{131}I^-$/ug I (~3.4 ug I/100 mCi $^{131}I^-$) ratios of the amount (ng) of iodide as $^{131}I^-$ administered to each animals are calculated as follows:

$$\left(uCi_O / \frac{ug_O}{V_O}\right) = \left(uCi_A / \frac{x}{V_A}\right) \quad [1]$$

$$\left(cpm_D / x\right) = \left(cpm_S / y\right) \quad [2]$$

where uCi_o equals number uCi's in the ordered solution, ug_o equals the number of micrograms in the purchased solution, V_o equals the volume of the purchased solution, uCi_A equals the number of mCi's administered to each animals, x equals the unknown ng of iodide in the dose, V_A equals the volume of the dose given, cpm_D equals counts per minute of the dose, cpm_S equals the counts per minute in each sample taken from the animals (ie. thyroid, serum, and urine), and y equal the unknown concentration of $^{131}I^-$ in ng/ml.

Urinary Excretion Data

Urine samples for the 1.5 and 15 hour experiments were analyzed for $^{131}I^-$, $^{127}I^-$, and ClO_4^-. Radioiodide parameters included ng excreted per ml of urine, total ng excreted, ng excreted per hour, and percent of $^{131}I^-$ excreted. Calculations for radioiodide parameters proceeded as follows:

$$ng\ excreted\ per\ ml = ((A/B) * C) * 1000 \quad [3]$$

$$TOT_{cml}\ (ng) = ng/ml * V_t \quad [4]$$

$$\%\ excreted = (TOT_{cml}/C) * 100 \quad [5]$$

where A equals counts/ml of urine, B equals the total number of counts administered to the animal, and C equals the dose of $^{131}I^-$ in the dosing solution, and V_t equals cumulative volume of urine excreted over a specific time interval. Perchlorate and stable iodide parameters included ug excreted per ml of urine (reported from IC analysis), total ug excreted, ug excreted per hour, and percent of prophylactic excreted. Calculations for stable iodide and perchlorate endpoints proceeded as follows:

$$TOT_{cml,ClO_4}\ (ug) = ug/ml * V_t \quad [6]$$

$$\%\ excreted = (TOT_{cml,ClO_4}/C_0) * 100 \quad [7]$$

where V_t equals cumulative volume of urine excreted over a specific time interval and C_0 equals dose of prophylactic administered to the animals.

Statistical Analysis

Single factor ANOVA was used initially to determine significance between the three treatment groups with significance set at $p<0.05$. Once significance was determined a two-

sample t-test assuming equal variance was used for comparison of statistical significance between each dose group ($p<0.05$).

Results

The 1.5 and 15 hour thyroid data for animals treated with perchlorate and stable iodide indicated appreciably less thyroidal $^{131}I^-$ accumulation relative to saline treatments. Saline, KI, and perchlorate dosed animals had thyroidal $^{131}I^-$ concentrations of 0.0026 ± 0.0004, 0.0009 ± 0.0002, and 0.0008 ± 0.0003 ng/mg at 1.5 hours and 0.0184 ± 0.0039, 0.0043 ± 0.0008, and 0.0048 ± 0.0011 ng/mg at 15 hours (Figure 2.2). These results signify a highly significant and equivalent decrease ($p<0.001$) in thyroidal $^{131}I^-$ concentration of animals that were put on treatment compared to those that received a saline dose.

Control experiments at 0.5 and 3 hours post radioiodide dose accumulated concentrations of 0.0010 ± 0.0002 and 0.0087 ± 0.0016 ng/mg of $^{131}I^-$ in the thyroid respectively. Comparing control experiments to their saline and prophylactic counterparts revealed that saline treatments accumulated three times the concentration of $^{131}I^-$ in the thyroid (0.0010 to 0.0026 ng/mg) at 1.5 hours and two times the concentration of $^{131}I^-$ in the thyroid (0.0087 to 0.0184 ng/mg) at 15 hours. Saline animals at each time-point had a highly significant increase in thyroidal $^{131}I^-$ concentration when compared to control experiments ($p<0.001$). An immediate cessation of further accumulation of $^{131}I^-$ in the thyroid was determined as soon as treatment with KI and perchlorate was initiated. Thyroidal $^{131}I^-$ concentrations for 0.5 hour control animals, 1.5 hour KI animals, and 1.5 hour perchlorate animals were 0.0010, 0.0009, and 0.0008 ng/mg, respectively. Concentrations for 3 hour control animals and 15 hour KI and perchlorate treatment groups were

0.0087, 0.0043, and 0.0048 ng/mg, respectively, with KI and perchlorate dose groups containing a significantly lower concentration of $^{131}I^-$ ($p<0.001$).

The percent organification of iodide in the thyroid as bound and free fractions for experimental and control animals are shown in Table 2.1. For the 1.5 and 15 hour time-points >90% of the total radioiodide located in the thyroid had been organified in all three dose groups. No significance was determined between the saline, KI, or perchlorate treatments for the bound and free fractions of the $^{131}I^-$ challenge at either time-point. At the 1.5 hour time-point a statistically significant increase ($p<0.05$) in intrathyroidal inorganic iodide was ascertained in animals that were dosed with high concentrations of KI. Comparisons between the 1.5 and 15 hour time-points for similar dose groups revealed that saline dosed animals organified 3.2% more $^{131}I^-$ at the late time-point, KI dosed animals organified 6.5% more $^{131}I^-$ at the late time-point, and perchlorate dosed animals organified 1.6% more $^{131}I^-$ at the late time-point. Bound and free fraction measurements in control experiments were 86.8 ± 1.3% organified at 0.5 hours post radioiodide dose and 94.8 ± 1.0% organified at three hours post $^{131}I^-$ dose.

Serum and urine samples were analyzed for radioiodide, perchlorate, and stable iodide concentrations. The serum data for radioiodide concentration are shown in Figure 2.3. At each time-point the lowest concentration of serum radioiodide was in the saline dosed animals. For the 15 hour study there was a highly significant decrease of 35% in serum concentration for perchlorate and saline dosed animals compared to KI dosed animals ($p < 0.001$). In the 1.5 hour study, both KI and perchlorate treated animals had equivalent serum concentrations and a highly significant increase in $^{131}I^-$ of 20% relative to the saline treatment ($p < 0.001$).

Stable iodide and perchlorate concentrations were measured by IC analysis in the serum and are displayed in Figure 2. 4. Concentrations of stable iodide and perchlorate were 86.9 ±

48.3 and 70.2 ± 27.1 ug/ml at 1.5 hours and 47.7 ± 17.3 and 11.9 ± 3.3 ug/ml at 15 hours. Across each time-point perchlorate dosed animals exhibited lower serum concentrations of the prophylactic dose than did the animals that received stable iodide. The 1.5 hour study had a 20% decrease in serum perchlorate concentration, though it was not statistically significant. The 15 hour data demonstrated a 75% decrease in serum perchlorate concentration relative to stable iodide ($p < 0.001$).

Renal $^{131}I^-$ elimination data are shown in Table 2.2. No significance was determined between the void volumes for any of the treatment groups at either time-point. By the termination of the 15 hour study the saline, KI, and perchlorate dosed animals had excreted 16%, 30%, and 43%, respectively of the total administered $^{131}I^-$. Both the 1.5 and 15 hours studies revealed that animals administered perchlorate had a significant increase in excretion of the cumulative amount of $^{131}I^-$ when compared to animals who received a control dose ($p<0.001$ for 15 hour and $p<0.05$ for 1.5 hours). Perchlorate dosed animals also demonstrated a significant increase in excreted $^{131}I^-$ relative to the KI dosings during the 15 hour study ($p<0.001$). KI dosed animals had a significant increase in excreted $^{131}I^-$ concentration compared to saline dosed animals at the 15 hour time-point ($p<0.001$), but at the 1.5 hour time-point no statistical significance was noted.

Percent excretion of the prophylactic dose, ug/ml, cumulative amounts, and ug excreted per hour are shown in Table 2.3 for each study. These results confirm that perchlorate was excreted more rapidly in the urine than iodide. In total, perchlorate and KI dosed animals excreted 12% and 6% of the administered prophylactic dose at 1.5 hours and 56% and 47% of the administered prophylactic dose at 15 hours, respectively. A statistically significant increase ($p<0.05$) in the prophylactic excretion of perchlorate versus stable iodide was determined at 15

hours, but not at 1.5 hours. Across both studies, animals administered perchlorate excreted 21% and 95% more of the dose than did animals who received KI. Considerations of the time variation across similar dosings demonstrated that 86% and 77% of the stable iodide and perchlorate were excreted after the 1.5 hour time-point, respectively.

The thyroid:serum, thyroid:urine, and urine:serum radioiodide concentration ratios are shown in Figure 2.5 A, B, and C for both studies. These results indicate that most of the radioiodide across experiments and dosing groups was located in the urine when compared to the other measured endpoints. Considering prophylactic dosing time differences, thyroid:serum ratios increased by 90-95% for each similar dose group from the 1.5 to the 15 hour time-points. Thyroid:urine ratios increased by 81%, 57%, and 77% for saline, KI, and perchlorate dosed animals, respectively, from the early to the late time-points. Urine:serum ratios increased by 59%, 78%, and 84%, respectively from the early to the late time-point. At the 1.5 hour time-point all three dose groups had higher concentrations of the radioiodide in the serum relative to the thyroid. However, by the 15 hour time-point only those animals dosed with KI had a higher concentration of radioiodide in the serum relative to the thyroid. Perchlorate treated animals had approximately a one to one thyroid:serum ratio at 15 hours, while saline dosed animals contained over four times the concentration of the radioiodide in the thyroid relative to the serum.

Discussion

The objective of this study was to compare the relative inhibition of radioiodide uptake into the thyroid gland following large doses of either potassium iodide or perchlorate. In the unblocked gland, animals at the 1.5 and 15 hour time-points accumulated approximately 2.1% and 15.9% of the total orally administered $^{131}I^-$ dose. However, these numbers declined

significantly following the administration of stable iodide or perchlorate. For iodide and perchlorate dosings at 1.5 and 15 hour time-points the total percent uptake of the radioiodide plummeted to 0.756% and 3.4% for iodide and 0.698% and 3.7% for perchlorate respectively ($p < 0.001$). The remaining radioiodide was predominantly excreted in the urine or dispersed in small amounts throughout the carcass.

The effectiveness of preventing radiation poisoning using prophylactic doses of iodide or perchlorate is both time and dose-dependent (Wolff 1980). To achieve the highest levels (>90%) of thyroid protection, it is necessary to take a loading dose of either iodide or perchlorate before any threat of radioiodide reaches the general population. Ample evidence exists in the literature that taking loading doses of either prophylactic blocks >98% of the ingested radioiodide (Ramsden, Passant *et al.* 1967; Wolff 1980; Zanzonico and Becker 2000). However, unanticipated radioiodide exposure dictates that taking a loading dose would not be feasible. Iodide administered up to four hours after ingestion of $^{131}I^-$ almost immediately halted the further accumulation of the isotope into the thyroid gland (Lengemann and Swanson 1957; Adams and Bonnell 1962; Pochin and Barnaby 1962; Blum and Eisenbud 1967; Pahuja, Rajan *et al.* 1993). These same studies also reported that delaying iodide prophylaxis for to 2-4 hours inhibited 60-80% of the radioiodide from entering the thyroid. When comparisons were made in the current study between controls and therapeutic dosings, there was a trend of inhibition of further up-regulation of radioiodide into the thyroid at both the early and late time-points employed. Control animals at three hours up-regulated approximately twice the concentration of $^{131}I^-$ in the thyroid compared to animals dosed with either iodide or perchlorate 12 hours later. The 0.5 hour control and 1.5 hour iodide and perchlorate dosed animals had an equivalent concentration of $^{131}I^-$ which provided evidence for immediate blocking of the isotope.

Characterization of bound and free fractions of total thyroidal iodide proved to be a viable determinant of the relevance of liberating the previously accumulated radioiodide after the therapeutics had been delivered. At the earliest time-point (+0.5 hours) only 13% of the total thyroidal iodide that had accumulated in the gland was available for displacement, and one hour later that percentage fell to 5%. Under euthyroid conditions the thyroid is able to bind iodide at a faster rate than it can be transported in, resulting in low levels of free intrathyroidal iodide pools (Berson and Yalow 1955). However, when binding is impaired, as is the case with autoimmune thyroiditis and to a smaller extent when large amounts of iodide are ingested, a pool of inorganic iodide starts to accumulate in the intrathyroidal space (Wolff and Chaikoff 1948; Dayan and Daniels 1996). In the current study, at the 1.5 hour time-point KI dosed animals had significantly more intrathyroidal free iodide than did the perchlorate and saline dosed animals at the same time. This finding can be explained by the Wolff-Chaikoff effect, an autoregulatory phenomenon which inhibits formation of thyroid hormones when plasma levels exceed 0.25-0.35 ug/ml (Wolff and Chaikoff 1948; Yu, Narayanan *et al.* 2002). At the 1.5 hour time-point serum iodide levels for KI dosed animals were 87 ± 48 ug/ml. Consequently, the organification of iodide is slowed down and a larger concentration of iodide in the thyroid is in the free form. This would also explain why there was no significance at the 15 hour time-point. Approximately 50% of the KI dose had been excreted in the urine by the termination of the 15 hour experiment. The excretion of KI considerably lowered circulating serum concentrations of iodide and allowed the thyroid to once again organify as much iodide as was being up-regulated, resulting in a lower concentration of inorganic iodide in the thyroid.

Perchlorate has been shown to be able to displace inorganic iodide from the thyroid when abnormalities in binding and organification are present, i.e. the perchlorate discharge test

(Stewart and Murray 1966; Gray, Hooper *et al.* 1972; Gray, Hooper *et al.* 1973). Displacement of inorganic iodide is accomplished due to the higher affinity of NIS for perchlorate than iodide ($ClO_4^- > ReO_4^- > I^- \geq SCN^- > ClO_3^- > Br^-$) (Van Sande, Massart *et al.* 2003). Perchlorate also has a potential displacement potential on free intrathyroidal iodide. Oral dosing of perchlorate in the perchlorate discharge test has been verified to be insensitive and inaccurate, especially in the case of small intrathyroidal iodide pools (Gray, Hooper *et al.* 1972). Since small pools of inorganic iodide existed in the thyroid at the time of perchlorate treatment and perchlorate possesses a mechanism of accelerating the loss of free iodide in the thyroid, it is feasible that the perchlorate dose would have led to lower free iodide concentrations in the thyroid and a subsequently higher excretion rate, though it cannot be stated to an absolute certainty.

The animals were fasted before the experiment was conducted to ensure complete oral absorption of the isotope and the therapeutic interventions. This meant that the animals in the 15 hour study were fasted for approximately 26 hours. Most of the literature involving fasting in thyroid studies had a minimum of a two-day fasting period before thyroid hormones and other end-points were measured, so correlation to these experiments are inadequate. It is therefore uncertain whether fasting the animals for 26 hours had any significant impact on thyroid function. One study involving a two-day fasting, concluded that rats lost 12-15% of their body weight, but had no effect on hypothalamic TRH, but did have a significant decrease in pituitary TSH, serum TSH, serum T_4, free T_4, and free T_3 (Harris, Fang *et al.* 1978). Serum T_3 loss during the study was likely a secondary response to the reduction in serum T_4. In the rat, 60% of circulating T_3 comes from conversion of T_4 to T_3 by 5'-deiodinase activity in the peripheral tissues (Abrams and Larsen 1973). Similar results have also been reported in longer fasting

studies in rats and in humans (D'Angelo 1951; Portnay, O'Brian *et al.* 1974; Campbell, Kurcz *et al.* 1977; Carlson, Drenick *et al.* 1977).

Serum $^{131}I^-$ data in this study reflected interesting relationships in the state of the thyroid gland and urinary excretion rates. At the 15 hour time-point, the lowest concentrations of radioiodide were located in the saline and perchlorate dosed animals, whereas KI dosed animals had a significantly higher serum concentration. These results can be attributed to the increased uptake of $^{131}I^-$ in the unblocked gland for saline dosed animals and the enhanced urinary excretion of $^{131}I^-$ in perchlorate dosed animals. The magnitude of thyroidal uptake of iodide (or perchlorate) depends on its serum concentration (Chow, Chang *et al.* 1969). In our study, KI blocked 75% of the radioiodide from entering the thyroid but had significantly lower excretion rate, which relegated the $^{131}I^-$ to the serum. At the 1.5 hour time-point, the lowest concentration of radioiodide was in saline dosed animals (probably due to the unblocked gland), while at this time-point KI and perchlorate dosed animals had equivalent $^{131}I^-$ concentration (and higher than controls). However, at 1.5 hours KI and perchlorate dosed animals had equivalent blocking efficiencies of radioiodide entering the thyroid, and no significance in urinary excretion of $^{131}I^-$ between the two doses was determined.

Excretion of $^{131}I^-$ proved to be the most efficient determinant of prophylaxis between KI and perchlorate dosed animals in the current study. When all end points were considered across iodide and perchlorate dosings, the only major difference occurred in the urine. Indeed, it has long been established that urinary excretion is the predominant clearance of $^{131}I^-$ (Johnson and Albert 1951). Perchlorate urinary excretion far exceeded that of the KI data with 43% versus 31%, respectively of the total radioactive dose excreted by 15 hours. Previous studies have demonstrated that approximately 90% of the radioiodine dose was excreted in the urine by 24

hours of animals receiving perchlorate (Sinadinovic and Jovanovic 1971). In that study, iodide and perchlorate prophylaxis were able to excrete approximately the same quantity of radioiodide in the urine, though iodide prophylaxis had a marked decrease in the excretion rate of $^{131}I^-$ in the early hours. The 1.5 hour time-point was a relatively short time frame to be able to consider urinary output, and no statistical difference was determined between the perchlorate and the KI dosing. This early time-point data contradicted the data of Halmi et al. (1958) who administered subcutaneous loading doses of KI and perchlorate and determined that iodide dosed animals excreted a higher concentration of $^{131}I^-$ than perchlorate dosed animals at one hour after isotope delivery.

It was significant that a high proportion of the therapeutic intervention doses were excreted following 12 hours in the current study. Excessive doses of perchlorate and iodide with continued administration over long periods of time have been shown to cause various thyroid related disorders from hypothyroidism, goiter, nodules, thyrotoxicosis, and aplastic anemia (Adams and Bonnell 1962; Krevans, Asper *et al.* 1962; Meck, Chen *et al.* 1985; Zanzonico and Becker 2000). Previous studies have reported that perchlorate does not appear to be metabolized in the body and is excreted unchanged in the urine (Anbar, Guttmann *et al.* 1959; Eichler and Hackenthal 1962). For the large doses administered in the current study, 47% of stable iodide and 56% of perchlorate had been excreted by the 15 hour time-point 12 hours after the dose). Similar studies have revealed that when 0.1-3.0 mg/kg of perchlorate were administered to rats by intravenous (iv) injection that 72-97% of the total dose of perchlorate was excreted in the urine by 24-26 hours (Fisher, Todd *et al.* 2000; Yu, Narayanan *et al.* 2002).

In conclusion, the primary goal of these studies was to evaluate the relative prophylactic efficacies of KI and perchlorate on the thyroid gland in response to $^{131}I^-$ contamination. Both

therapeutics were efficient in blocking the uptake of $^{131}I^-$ from entering the thyroid when compared to the saline dosings. However, neither therapeutic approach demonstrated a statistically significant enhancement of the ability to block the uptake of radioiodide relative to the other treatment. Since perchlorate dosed animals excreted significantly higher levels of radioiodide than the iodide dosed animals, future studies should include a primary focus on urinary excretion profiles of radioiodide following administration of potassium iodide or perchlorate.

Acknowledgements

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

References

Abrams, G. M. and P. R. Larsen (1973). "Triiodothyronine and thyroxine in the serum and thyroid glands of iodine-deficient rats." J Clin Invest **52**(10): 2522-31.

Adams, C. A. and J. A. Bonnell (1962). "Administration of stable iodide as a means of reducing thyroid irradiation resulting from inhalation of radioactive iodine." Hisp Med **7**: 127-49.

Amitai, Y., G. Winston, et al. (2007). "Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels." Thyroid **17**(9): 843-50.

Anbar, M., S. Guttmann, et al. (1959). "The mode of action of perchlorate ions on the iodine uptake of the thyroid gland." Int J Appl Radiat Isot **7**: 87-96.

Balonov, M. (2007). "Third annual Warren K. Sinclair keynote address: retrospective analysis of impacts of the Chernobyl accident." Health Phys **93**(5): 383-409.

Bartalena, L., S. Brogioni, et al. (1996). "Treatment of amiodarone-induced thyrotoxicosis, a difficult challenge: results of a prospective study." J Clin Endocrinol Metab **81**(8): 2930-3.

Berson, S. A. and R. S. Yalow (1955). "The iodide trapping and binding functions of the thyroid." J Clin Invest **34**(2): 186-204.

Blum, M. and M. Eisenbud (1967). "Reduction of thyroid irradiation from 131-I by potassium iodide." Jama **200**(12): 1036-40.

Brown, R. S., S. Bloomfield, et al. (1997). "Routine skin cleansing with povidone-iodine is not a common cause of transient neonatal hypothyroidism in North America: a prospective controlled study." Thyroid **7**(3): 395-400.

Campbell, G. A., M. Kurcz, et al. (1977). "Effects of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin; response to LH-releasing hormone and thyrotropin-releasing hormone." Endocrinology **100**(2): 580-7.

Carlson, H. E., E. J. Drenick, et al. (1977). "Alterations in basal and TRH-stimulated serum levels of thyrotropin, prolactin, and thyroid hormones in starved obese men." J Clin Endocrinol Metab **45**(4): 707-13.

Chow, S. Y., L. R. Chang, et al. (1969). "A comparison between the uptakes of radioactive perchlorate and iodide by rat and guinea-pig thyroid glands." J Endocrinol **45**(1): 1-8.

Crocker, D. G. (1984). "Nuclear reactor accidents--the use of KI as a blocking agent against radioiodine uptake in the thyroid--a review." Health Phys **46**(6): 1265-79.

D'Angelo, S. A. (1951). "The effect of acute starvation on the thyrotrophic hormone level in the blood of the rat and mouse." Endocrinology **48**(3): 341-3.

Dayan, C. M. and G. H. Daniels (1996). "Chronic autoimmune thyroiditis." N Engl J Med **335**(2): 99-107.

Dunn, J. T., M. J. Semigran, et al. (1998). "The prevention and management of iodine-induced hyperthyroidism and its cardiac features." Thyroid **8**(1): 101-6.

Eichler, O. and E. Hackenthal (1962). "Uber Ausscheidung und Stoffwechsel von Perchlorat gemessen mit $^{36}ClO_4^-$ [Excretion and metabolism of perchlorate measured by $^{36}ClO_4^-$]." Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol. **243**: 554-565.

FDA (2001). Potassium Iodide as a Thyroid Blocking Agent in Radiation Emergencies. FDA. **66:** 1-15.

Fisher, J., P. Todd, et al. (2000). "Preliminary development of a physiological model for perchlorate in the adult male rat: a framework for further studies." Drug Chem Toxicol **23**(1): 243-58.

Godley, A. F. and J. B. Stanbury (1954). "Preliminary experience in the treatment of hyperthyroidism with potassium perchlorate." J Clin Endocrinol Metab **14**(1): 70-8.

Goldman, S. J. and J. B. Stanbury (1973). "The metabolism of perchlorate in the rat." Endocrinology **92**(5): 1536-8.

Gray, H. W., L. A. Hooper, et al. (1973). "An evaluation of the 20 minute perchlorate discharge test." J Clin Endocrinol Metab **37**(3): 351-5.

Gray, H. W., L. A. Hooper, et al. (1972). "A twenty minute perchlorate discharge test." J Clin Endocrinol Metab **34**(3): 594-7.

Harris, A. R., S. L. Fang, et al. (1978). "Effect of starvation on hypothalamic-pituitary-thyroid function in the rat." Metabolism **27**(9): 1074-83.

Johnson, H. W. and A. Albert (1951). "The excretion and distribution of ^{131}I following administration of physiologic amounts of labeled iodide, diiodotyrosine and thyroxine in the rat." Endocrinology **48**(6): 669-81.

Krevans, J. R., S. P. Asper, et al. (1962). "Fatal Aplastic Anemia Following Use of Potassium Perchlorate in Thyrotoxicosis." JAMA **181**: 162-164.

Lengemann, F. W. and E. W. Swanson (1957). "A Study of the Secretion of Iodine in Milk of Dairy Cows, Using Daily Oral Doses of I^{131}." Journal of Dairy Science **40**: 216-224.

Lengemann, F. W. and J. C. Thompson, Jr. (1963). "Prophylactic and Therapeutic Measures for Radioiodine Contamination--a Review." Health Phys **9**: 1391-7.

Levin, R. J. (2008). "Incidence of thyroid cancer in residents surrounding the Three Mile Island nuclear facility." Laryngoscope **118**(4): 618-28.

Meck, R. A., M. S. Chen, et al. (1985). "Criteria for the administration of KI for thyroid blocking of radioiodine." Health Phys **48**(2): 141-57.

Merrill, E. A., R. A. Clewell, et al. (2003). "PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat." Toxicol Sci **73**(2): 256-69.

Morimoto, I., Y. Yoshimoto, et al. (1987). "Serum TSH, thyroglobulin, and thyroidal disorders in atomic bomb survivors exposed in youth: 30-year follow-up study." J Nucl Med **28**(7): 1115-22.

Nauman, J. and J. Wolff (1993). "Iodide prophylaxis in Poland after the Chernobyl reactor accident: benefits and risks." Am J Med **94**(5): 524-32.

Pahuja, D. N., M. G. Rajan, et al. (1993). "Potassium iodate and its comparison to potassium iodide as a blocker of 131I uptake by the thyroid in rats." Health Phys **65**(5): 545-9.

Pennington, J. A. (1990). "A review of iodine toxicity reports." J Am Diet Assoc **90**(11): 1571-81.

Pochin, E. E. and C. F. Barnaby (1962). "The effect of pharmacological doses of non-radioactive iodide on the course of radio-iodine uptake by the thyroid." Health Phys **7**: 125-6.

Portnay, G. I., J. T. O'Brian, et al. (1974). "The effect of starvation on the concentration and binding of thyroxine and triiodothyronine in serum and on the response to TRH." J Clin Endocrinol Metab **39**(1): 191-4.

Ramsden, D., F. H. Passant, et al. (1967). "Radioiodine uptakes in the thyroid. Studies of the blocking and subsequent recovery of the gland following the administration of stable iodine." Health Phys **13**(6): 633-46.

Robbins, J. and A. B. Schneider (1998). "Radioiodine-induced thyroid cancer: Studies in the aftermath of the accident at Chernobyl." Trends Endocrinol Metab **9**(3): 87-94.

Saxena, K. M., E. M. Chapman, et al. (1962). "Minimal dosage of iodide required to suppress uptake of iodine-131 by normal thyroid." Science **138**: 430-1.

Sinadinovic, J. P. and M. Jovanovic (1971). "Jticaj perhlorata i stabilnog jodida na eliminciju radioaktivnog joda (131 J) iz organizma [Effect of perchlorate and stabile iodide on the elimination of radioactive iodine (I 131) from the body]." Glas Srp Akad Nauka [Med](23): 29-36.

Stanbury, J. B., A. E. Ermans, et al. (1998). "Iodine-induced hyperthyroidism: occurrence and epidemiology." Thyroid **8**(1): 83-100.

Stanbury, J. B. and J. B. Wyngaarden (1952). "Effect of perchlorate on the human thyroid gland." Metabolism **1**(6): 533-9.

Stewart, R. D. and I. P. Murray (1966). "An evaluation of the perchlorate discharge test." J Clin Endocrinol Metab **26**(10): 1050-8.

Trotter, W. R. (1962). "The relative toxicity of antithyroid drugs." J New Drugs **2**: 333-43.

Valentin-Blasini, L., B. C. Blount, et al. (2007). "Quantification of iodide and sodium-iodide symporter inhibitors in human urine using ion chromatography tandem mass spectrometry." J Chromatogr A **1155**(1): 40-6.

Van Sande, J., C. Massart, et al. (2003). "Anion selectivity by the sodium iodide symporter." Endocrinology **144**(1): 247-52.

Verger, P., A. Aurengo, et al. (2001). "Iodine kinetics and effectiveness of stable iodine prophylaxis after intake of radioactive iodine: a review." Thyroid **11**(4): 353-60.

Wolff, J. (1980). "Physiological aspects of iodide excess in relation to radiation protection." Journal of Molecular Medicine **4**: 151-165.

Wolff, J. (1998). "Perchlorate and the thyroid gland." Pharmacol Rev **50**(1): 89-105.

Wolff, J. and I. L. Chaikoff (1948). "Plasma inorganic iodide as a homestatic regulator of thyroid function." J. Biol. Chem. **174**: 555-564.

Yu, K. O., L. Narayanan, et al. (2002). "The pharmacokinetics of perchlorate and its effect on the hypothalamus-pituitary-thyroid axis in the male rat." Toxicol Appl Pharmacol **182**(2): 148-59.

Zanzonico, P. B. and D. V. Becker (2000). "Effects of time of administration and dietary iodine levels on potassium iodide (KI) blockade of thyroid irradiation by 131I from radioactive fallout." Health Phys **78**(6): 660-7.

Table 2.1. Calculated percent bound and free iodide. Values for each time-point and dose are based on TCA precipitation data where the TCA pellet counts per minute divided by the total counts per minute of the pellet and supernatant represent the bound fraction and the supernatant divided by the total represents the free iodide portion in the thyroid. All values are based on averages where 15 and 1.5 hour animals have an n=6 and 4 hr and 0.5 hour animals have an n=4.

Time & Dose	% Bound	% Free
0.5 hr Control	86.8 ± 1.27	13.2 ± 1.27
1.5 hr Saline	93.8 ± 0.78	6.2 ± 0.78
1.5 hr KI	90.6 ± 2.30	9.4 ± 2.30
1.5 hr ClO_4^-	95.9 ± 1.24	4.1 ± 1.24
3 hr Control	94.8 ± 1.00	5.2 ± 1.00
15 hr Saline	96.9 ± 0.40	3.1 ± 0.40
15 hr KI	96.9 ± 0.47	3.1 ± 0.47
15 hr ClO_4^-	97.4 ± 0.23	2.6 ± 0.23

Table 2.1

Table 2.2 Total urinary radioiodide excretion data. Urine was collected via metabolism cages from male rats dosed via gavage with $^{131}I^-$ at time 0 followed by saline, KI (30 mg/kg), or perchlorate (30 mg/kg) at +0.5 hours for the 1.5 hour study and + 3 hours for the 15 hour study via gavage. For descriptions of calculations refer to methods section. Data are means ± SD (n=12 at 15 hrs, n=6 at 1.5 hrs). *Significantly different from control ($p<0.05$ at 1.5 hours and $p<0.001$ at 15 hours). $^{\#}$Significantly different from KI dose ($p<0.05$).

Time & Dose	$^{131}I^-$ 1.5 hour Saline	$^{131}I^-$ 1.5 hour Iodide	$^{131}I^-$ 1.5 hour ClO_4^-	$^{131}I^-$ 15 hour Saline	$^{131}I^-$ 15 hour Iodide	$^{131}I^-$ 15 hour ClO_4^-
volume excreted (ml)	1.2 ± 0.7	1.7 ± 0.5	1.6 ± 0.7	5.4 ± 2.4	5.0 ± 1.9	5.7 ± 3.1
ng/ml	0.058 ± 0.028	0.064 ± 0.031	0.114 ± 0.036*	0.060 ± 0.017	0.129 ± 0.036*	0.175 ± 0.070*#
total ng excreted	0.061 ± 0.047	0.104 ± 0.043	0.190 ± 0.099*	0.322 ± 0.137	0.593 ± 0.093*	0.844 ± 0.106*#
ng excreted/ hr	0.040 ± 0.031	0.069 ± 0.029	0.126 ± 0.066*	0.021 ± 0.009	0.040 ± 0.006*	0.056 ± 0.007*#
% excreted in urine	2.9 ± 2.2	5.0 ± 2.1	9.1 ± 4.8*	16.3 ± 6.9	29.9 ± 4.7*	42.6 ± 5.4*#

Table 2.2

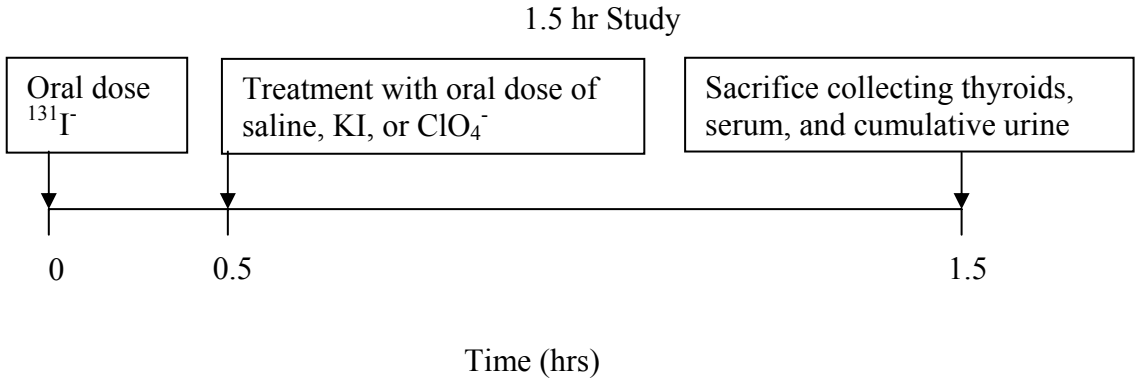
Table 2.3. Total urinary perchlorate and stable iodide excretion data. Urine was collected via metabolism cages for male rats dosed by gavage with 30 mg/kg of perchlorate or stable iodide at +0.5 hours for the 1.5 hour study and +3 hours for the 15 hour study. For descriptions of calculations refer to methods section. Data are means ± SD (n=6).

Time & Dose	1.5 hr Iodide	1.5 hr ClO_4^-	15 hr Iodide	15 hr ClO_4^-
volume excreted (ml)	1.7 ± 0.5	1.6 ± 0.7	5.0 ± 1.9	5.7 ± 3.1
ug/ml	317.8 ± 153.0	599.2 ± 264.3	758.0 ± 239.4	960.5 ± 603.5
total ug excreted	525.1 ± 227.2	1026.2 ± 620.4	3729.1 ± 331.5	4527.2 ± 629.8
ug excreted/hr	350.1 ± 151.5	684.1 ± 413.6	248.6 ± 22.1	301.8 ± 42.0
% excreted in urine	5.86 ± 2.64	11.54 ± 6.91	46.9 ± 3.3	56.2 ± 6.7

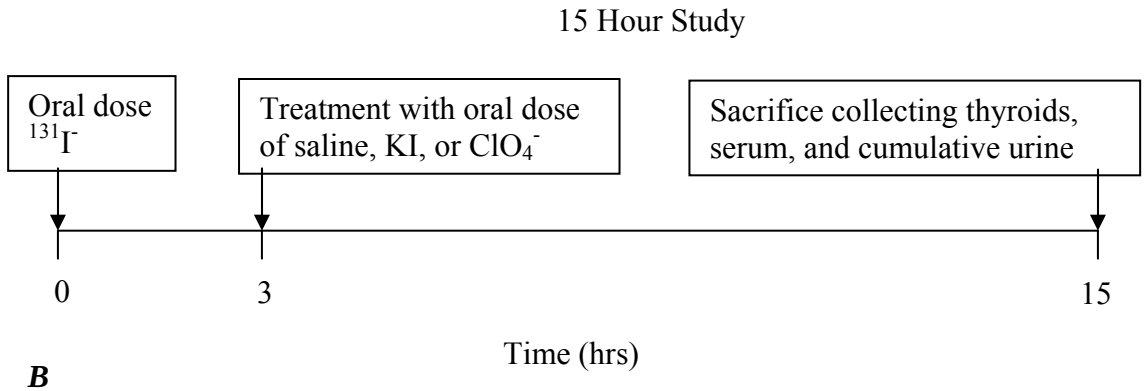
Table 2.3

Figure 2.1. Experimental Design and Dosing Schedule. Summary of radioiodide experiments in the rat to characterize the prophylactic nature of KI and ClO_4 to ameliorate $^{131}I^-$ exposure. (A) is the 1.5 hour experiment were $^{131}I^-$ was administered at time 0 followed by either 0.9% saline, 30 mg/kg of KI, or 30 mg/kg of perchlorate at + 0.5 hours and then sacrificed at +1.5 hours. (B) is the 15 hour experiment were $^{131}I^-$ was administered at time 0 followed by either 0.9% saline, 30 mg/kg of KI, or 30 mg/kg of perchlorate at +3 hours and then sacrificed at +15 hours

Experimental Design



A



B

Figure 2.1

Figure 2.2. $^{131}I^-$ concentrations in the thyroid of male rats dosed with $^{131}I^-$ via gavage followed by 0.9 % saline, KI (30 mg/kg), or perchlorate (30 mg/kg) at +0.5 hours for the 1.5 hour study and + 3 hours for the 15 hour study via gavage. Animals were then euthanized at 1.5 hours or 15 hours post $^{131}I^-$ dose. Data are means ± SD (n=12). *Significantly different from control ($p<0.001$).

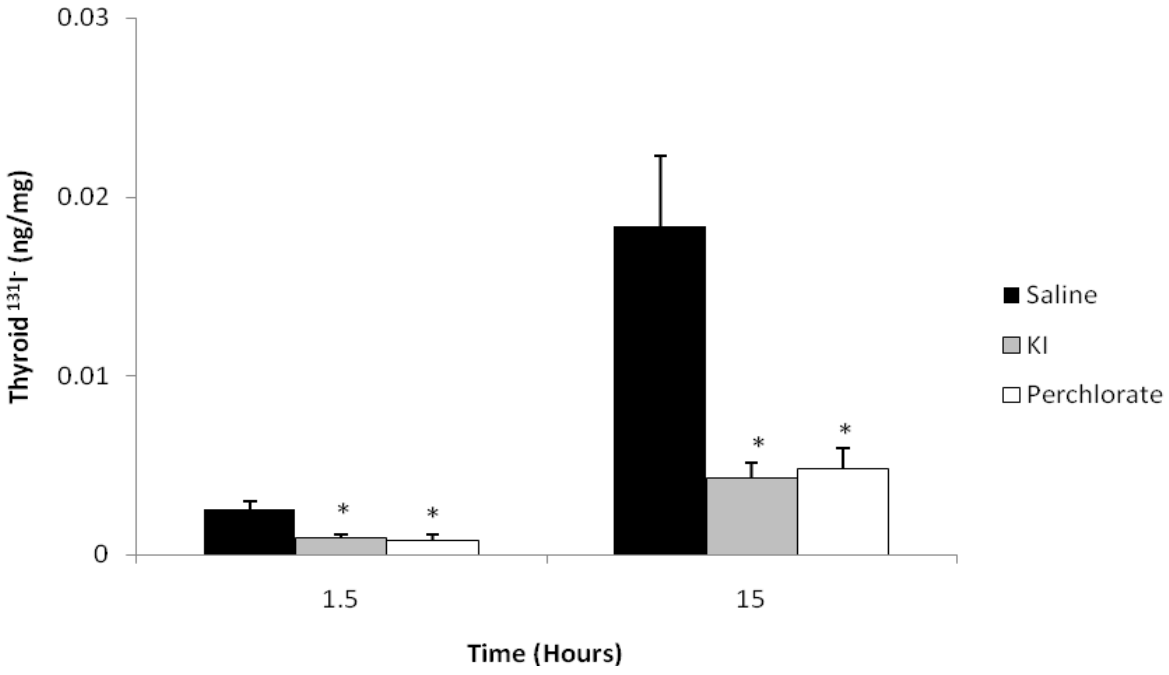

0.03
0.02
0.01
0
Thyroid $^{131}I^-$ (ng/mg)
*
*
*
*
1.5
15
Time (Hours)
Saline
KI
Perchlorate

Figure 2.2

Figure 2.3. $^{131}I^-$ concentrations in serum of male rats collected via cardiac puncture dosed with $^{131}I^-$ then 0.9% saline, KI (30 mg/kg), or perchlorate (30 mg/kg) as described in Figure 2. Data are means ± SD (n=12). *Significantly different from control ($p<0.001$). $^{\#}$Significantly different from KI dose ($p<0.001$).

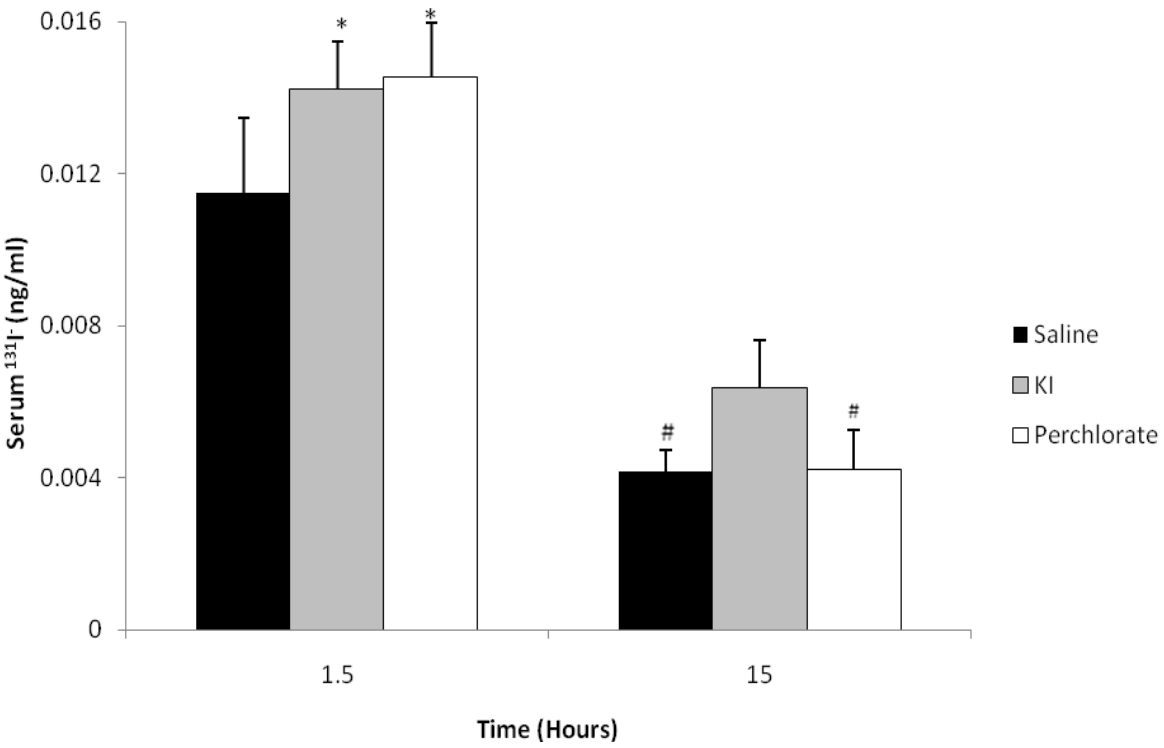

0.016
0.012
0.008
0.004
0
Serum $^{131}I^-$ (ng/ml)
*
*
#
#
1.5
15
Time (Hours)
Saline
KI
Perchlorate

Figure 2.3

Figure 2.4. Serum concentration for stable iodide and perchlorate in male rats 1 and 12 hours following treatment. Animals were dosed at +0.5 hours for 1.5 hour study or +3 hr for the 15 hour study via gavage with 30 mg/kg of either KI or perchlorate. Data are means ± SD (n=12).

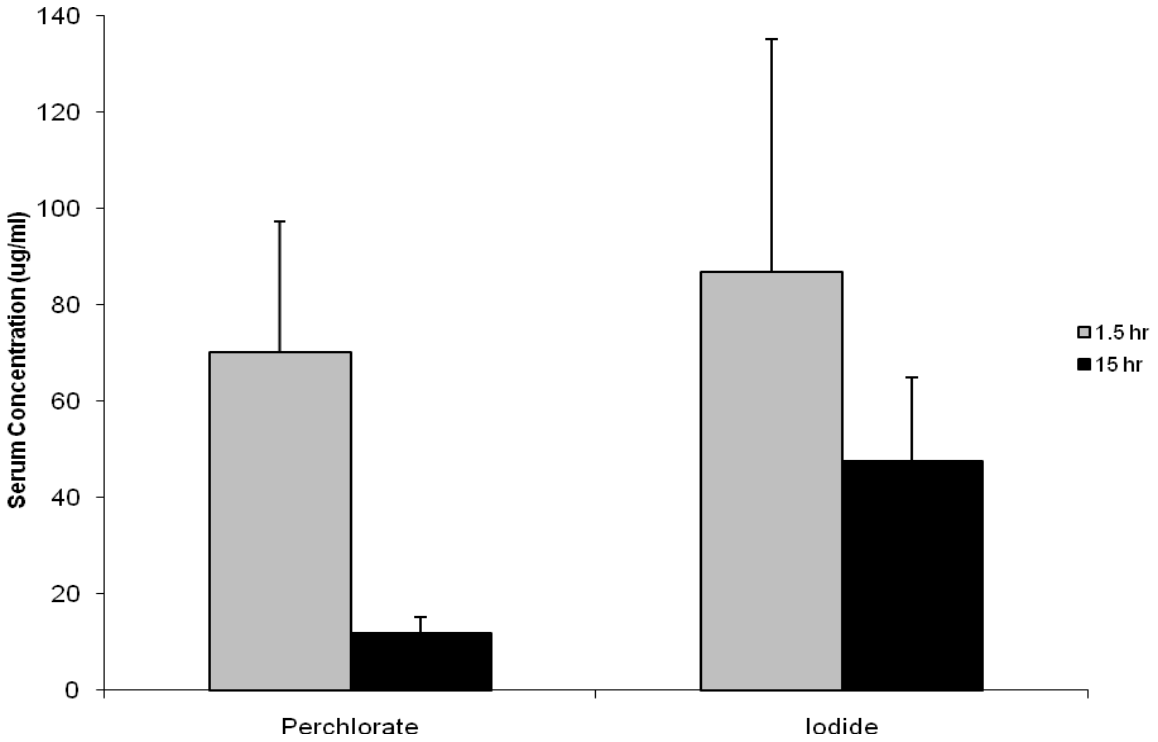

140
120
100
80
60
40
20
0
Serum Concentration (ug/ml)
1.5 hr
15 hr
Perchlorate
Iodide

Figure 2.4

Figure 2.5. (A) Ratio of the concentration of $^{131}I^-$ in the thyroid (ng/mg) and serum (ng/ml) in male rats for saline, KI, and perchlorate dosed animals (n=12). Procedures were the same as described in Figures 2 and 3. (B) Ratio of the concentration of $^{131}I^-$ in the thyroid (ng/mg) and urine (ng/ml) in male rats for saline, KI, and perchlorate dosed animals (n=12 for 15 hour study, n=6 for 1.5 hour study). Procedures were the same as described in Figure 2 and 5. (C) Ratio of the concentration of $^{131}I^-$ in the urine (ng/ml) and serum (ng/ml) in male rats for saline, KI, and perchlorate dosed animals (n=12 for 15 hour study, n=6 for 1.5 hour study). Procedures were the same as described in Figure 3 and 5.

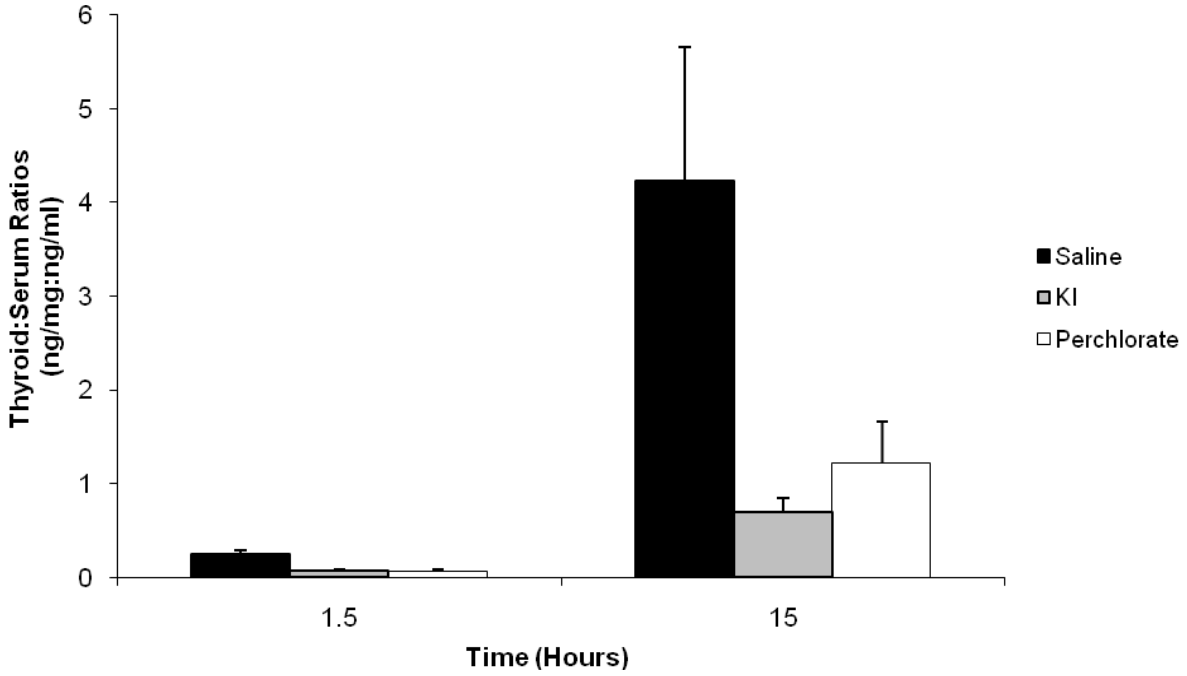


Figure 2.5 (A)

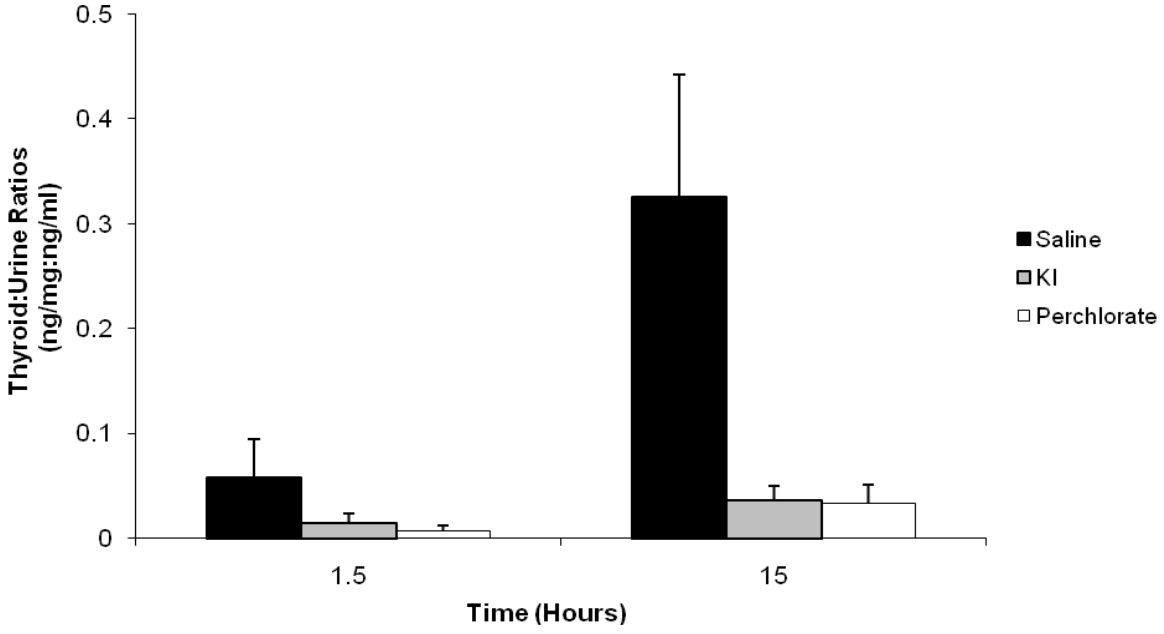


Figure 2.5 (B)

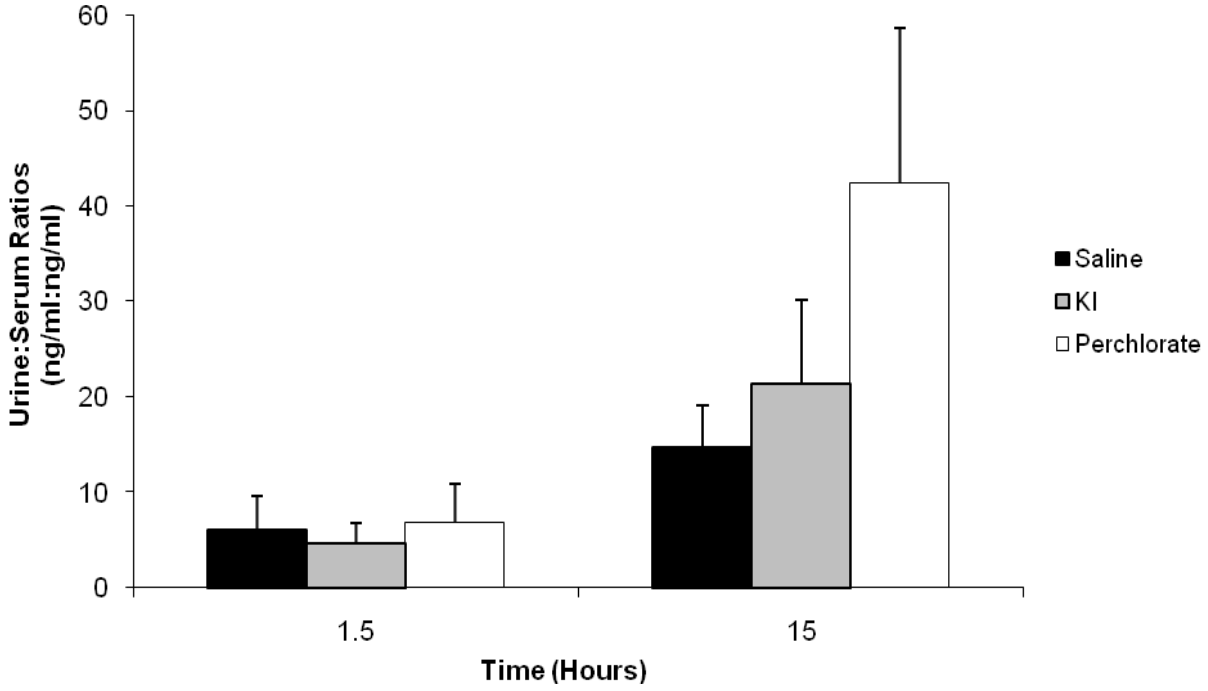

60
50
40
30
20
10
0
Urine:Serum Ratios
(ng/ml:ng/ml)
1.5
15
Time (Hours)
Saline
KI
Perchlorate

Figure 2.5 (C)

CHAPTER 3

RADIOACTIVE IODIDE ($^{131}I^-$) EXCRETION PROFILES IN RESPONSE TO POTASSIUM IODIDE (KI) AND AMMONIUM PERCHLORATE (NH_4ClO_4) PROPHYLAXIS[2]

[2] C. A. Harris, J. W. Fisher, E. A. Rollor III, C. A. White, B. C. Blount, L. Valentin-Blasini, and C. E. Dallas. To be Submitted to Environmental Research

Abstract

Radioactive iodide ($^{131}I^-$) protection studies have focused primarily on the thyroid gland and disturbances in the hypothalamic-pituitary-thyroid axis. Previous research by these authors has demonstrated that potassium iodide (KI) and ammonium perchlorate (NH_4ClO_4) had approximately equivalent blocking efficiencies in the thyroid when administered three hours after $^{131}I^-$ exposure. However, perchlorate-dosed animals had an enhanced urinary excretion of the isotope compared to KI and saline treatments. The objective of the current study was to establish urinary excretion profiles for saline, KI, and perchlorate dosings over a 75 hour time-course. To test the objective, animals were administered $^{131}I^-$ at time 0 followed by either 0.9% saline, 30 mg/kg KI, or 30 mg/kg perchlorate at +3 hours, and serial urine samples collected in six hour time intervals for 75 hours. A second study was also outlined following the same experimental protocols as previously described, but with the addition of hormone replacement therapy every 24 hours starting at the +3 hour time-point. Urinalysis of the first 36 hours of the time-course without hormone replacement revealed that perchlorate dosed animals excreted significantly more $^{131}I^-$ when compared to KI and saline dosings. However, at the final time-point of 75 hours, no significance was determined between any of the treatment groups for the urinary excretion of $^{131}I^-$. This seemed to indicate a time-dependent nature of the perchlorate effect and rapid excretion of perchlorate in the urine. Thyroid data after 75 hours revealed significantly less $^{131}I^-$ in iodide and perchlorate dosed animals relative to saline dosings and also significantly less $^{131}I^-$ in iodide dosed animals relative to perchlorate. Thyroxine (T_4) replacement therapy was then employed to see what would transpire if the thyroid was rendered virtually inactive. Following T_4 replacement, no significant changes were observed in cumulative excretion of $^{131}I^-$ by the termination of the experiment. However, during the 6-12

hour time interval, animals treated with perchlorate + T_4 excreted significantly more $^{131}I^-$ than did the other two treatment groups. Significant changes in $^{131}I^-$ accumulation occurred in the thyroids of animals who received T_4 when compared to the previous experiment. Thyroid data following hormone replacement indicated that KI + T_4 had the only significant decrease in $^{131}I^-$ concentration when compared to saline + T_4 animals. When similar treatment groups were compared between the T_4 and non-T_4 studies, all animals who had not received hormone replacement therapy had significantly less $^{131}I^-$ in the thyroid than did animals who had received hormone therapy. We concluded from these findings that perchlorate dosed animals excrete $^{131}I^-$ at a higher rate for the first day and a half before the bulk of the therapeutic had been excreted, and that hormone replacement therapy works to the detriment of the thyroid causing it to store the previously accumulated $^{131}I^-$ before prophylaxis had begun.

Introduction

Potassium iodide (KI) and perchlorate have been the focus of many researchers for the treatment of Grave's disease and as a thyroid radioprotectant since the early 1900's. Most of these studies have focused primarily on the thyroid gland and perturbations of the hypothalamus-pituitary-thyroid (HPT) axis. Though blocking the radioiodide (for the purposes of this paper radioiodide will be limited to $^{131}I^-$) from uptake into the thyroid is the measure of whether or not the therapeutics are successful, purging the body of the radionuclide then becomes essential. Human and animal physiologies contain no mechanism of detection between radioactive and non-radioactive iodide. This allows for the radioactive iodide to be reabsorbed in the kidneys and returned to the systemic circulation where the radiation can once again be up-regulated into the thyroid.

Studies have shown that approximately 95% of filtered $^{131}I^-$ was reabsorbed at a tubular site proximal to final water reabsorption, and approximately 76% of the total administered dose was excreted in the urine after 48 hours (Johnson and Albert 1951; Giebisch, Macleod *et al.* 1956; Halmi, King *et al.* 1958). The reabsorption occurred by passive diffusion and possibly an active transport mechanism that was capable of being saturated by iodide, perchlorate, and other competitive anions, i.e. chloride, bromide, etc (Halmi, King *et al.* 1958). Enhancement of the renal clearance of $^{131}I^-$ was also manifested when salts of perchlorate, iodide, and chloride were administered prior to tracer and helped further the notion of an active transport mechanism (Halmi, King *et al.* 1958). If the salts of the various anions were able to saturate the renal iodide carriers, as perchlorate has already been demonstrated to do in the thyroid and stomach (Halmi, Stuelke *et al.* 1956; Schonbaum, Sellers *et al.* 1965), then $^{131}I^-$ cannot be reabsorbed by the tubules and is excreted into the urine. The problem with these studies is that treatment occurred hours before the radiation exposure. If a larger dose of isotope is given prior to the anions mentioned in the Halmi *et al.* (1958) study, then it is conceivable that the tracer could saturate the active transport mechanism and promote the excretion of the therapeutics instead of vice versa, as would be observed with competitive inhibition. However, perchlorate has demonstrated the ability to displace accumulated iodide in the thyroid gland that has not been organified as demonstrated by the perchlorate discharge test (Stewart and Murray 1966; Gray, Hooper *et al.* 1972; Gray, Hooper *et al.* 1973). So, if perchlorate has a higher affinity for the receptor sites in the kidney of the active transport mechanism, then it may also possess the ability to displace $^{131}I^-$ from those receptor sites and promote its elimination.

Perchlorate has been confirmed to have a similar clearance rate from the plasma and thyroid to that of iodide in rats, and is rapidly and almost completely excreted in the urine

(Johnson and Albert 1951; Goldman and Stanbury 1973). Perchlorate was able to remove $^{131}I^-$ from the plasma and into the urine more rapidly than in controls by interfering with the renal preservation of iodide and blocking gastric secretion of $^{131}I^-$ (Halmi, Stuelke *et al.* 1956; Ullberg and Ewaldsson 1964; Schonbaum, Sellers *et al.* 1965). Inhibiting iodide crossing over from the blood into the GI, as well as other tissues, restricted the amount of total iodide space and recycling of iodinated compounds. This led to increased serum concentration of $^{131}I^-$ in perchlorate dosed animals versus that of control animals and rendered greater concentration of $^{131}I^-$ available for clearance in the urine. Schonbaum *et al.* (1965) reported that at all time-points, perchlorate dosed animals had decreased serum counts, increased urine counts, and much lower gastric counts than in their control counterparts. Urinalysis of perchlorate over a 24 hr period revealed that when 0.1-3.0 mg/kg of perchlorate was administered by intravenous (iv) injection, approximately 83% of the total dose was excreted in first 24 hours (Fisher, Todd *et al.* 2000). A similar study using 3.3 mg/kg of labeled perchlorate ($^{36}ClO_4^-$) administered by iv injection reported that 96% of the total dose was excreted in the first 24 hours and 99.5% was excreted in 48 hours (Yu, Narayanan *et al.* 2002). Yu *et al.* (2002) also gave loading doses of $^{36}ClO_4^-$ as a radioprotectant for $^{125}I^-$ in iv doses of 0.01-3.0 mg/kg and over a 26 hr period a range of 72-97% of the $^{36}ClO_4^-$ had been excreted in the urine (Yu, Narayanan *et al.* 2002). Since small doses of perchlorate, and to a lesser extent iodide, have proven effective in blocking of $^{131}I^-$ from up-regulation into the thyroid and in promoting the excretion of the isotope when administered prior to tracer, the question remains: what happenes if the tracer has a head start? This question motivated the researchers of this paper to look at a urine time-course of $^{131}I^-$, perchlorate, and iodide when large doses of the therapeutics are administered post radioiodide ingestion.

Exposure to the thyroid resulting from perchlorate and stable iodide cause the accumulation of iodide into the gland to be inhibited to some measure (Ramsden, Passant *et al.* 1967; Wolff 1980). Thyroidal iodide inhibition can lead to low circulating levels of thyroid hormones thyroxine (T_4), triiodothyronine (T_3), or both (Mannisto, Ranta *et al.* 1979). These low levels then stimulate the feedback loops of the HPT axis to produce higher concentrations of thyroid stimulating hormone (TSH) (Kapitola, Schullerova *et al.* 1971). Up-regulation of TSH can compensate for the partial inhibition of iodide as a function of the HPT axis, but studies have shown that continuous stimulation of the thyroid by TSH has resulted in goiter and hypothyroidism (Wyngaarden, Wright *et al.* 1952; Gerber, Studer *et al.* 1981). To compensate for the up-regulation of TSH, replacement doses of T_4 can be administered in order to deactivate the feed-back loops that stimulate TSH production.

The objective of the present study was to evaluate the $^{131}I^-$ excretion profiles over three days following a single dose of either KI or perchlorate in rats with and without hormone replacement therapy. To test the objective, rats were orally administered $^{131}I^-$ followed by oral administration of saline, 30 mg/kg KI, or 30 mg/kg NH_4ClO_4 at designated time intervals post radioiodide dose with urine being collected via metabolism cages in designated time intervals. After sacrifice, thyroids, serum, and urine were analyzed for $^{131}I^-$ content and expressed as percentages of the saline dosings.

Materials and Methods

Chemicals

Ammonium perchlorate (99.8%), 100% ethanol, and sodium hydroxide were purchased from Aldrich (Milwaukee, WI). Nonradioactive thyroxine was purchased from Sigma Chemical

Corporation. Potassium iodide was purchased from J. T. Baker. Carrier-free iodine-131 was purchased from Amersham Biosciences (29.4 mCi/ug). Isoflurane (99.9%) was purchased from Abbott Laboratories. Acepromazine maleate (10 mg/ml) and ketamine HCL (100 mg/ml) were purchased from Fort Dodge Animal Health (Fort Dodge, Iowa). Xylazine (20 mg/ml) was purchased from Ben Venue Laboratories (Bedford, Ohio).

Animals

Male Sprague-Dawley rats (330 ± 30 g) were used throughout the experiments and were obtained from Harlan Laboratories (Indianapolis, Indiana). The animals were individually housed in metabolism cages for urine collection (a one week acclimation period in the animal facility was allowed before moving into the metabolism cages). The cages were stored in an environmentally controlled room (12 h light/12 h dark cycle, 22 ± 2°C room temperature, 50 ± 20% relative humidity, 10-15 air changes/hr). Animals were provided LabDiet Laboratory Rodent Diet 5001 rat chow and water *ad libitum*. Sera, urine, and tissue samples were stored at -80°C until analysis. The animals used in this study were handled in accordance with the procedures of The University of Georgia Institutional Animal Care and Use Committee (IACUC), AUP# A2005-10110-0.

Experimental Design

Fifty-four animals were used for each experimental study. A summary of the experiments is shown in Table 3.1. Animals were randomly assigned to individual metabolism cages. The night before the experiments food, but not water, was removed from the animals. The following morning animals were weighed and gavaged with 1 ml of a 2.91 μCi (6 ng/kg)

$^{131}I^-$ solution at time 0 hours. Animals were later gavaged with a 1 ml solution of either 0.9% saline, KI (30 mg/kg), or perchlorate (30 mg/kg) at +3 hours and urine was collected from the metabolism cages. Urine was again collected at +6 hours and food was returned to the animals, and then serial urine samples were collected in 6 hour intervals starting at +12 through +72 and finally at +75 hours when the animals were sacrificed. At +15 hours a blood sample was taken from each animal via a tail artery bleed, with animals anesthetized via inhalation with isoflurane. Approximately 1 ml of blood was removed from the tail to determine the number of radioactive counts in the blood. The animals were anesthetized with a ketamine cocktail (0.1 ml per 100 g body weight) and moved from the experimental room to a surgical room where they were subsequently euthanized at +75 hours by CO_2 asphyxiation, blood was withdrawn via cardiac puncture, thyroids were removed and weighed, and urine was collected from the metabolism cages and bladder. Serum was prepared by centrifugation of the blood at 1500 rpm at 4°C for 15 minutes. Each experiment was repeated three times and in each experiment 2 animals received saline, 2 received KI, and 2 received perchlorate.

Hormone replacement studies were conducted identically to the non-hormone replacement studies except that 0.1 ml ip doses of either 0.1 M NaOH (controls) or 15 ug/kg of T_4 dissolved in 0.1 M NaOH were administered at 3, 27, and 51 hours following $^{131}I^-$ administration. Each experiment was repeated 6 times and in each experiment 1 animal received saline + NaOH, 1 animal received saline + T_4, 1 animal received KI + NaOH, 1 animal received KI + T_4, 1 animal received perchlorate + NaOH, and 1 animal received perchlorate + T_4.

Sample Analysis

Serial urine samples were counted immediately after collection during the experiment on a 1470 Wallac Wizard Gamma Counter equipped with one detector to get raw $^{131}I^-$ counts/minute (cpm). After sacrifice, thyroids, urine, and serum were also immediately counted on the gamma counter. Urine and sera were then stored at -80°C for no less than 80 days (10 half-lives for $^{131}I^-$) in order for the radioactivity to decay.

Serum TSH measurements were made using a rat thyroid stimulating hormone radioimmunoassay kit from A. F. Parlow and the National Hormone & Peptide Program (lot numbers AFP329691Rb, AFP11542B, and AFP5512B).

$^{127}I^-$ and ClO_4^- Analysis

Non-radioactive analytes ($^{127}I^-$ and ClO_4^-) were quantified using ion chromatography coupled with mass spectrometry. Serum samples were spiked with internal standard ($^{129}I^-$ and $Cl^{18}O_4^-$), treated to remove proteins and analyzed by ion chromatography electrospray ionization mass spectrometry (Amitai, Winston *et al.* 2007). Urine samples were spiked with internal standard ($^{129}I^-$ and $Cl^{18}O_4^-$) and analyzed by ion chromatography electrospray ionization mass spectrometry (Valentin-Blasini, Blount *et al.* 2007).

Converting counts per minute to concentration

For solutions containing a specific activity of approximately 29.5 mCi $^{131}I^-$/ug I (~3.4 ug I/100 mCi $^{131}I^-$) ratios of the amount (ng) of iodide as $^{131}I^-$ administered to each animals are calculated as follows:

$$\left(uCi_Q \Big/ \frac{ng_Q}{V_Q}\right) = \left(uCi_A \Big/ \frac{x}{V_A}\right) \qquad [1]$$

$$(cpm_D/x) = (cpm_S/y) \quad [2]$$

where uCi_o equals number uCi's in the ordered solution, ug_o equals the number of micrograms in the purchased solution, V_o equals the volume of the purchased solution, uCi_A equals the number of mCi's administered to each animals, x equals the unknown ng of iodide in the dose, V_A equals the volume of the dose given, cpm_D equals counts per minute of the dose, cpm_S equals the counts per minute in each sample taken from the animals (ie. thyroid, serum, and urine), and y equal the unknown concentration of $^{131}I^-$ in ng/ml.

Urinary Excretion Data

Urine samples for the 75 hour and 75 hour + T_4 experiments were analyzed for $^{131}I^-$, $^{127}I^-$, and ClO_4. Radioiodide parameters included ng excreted per ml of urine, total ng excreted, ng excreted per hour, and percent of $^{131}I^-$ excreted. Calculations for radioiodide parameters proceeded as follows:

$$ng\ excreted\ per\ ml = ((A/B) * C) * 1000 \quad [3]$$

$$TOT_{cml}\ (ng) = ng/ml * V_t \quad [4]$$

$$\%\ excreted = (TOT_{cml}/C) * 100 \quad [5]$$

where A equals counts/ml of urine, B equals the total number of counts administered to the animal, and C equals the dose of $^{131}I^-$ in the dosing solution, and V_t equals cumulative volume of urine excreted over a specific time interval. Perchlorate and stable iodide parameters included ug excreted per ml of urine (reported from Ion Chromatography (IC) analysis), total ug excreted, ug excreted per hour, and percent of prophylactic excreted. Calculations for stable iodide and perchlorate endpoints proceeded as follows:

$$TOT_{cml,ClO_4}\ (ug) = ug/ml * V_t \quad [6]$$

$$\% \ excreted = \left(TOT_{cml,ClO_4} / C_0\right) * 100 \qquad [7]$$

where V_t equals cumulative volume of urine excreted over a specific time interval and C_0 equals dose of prophylactic administered to the animals.

All half-life calculations were made using modeling software Win Non Lin 5.2.

Statistical Analysis

Single factor ANOVA was used initially to determine significance between the three dose groups with significance set at $p < 0.05$. Once significance was determined a two-sample t-test assuming equal variance was used for comparison of statistical significance between each dose group ($p < 0.05$).

Results

75 Hour Time-Course Urine Experiment

Previous research by the authors of this paper has led to an increased interest in the urinary output of $^{131}I^-$ following the administration of iodide or perchlorate. A 75 hour time-course urine study was outlined with $^{131}I^-$ administered at time zero followed by either saline, KI, or perchlorate at +3 hours. Urine was collected every six hours from metabolism cages. After 75 hours had lapsed, there was no statistical significance ($p > 0.05$) in the urine output from any of the treatment groups. An average volume of 39.7 ± 7.7 ml of urine was collected from each animal during the experiment.

The cumulative urinary excretion of $^{131}I^-$ is shown in Figure 3.1. The excretion of the total administered $^{131}I^-$ was 71%, 63%, and 62% for the perchlorate, KI, and saline treated animals respectively. No significance between the dose groups was observed for $^{131}I^-$ by the

termination of the study ($p > 0.05$). However, after 18 hours, perchlorate dosed animals on average excreted 50% and 28% more $^{131}I^-$ when compared to the saline and KI dosed animals at the same time-point, respectively ($p < 0.01$). These numbers dropped significantly by the termination of the study when perchlorate dosed animals had only a 15.0% and 12.3% increase over saline and KI studies.

Urine samples were also analyzed using ion chromatography (IC) for perchlorate and iodide concentration, total ug excreted, percent excreted, and ug excreted per hour at each time-interval (Table 3.2 a and b). Urinalysis indicated that iodide and perchlorate treated animals excreted >90% of the total administered dose by the termination of the experiment. Furthermore, 96% of the total perchlorate and 87% of the total iodide excreted occurred during the first 18 hours of the time-course. Distribution and terminal half-lives were determined for $^{131}I^-$, stable iodide, and perchlorate and are shown in Table 3. The distribution half-life of $^{131}I^-$ in perchlorate dosed animals relative to the saline and KI treatment groups was significantly shorter ($p < 0.001$), while no significant differences were noted for the terminal half-lives among the three treatment groups. Perchlorate also had a significantly lower half-life relative to stable iodide in the distribution phase ($p < 0.01$) with no significance during the terminal phase.

Two serum time-points were chosen to compare serum concentration of the radioiodide early and at the end of the study. The first sampling was withdrawn via the tail vein at +15 hr and the final sampling was withdrawn via cardiac puncture at +75 hr when the animal was sacrificed. The serum $^{131}I^-$ concentrations for both time-points are shown in Figure 3.2. At +15 and +75 hrs the perchlorate and KI dosed animals had a significant reduction ($p < 0.05$) in the serum concentration of $^{131}I^-$ as compared to the saline dosed animals. IC analysis of free iodide and perchlorate in the serum revealed concentrations of 200.4 ± 199.8 and 81.3 ± 31.6 ng/ml

respectively. TSH values were also measured in serum and are shown in Figure 3.3. An average concentration of 3.8 ± 1.5 ng/ml, with no statistical significance ($p > 0.05$) between them, was determined across all treatment groups.

The results for concentration of $^{131}I^-$ in the thyroid gland are shown in Figure 3.4. At 75 hours post $^{131}I^-$ dosing there was still a statistically significant percent inhibition ($p < 0.05$) in $^{131}I^-$ concentration in the thyroid of perchlorate (65%) and KI (76%) dosed animals versus saline. Thyroid comparisons between perchlorate and KI treatment groups revealed that animals administered KI had a statistically significant reduction ($p < 0.05$) in $^{131}I^-$ concentration relative to its perchlorate counterpart.

75 Hour Time-Course Urine with Replacement T_4 Experiment

Intraperitoneal injections of replacement T_4 doses were administered to all treatment groups at +3, +27, and +51 hrs. Urine, serum, and thyroids were collected as previously described in the 75 hour study. An average void volume of 41.3 ± 5.7 ml was measured across all treatment groups with no significant difference between groups. The results for $^{131}I^-$ content in urine are shown in Figure 3.5. Excretion percentages of 63%, 71%, and 72% of the total administered radioiodide were measured for saline, KI, and perchlorate dosed animals respectively. No significance in cumulative amount of $^{131}I^-$ excreted was determined in urine over the 75 hour time-course between the treatment groups. Approximately 80-90% of the total excreted $^{131}I^-$ occurred in the first 18 hours, though no significance was determined. The only significant increase in $^{131}I^-$ urinary excretion occurred during the first 12 hours of collection when perchlorate dosed animals excreted 36% and 20% more $^{131}I^-$ than did the saline and KI dosed animals ($p < 0.05$). IC urinalysis of the anions revealed that >85% of the total

administered KI and perchlorate were excreted by the termination of the experiment, and 84% and 96% of the total iodide and perchlorate excreted occurred during the first 18 hours of collection (Table 3.4 a and b).

Distribution and terminal half-lives for $^{131}I^-$, stable iodide, and perchlorate in the hormone replacement study are shown in Table 3.5. Statistical significance was determined for the half-life distribution phase of perchlorate dosed animals relative to saline and KI treatment groups ($p < 0.001$). No significant change was found for the half-life terminal phase between any of the treatment groups for radioiodide. Comparisons between the T_4 and non-T_4 study revealed that the addition of replacement T_4 doses had no effect on the distribution half-life for $^{131}I^-$, but during the terminal phase KI treated animals possessed a significantly lower $^{131}I^-$ half-life relative to the saline or perchlorate treatment.

Serum samples demonstrated more of a response to T_4 at the various doses than did the urine for the same study (Figure 3.6). At the +15 hour time-point only perchlorate had a significant decrease in serum $^{131}I^-$ concentration ($p < 0.05$) over saline dosed animals. However, at the +75 hour time-point both KI and perchlorate treated animals had a significant decrease in $^{131}I^-$ concentration ($p < 0.05$) over saline dosed animals. Stable iodide and perchlorate anion data were analyzed by IC for the same end-points as described in the non-T_4 study. Free iodide and perchlorate levels in serum measured 56.7 ± 15.9 ng/ml and 26.3 ± 14.6 ng/ml respectively.

Comparisons of the T_4 study revealed that KI had the only statistically significant effect on the thyroid concentration of $^{131}I^-$. The KI and perchlorate treatments were proficient in blocking the up-regulation of $^{131}I^-$ by 75% and 50% (Figure 3.7). However, when comparisons were made between the T_4 and non-T_4 study it was revealed that in all dose groups there was a

statistically significant reduction in $^{131}I^-$ in the thyroid of animals that did not receive the T_4 (Figure 3.8).

Discussion

The objective of this study was to compare the relative abilities of stable iodide and perchlorate to excrete previously accumulated $^{131}I^-$ in the urine. The magnitude of the stable iodide and perchlorate effect on $^{131}I^-$ uptake and excretion have been found to be time dependent (Sinadinovic and Jovanovic 1971; Zanzonico and Becker 2000). Over time, iodide and perchlorate prophylaxis excrete approximately the same quantity of radioiodide in the urine, though iodide prophylaxis has a marked decrease in the excretion rate of $^{131}I^-$ in the early hours (Sinadinovic and Jovanovic 1971). These results were confirmed in the current study. In evaluating this relationship using T_4 hormone intervention, no significant changes were determined in any of the treatment groups with or without T_4 over a 75 hour time course. However, in the early collection times (3-18 hours following $^{131}I^-$ exposure) of the non-T_4 study, perchlorate dosed animals had a significant increase in excretion over KI and saline dosings. This finding proved to be important since in all dose groups 80-90% of the isotope was excreted within the first 18 hours, which is where significant increases in cumulative amounts of $^{131}I^-$ were determined with the perchlorate treatment. Similar results were also determined from the half-lives of radioiodide for saline, KI, and perchlorate treatment groups. During the distribution phase significantly lower radioiodide half-lives were determined among animals administered perchlorate compared to the other two treatment groups, though the significance was not maintained through the terminal phase. Comparisons between the non-T_4 and the T_4 studies

revealed that there was no significant change in cumulative amount of $^{131}I^-$ excreted from similar treatment groups at any time-point throughout the study.

The time dependent nature of perchlorate was also manifested in its urinary excretion. Perchlorate has a biological half-life of 6-8 hours (Yu, Narayanan *et al.* 2002; NRC 2005), and during the time-course over 95% of the total perchlorate excreted occurred within the first 18 hours of collection. The perchlorate animals were thus similar to the saline animals after the 18 hour collection, with the KI and saline treated animals essentially catching up in their excretion profiles. Virtually all of the administered iodide was excreted by the conclusion of the experiment. Stable iodide excretion percentage was also high by the 18 hour collection with 87% and 84% excreted for the non-T_4 and T_4 studies, respectively. In a previous study, the current authors found that 12 hours after a 30 mg/kg dose of iodide, fasted animals excreted approximately 50% of the administered iodide dose (Harris, Fisher *et al.* 2008). The 30-40 % increase in the current study can be attributed to ingestion of iodide in the chow and possibly from the chow falling into the catch cups in the metabolism cages and liberating the iodide from the chow into the urine.

The results for serum $^{131}I^-$ concentration in the T_4 and non-T_4 studies were expected based on the urinary excretion and thyroid data. In the non-T_4 study, both KI and perchlorate dosed animals had significant decreases in serum $^{131}I^-$ concentration at +15 and + 75 hours when compared to saline dosed animals. However, contrasting results appeared in the serum concentration of animals that received T_4 injections. At +15 hours only perchlorate had a significant decrease in serum concentration due to its enhanced urinary excretion during the first 18 hours. At +75 hours KI and perchlorate treated animals had significant decreases in serum $^{131}I^-$ concentration compared to saline treated animals by excreting greater amounts of $^{131}I^-$.

Reports in the literature for TSH values in the rat range from 4.6- 8.7 ng/ml (McLanahan, Campbell *et al.* 2007), 15-20 ng/ml (Siglin, Mattie *et al.* 2000), 327 ng/ml (Okamura, Taurog *et al.* 1981), and 220 ng/ml (Lemarchand-Beraud and Berthier 1981). These highly variable values can be attributed to the type of RIA kit used for analysis, sample collection time, and the sensitivity and efficiency of the detector used for counting. Also, perchlorate and iodide have been shown to significantly increase serum TSH levels (Siglin, Mattie *et al.* 2000; McLanahan, Campbell *et al.* 2007). The 75 hour results did not reflect any perturbations in TSH concentration based on perchlorate and iodide treatments by the termination of the study and demonstrated agreement with McLanahan *et al.* (2007) of an average TSH concentration of ~4.0 ng/ml. Since the vast majority of the therapeutics were eliminated within the first 18 hours of the experiment, the thyroid had plenty of time to recover from the excess KI and perchlorate dose to regulate hormone levels back to normal by the end of the experiment.

The thyroid gland proved to be the main source of variation when making comparisons between the therapeutic dose groups and comparisons between similar dose groups of the non-T_4 and replacement T_4 studies. In the previous study, perchlorate and iodide had approximately equivalent blocking effects of $^{131}I^-$ with no significance between the dose groups at +15 hours when same experimental protocols were followed (Harris, Fisher *et al.* 2008). In the current study, at +75 hours there was a significant increase in the percent inhibition of $^{131}I^-$ in the thyroid of KI dosed animals versus that of perchlorate dosed animals. This was the first time for any marked discrepancies in thyroidal $^{131}I^-$ inhibition between the KI and perchlorate treatments.

Studies have shown that exposure to high concentrations of iodine *in vivo* and *in vitro* reduce iodine transport and its organification into proteins, but only for approximately 24 hours (Yamada, Iino *et al.* 1963; Ferreira, Lima *et al.* 2005). From these studies it was ascertained that

initially the excess doses of iodide greatly limited the function of the thyroid due to low thyroid:serum ratios. However, once the iodide began being excreted in the urine, the concentration of iodide in the serum was still high but not sufficiently high so that organification was blocked. This led to the thyroid regaining its normal function and production of thyroid hormones at a higher rate to make up for the low concentrations of circulating levels during the organification block. This meant that all the previously accumulated $^{131}I^-$ that had been incorporated into thyroid hormone prior to iodide prophylaxis was liberated from the thyroid gland and resulted in a lower concentration of $^{131}I^-$ in the thyroid of animals that received KI treatment versus saline or perchlorate.

These results led to a separate study to see what thyroid and urinary excretion affects would manifest if daily replacement doses of thyroxine (T_4) were administered in addition to following the 75 hr experimental protocol. In all similar dose groups, i.e. saline and saline + T_4, KI and KI + T_4, or perchlorate and perchlorate + T_4, there was a statistically significant increase in the percent inhibition of animals that did not receive T_4 versus those animals where T_4 was administered. It has been shown shown that when thyroxine was administered 40 hours following $^{131}I^-$ administration that the biological half-life of $^{131}I^-$ in the thyroid escalated from 1.4 to approximately 26 days (Wolff 1951). Thyroxine has a half-life of approximately 12 hours in the rat (Abrams and Larsen 1973) and urinary excretion of administered radiothyroxine data have revealed that only 30% of the administered dose was excreted as $^{131}I^-$ in the urine after 48 hours (Johnson and Albert 1951). At three hours post $^{131}I^-$ dosing over 95% of the isotope that had been up-regulated by the thyroid had already been organified and incorporated into hormone (Harris, Fisher *et al.* 2008). Administration of replacement doses of T_4 caused the feedback loops of the HPT axis to shut down and rendered the thyroid inactive. Once inactive, all

previously accumulated $^{131}I^-$ that had been up-regulated into the thyroid gland was sequestered until the negative feedback loop of the HPT axis determined that there was a need for more hormone (Yu, Narayanan *et al.* 2002). Based on these findings and the Harris *et al.* (2008) study, it was implied that any $^{131}I^-$ that was liberated from the thyroid would have to come from leaching of $^{131}I^-$ in the inorganic form from the follicular cell back into the systemic circulation.

In conclusion, the primary goal of these studies was to evaluate the urinary radioiodide excretion profiles of animals that were administered saline, KI, or perchlorate. All three treatment groups were efficient in their excretion of $^{131}I^-$ by the termination of the 75 hour experiment with no significance determined between the treatment groups. However, excretion significance was determined for perchlorate dosed animals relative to saline and KI dosings during the first 36 hours of the non-T_4 experiments. We concluded that perchlorate prophylaxis operates in a more efficient therapeutic nature during the onset of administration, but that the majority of perchlorate is excreted within the first 18 hours and the perchlorate effect was nullified. Future studies in this area should include multiple dosings of perchlorate to see if the intensity of the perchlorate effect can be maintained over the time-course and allow for a higher cumulative excretion of the $^{131}I^-$.

Based on the available data, hormone replacement therapy worked to the detriment of the thyroid and should not be employed in this therapeutic regime unless administration can take place prior to $^{131}I^-$ contamination, which negates its utility in true crisis exposure. While T_4 replacement almost certainly prevented further accumulation of the $^{131}I^-$ from entering the thyroid by rendering the thyroid inactive, it also prevented the thyroid from being able to discharge the stored $^{131}I^-$ that had been organified and incorporated into thyroid hormones. This

allowed for higher concentrations of $^{131}I^-$ to be located in the thyroid of all treatment groups that received T_4 versus those that did not.

Acknowledgements

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

References

Abrams, G. M. and P. R. Larsen (1973). "Triiodothyronine and thyroxine in the serum and thyroid glands of iodine-deficient rats." J Clin Invest **52**(10): 2522-31.

Amitai, Y., G. Winston, et al. (2007). "Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels." Thyroid **17**(9): 843-50.

Ferreira, A. C., L. P. Lima, et al. (2005). "Rapid regulation of thyroid sodium-iodide symporter activity by thyrotrophin and iodine." J Endocrinol **184**(1): 69-76.

Fisher, J., P. Todd, et al. (2000). "Preliminary development of a physiological model for perchlorate in the adult male rat: a framework for further studies." Drug Chem Toxicol **23**(1): 243-58.

Gerber, H., H. Studer, et al. (1981). "Reaccumulation of thyroglobulin and colloid in rat and mouse thyroid follicles during intense thyrotropin stimulation. A clue to the pathogenesis of colloid goiters." J Clin Invest **68**(5): 1338-47.

Giebisch, G., M. B. Macleod, et al. (1956). "Renal excretion of radioiodide in the dog." Am J Physiol **187**(3): 529-35.

Goldman, S. J. and J. B. Stanbury (1973). "The metabolism of perchlorate in the rat." Endocrinology **92**(5): 1536-8.

Gray, H. W., L. A. Hooper, et al. (1973). "An evaluation of the 20 minute perchlorate discharge test." J Clin Endocrinol Metab **37**(3): 351-5.

Gray, H. W., L. A. Hooper, et al. (1972). "A twenty minute perchlorate discharge test." J Clin Endocrinol Metab **34**(3): 594-7.

Halmi, N. S., L. T. King, et al. (1958). "Renal excretion of radioiodide in rats." Am J Physiol **193**(2): 379-85.

Halmi, N. S., R. G. Stuelke, et al. (1956). "Radioiodide in the thyroid and in other organs of rats treated with large doses of perchlorate." Endocrinology **58**(5): 634-50.

Harris, C. A., J. W. Fisher, et al. (2008). "Prophylactic Evaluation of Potassium Iodide (KI) and Ammonium Perchlorate (NH_4ClO_4) to Ameliorate $^{131}I^-$ Exposure in the Rat." Submitted to the Journal of Toxicology and Environmental Health.

Johnson, H. W. and A. Albert (1951). "The excretion and distribution of ^{131}I following administration of physiologic amounts of labeled iodide, diiodotyrosine and thyroxine in the rat." Endocrinology **48**(6): 669-81.

Kapitola, J., M. Schullerova, et al. (1971). "Blood flow and radioiodine uptake in the thyroid gland of rats after administration and discontinuation of carbimazole and perchlorate." Acta Endocrinol (Copenh) **68**(4): 817-25.

Lemarchand-Beraud, T. and C. Berthier (1981). "Effects of graded doses of triiodothyronine on TSH synthesis and secretion rates in hypothyroid rats." Acta Endocrinol (Copenh) **97**(1): 74-84.

Mannisto, P. T., T. Ranta, et al. (1979). "Effects of methylmercaptoimidazole (MMI), propylthiouracil (PTU), potassium perchlorate (KClO4) and potassium iodide (KI) on the serum concentrations of thyrotrophin (TSH) and thyroid hormones in the rat." Acta Endocrinol (Copenh) **91**(2): 271-81.

McLanahan, E. D., J. L. Campbell, Jr., et al. (2007). "Low-dose effects of ammonium perchlorate on the hypothalamic-pituitary-thyroid axis of adult male rats pretreated with PCB126." Toxicol Sci **97**(2): 308-17.

NRC (2005). Health Implications of Perchlorate Ingestion, National Research Council of National Academies. National Academies Press, Washington, D.C.

Okamura, K., A. Taurog, et al. (1981). "Strain differences among rats in response to Remington iodine-deficient diets." Endocrinology **109**(2): 458-63.

Ramsden, D., F. H. Passant, et al. (1967). "Radioiodine uptakes in the thyroid. Studies of the blocking and subsequent recovery of the gland following the administration of stable iodine." Health Phys **13**(6): 633-46.

Schonbaum, E., E. A. Sellers, et al. (1965). "Some effects of perchlorate on the distribution of 131-iodide." Acta Endocrinol (Copenh) **50**(2): 195-201.

Siglin, J. C., D. R. Mattie, et al. (2000). "A 90-day drinking water toxicity study in rats of the environmental contaminant ammonium perchlorate." Toxicol Sci **57**(1): 61-74.

Sinadinovic, J. and M. Jovanovic (1971). "Jticaj perhlorata i stabilnog jodida na eliminciju radioaktivnog joda (131 J) iz organizma [Effect of perchlorate and stabile iodide on the elimination of radioactive iodine (I 131) from the body]." Glas Srp Akad Nauka [Med] **23**: 29-36.

Stewart, R. D. and I. P. Murray (1966). "An evaluation of the perchlorate discharge test." J Clin Endocrinol Metab **26**(10): 1050-8.

Ullberg, S. and B. Ewaldsson (1964). "Distribution of Radio-Iodine Studied by Whole-Body Autoradiography." Acta Radiol Ther Phys Biol **2**: 24-32.

Valentin-Blasini, L., B. C. Blount, et al. (2007). "Quantification of iodide and sodium-iodide symporter inhibitors in human urine using ion chromatography tandem mass spectrometry." J Chromatogr A **1155**(1): 40-6.

Wolff, J. (1951). "Some factors that influence the release of iodine from the thyroid gland." Endocrinology **48**(3): 284-97.

Wolff, J. (1980). "Physiological aspects of iodide excess in relation to radiation protection." Journal of Molecular Medicine **4**: 151-165.

Wyngaarden, J. B., B. M. Wright, et al. (1952). "The effect of certain anions upon the accumulation and retention of iodide by the thyroid gland." Endocrinology **50**(5): 537-49.

Yamada, T., S. Iino, et al. (1963). "Inhibitory effect of excess iodide on thyroidal radioiodine release in the rat." Endocrinology **72**: 83-90.

Yu, K. O., L. Narayanan, et al. (2002). "The pharmacokinetics of perchlorate and its effect on the hypothalamus-pituitary-thyroid axis in the male rat." Toxicol Appl Pharmacol **182**(2): 148-59.

Zanzonico, P. B. and D. V. Becker (2000). "Effects of time of administration and dietary iodine levels on potassium iodide (KI) blockade of thyroid irradiation by 131I from radioactive fallout." Health Phys **78**(6): 660-7.

Table 3.1. Experimental Design and Dosing Schedule. Summary of experiments in the rat to characterize excretion profiles of $^{131}I^-$ following prophylactic administration of saline, KI, or perchlorate with and without T_4 hormone replacement.

Experiment Number	Description	Dose (mg/kg)	Urine Collection Times (hours)	T_4 Injection Times (hours)	Serum and Thyroid Collection Time (hours)
I. Urinalysis of radioiodide following saline, KI, or perchlorate treatments	Single oral dose of $^{131}I^-$ followed by single oral doses of saline, KI, or perchlorate	$^{131}I^-$: 6×10^{-6} KI: 30 ClO_4: 30	3, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, and 75	-	Serum: 15 and 75 Thyroid: 75
II. Urinalysis of radioiodide following saline, KI, or perchlorate treatments and hormone replacement therapy	Single oral dose of $^{131}I^-$ followed by single oral doses of saline, KI, or perchlorate and ip replacement T_4 doses every 24 hours	$^{131}I^-$: 6×10^{-6} KI: 30 ClO_4: 30 T_4: 0.015	3, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, and 75	3, 27, and 51	Serum: 15 and 75 Thyroid: 75

Table 3.1

Table 3.2. Total urinary perchlorate and stable iodide excretion data collected via metabolism cages for male Sprague-Dawley rats dosed by gavage with 30 mg/kg of perchlorate or stable iodide at +3 hours and collections every 6 hours from +6 to +75 hours. For descriptions of calculations refer to methods section. Data are means ± SD (n=12).

(a)

75 hr Experiment Perchlorate Averages				
Time	**ug/ml**	**ug excreted**	**% excreted in urine**	**ug excreted/hr**
6	2410 ± 1208	2285 ± 911	23 ± 8.6	761 ± 304
12	2117 ± 752	5743 ± 2413	58 ± 23	957 ± 402
18	154 ± 67	596 ± 176	6.3 ± 2.3	99 ± 29
24	60 ± 34	129 ± 74	1.3 ± 0.8	21 ± 12
30	41 ± 25	98 ± 87	0.99 ± 0.85	16 ± 14
36	16 ± 10	45 ± 30	0.52 ± 0.51	7.5 ± 5.1
42	9.4 ± 5.6	36 ± 20	0.43 ± 0.19	6.8 ± 2.8
48	8.1 ± 5.0	28 ± 14	0.29 ± 0.13	4.8 ± 2.4
54	5.0 ± 5.0	12 ± 12	0.14 ± 0.13	2.1 ± 2.0
60	4.3 ± 2.6	16 ± 10	0.18 ± 0.09	2.8 ± 1.6
66	6.3 ± 5.5	21 ± 11	0.23 ± 0.11	3.8 ± 1.9
72	5.0 ± 5.4	14 ± 14	0.15 ± 0.14	2.5 ± 2.4
75	3.2 ± 1.9	5.8 ± 5.2	0.06 ± 0.05	1.9 ± 1.8

(b)

75 hr Experiment Iodide Averages				
Time	**ug/ml**	**ug excreted**	**% excreted in urine**	**ug excreted/hr**
6	1004 ± 475	691 ± 399	7.6 ± 4.3	230 ± 133
12	3352 ± 761	8686 ± 2147	89 ± 22	1447 ± 357
18	400 ± 102	1343 ± 478	14 ± 5.2	208 ± 69
24	241 ± 84	720 ± 325	7.3 ± 2.9	120 ± 54
30	112 ± 33	271 ± 78	2.8 ± 0.7	45 ± 13
36	75 ± 47	203 ± 89	2.3 ± 1.2	33 ± 14
42	32 ± 15	123 ± 47	1.3 ± 0.5	20 ± 7.9
48	24 ± 14	68 ± 28	0.76 ± 0.43	11 ± 4.7
54	17 ± 8.8	37 ± 16	0.42 ± 0.26	6.3 ± 2.8
60	13 ± 7.1	39 ± 25	0.46 ± 0.18	7.4 ± 3.6
66	8.2 ± 2.4	38 ± 13	0.41 ± 0.17	6.4 ± 2.2
72	9.6 ± 3.5	34 ± 12	0.37 ± 0.13	5.5 ± 2.0
75	9.3 ± 5.6	13 ± 6.8	0.13 ± 0.07	4.3 ± 2.7

Table 3.2

Table 3.3. Half-lives for $^{131}I^-$, stable iodide, and perchlorate were based on urinary excretion rates for each time-point. Rats were dosed with $^{131}I^-$ followed by saline, KI (30 mg/kg), or perchlorate (30 mg/kg) at +3 hours with urine collections every 6 hours for 75 hours (n=12). * is statistically significantly from saline treatment ($p<0.001$) and # is statistically significantly from KI treatment ($p<0.001$) for the distribution phase. No significance was determined for the terminal phase half-life. Half-lives for stable iodide and perchlorate are based on excretion rates for each time-point following the +3 hour dose. Half-lives were calculated using Win Non Lin5.2.

75 hour Time Course Urine Half-Lives						
	$^{131}I^-$		Stable Iodide		Perchlorate	
	Distribution	Terminal	Distribution	Terminal	Distribution	Terminal
Saline	4.0 ± 0.7	17.7 ± 4.9	-	-	-	-
KI	3.5 ± 0.5	16.1 ± 4.7	3.5 ± 0.6	13.8 ± 4.4	-	-
ClO_4^-	2.6 ± 0.4*#	25.4 ± 15.2	-	-	2.5 ± 0.5	15.6 ± 6.8

Table 3.3

Table 3.4. Total urinary perchlorate and stable iodide excretion data collected via metabolism cages for male rats dosed by gavage with 30 mg/kg of perchlorate or stable iodide at +3 hours and replacement T_4 doses at +3, +27, and +51 hours with urine collections every 6 hours from +6 to +75 hours. For descriptions of calculations refer to methods section. Data are means ± SD (n=6).

(a)

75 hr + T_4 Experiment Perchlorate Averages				
Time	**ug/ml**	**ug excreted**	**% excreted in urine**	**ug excreted/hr**
6	1048 ± 831	1375 ± 1410	14 ± 12	458 ± 470
12	1796 ± 321	5387 ± 1806	60 ± 17	897 ± 301
18	152 ± 40	661 ± 320	7.4 ± 3.5	110 ± 53
24	43 ± 19	120 ± 19	1.4 ± 0.4	20 ± 3.3
30	15 ± 9.3	40 ± 17	0.47 ± 0.30	6.7 ± 2.9
36	15 ± 11	47 ± 21	0.55 ± 0.28	8.0 ± 3.7
42	7.9 ± 4.0	34 ± 14	0.39 ± 0.17	5.7 ± 2.4
48	5.9 ± 2.2	21 ± 11	0.24 ± 0.12	3.5 ± 1.8
54	5.1 ± 2.8	9.6 ± 2.9	0.11 ± 0.03	1.6 ± 0.49
60	3.2 ± 2.4	8.9 ± 4.4	0.10 ± 0.05	1.5 ± 0.74
66	3.5 ± 1.8	17 ± 9.3	0.18 ± 0.08	2.8 ± 1.5
72	2.4 ± 1.1	10 ± 4.9	0.12 ± 0.07	1.7 ± 0.82
75	1.0 ± 0.5	1.6 ± 0.9	0.02 ± 0.01	0.53 ± 0.29

(b)

75 hr + T_4 Experiment Iodide Averages				
Time	**ug/ml**	**ug excreted**	**% excreted in urine**	**ug excreted/hr**
6	1148 ± 1185	810 ± 845	9.0 ± 9.5	270 ± 281
12	1960 ± 832	5448 ± 2400	58 ± 26	908 ± 400
18	485 ± 161	1958 ± 604	21 ± 6	326 ± 100
24	220 ± 76	758 ± 157	8.1 ± 0.9	126 ± 26
30	115 ± 35	206 ± 118	2.3 ± 1.4	34 ± 19
36	73 ± 33	301 ± 133	3.1 ± 1.1	50 ± 22
42	28 ± 14	140 ± 61	1.5 ± 0.46	23 ± 10
48	18 ± 9.5	67 ± 38	0.71 ± 0.33	11 ± 6.4
54	15 ± 6.1	31 ± 15	0.33 ± 0.13	5.3 ± 2.5
60	9.9 ± 5.2	34 ± 21	0.40 ± 0.30	5.8 ± 3.6
66	7.3 ± 3.6	34 ± 10	0.36 ± 0.09	5.7 ± 1.8
72	7.4 ± 2.2	28 ± 9.6	0.32 ± 0.17	4.7 ± 1.6
75	6.5 ± 3.8	13 ± 12	0.13 ± 0.11	4.3 ± 4.0

Table 3.4

Table 3.5 Half-lives for $^{131}I^-$, stable iodide, and perchlorate were based on urinary excretion rates for each time point. Rats were dosed with $^{131}I^-$ followed by saline, KI (30 mg/kg), or perchlorate (30 mg/kg) at +3 hours and replacement T_4 doses at +3, +27, and +51 hours with urine collections every 6 hours for 75 hours (n=12). * is statistically significantly from saline treatment ($p<0.001$) and # is statistically significantly from KI treatment ($p<0.001$) for the distribution phase. * is statistically significantly from saline treatment ($p<0.05$) and † is statistically significantly from perchlorate treatment ($p<0.05$) for the terminal phase. Half-lives were calculated using Win Non Lin5.2.

75 hour + Hormone Replacement Time Course Urine Half-Lives						
	$^{131}I^-$		Stable Iodide		Perchlorate	
	Distribution	Terminal	Distribution	Terminal	Distribution	Terminal
Saline	3.7 ± 1.2	25.2 ± 10.2	-	-	-	-
KI	3.8 ± 0.3	13.5 ± 2.7*†	3.9 ± 0.3	12.3 ± 4.8	-	-
ClO_4^-	2.6 ± 0.4*#	22.1 ± 6.9	-	-	2.5 ± 0.4	19.9 ± 9.7

Table 3.5

Figure 3.1. Cumulative $^{131}I^-$ in urine of male rats collected via metabolism cages dosed with $^{131}I^-$ followed by 0.9% saline, KI (30 mg/kg), or perchlorate (30 mg/kg) at +3 hours with urine collections every 6 hours for 75 hours (n=12). *Significantly different from control ($p<0.05$). $^{\#}$Significantly different from KI dose ($p<0.05$).

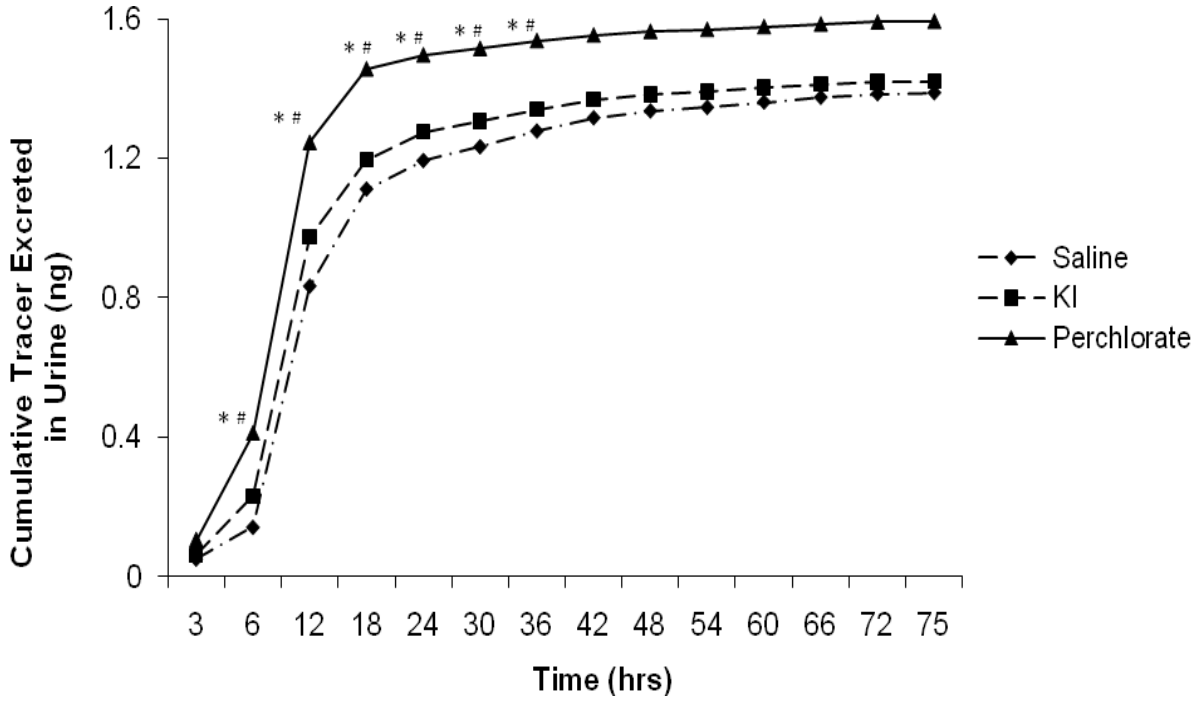

1.6
1.2
0.8
0.4
0
Cumulative Tracer Excreted in Urine (ng)
3
6
12
18
24
30
36
42
48
54
60
66
72
75
Time (hrs)
Saline
KI
Perchlorate
* #

Figure 3.1

Figure 3.2. $^{131}I^-$ concentrations in serum of male rats collected via tail vein bleed at +15 hours and via cardiac puncture at + 75 hours. Animals were dosed with $^{131}I^-$ followed by 0.9% saline, KI (30 mg/kg), or perchlorate (30 mg/kg) as described in Figure 1. Data are means ± SD (n=12). *Significantly different from control ($p<0.05$).

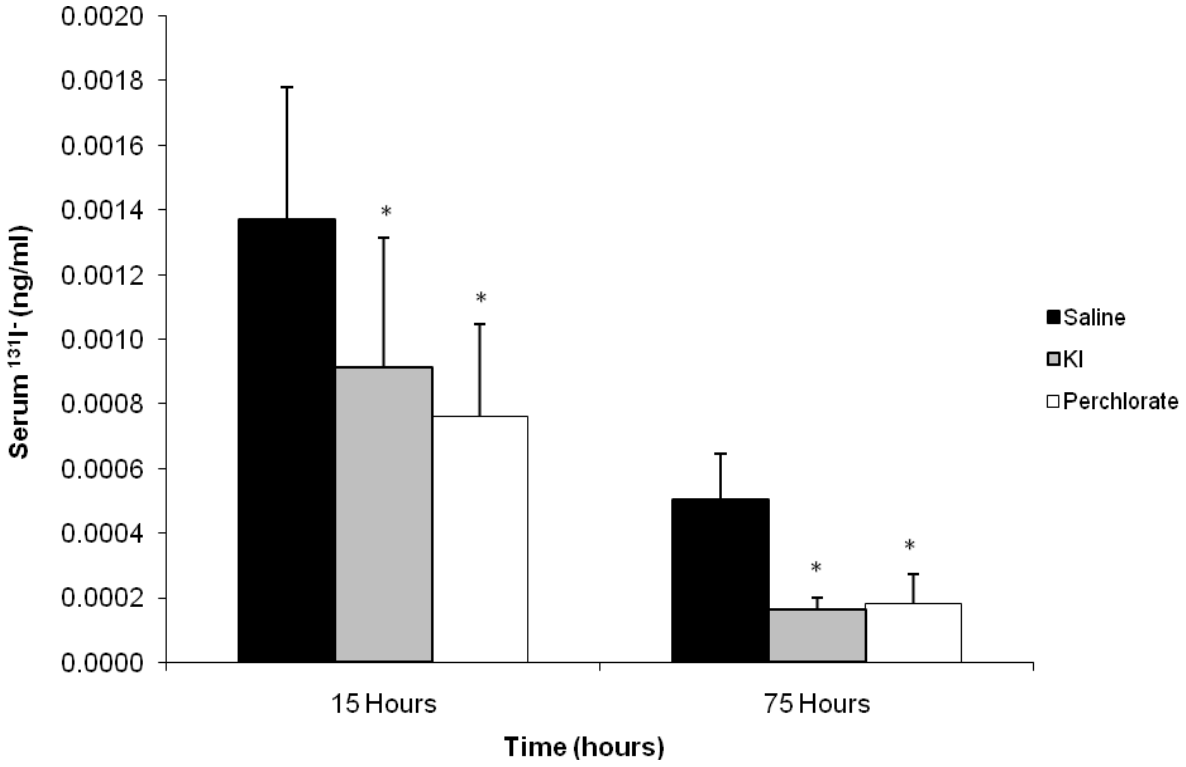

0.0020
0.0018
0.0016
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
Serum $^{131}I^-$ (ng/ml)
*
*
*
*
15 Hours
75 Hours
Time (hours)
Saline
KI
Perchlorate

Figure 3.2

Figure 3.3. 75 hour serum TSH values. Serum from the 75 hour cardiac puncture was measured for TSH concentration using TSH-RIA kits. Serum values based on averages from similar dose groups (n=11). No significance was determined between the various treatments ($p > 0.05$).

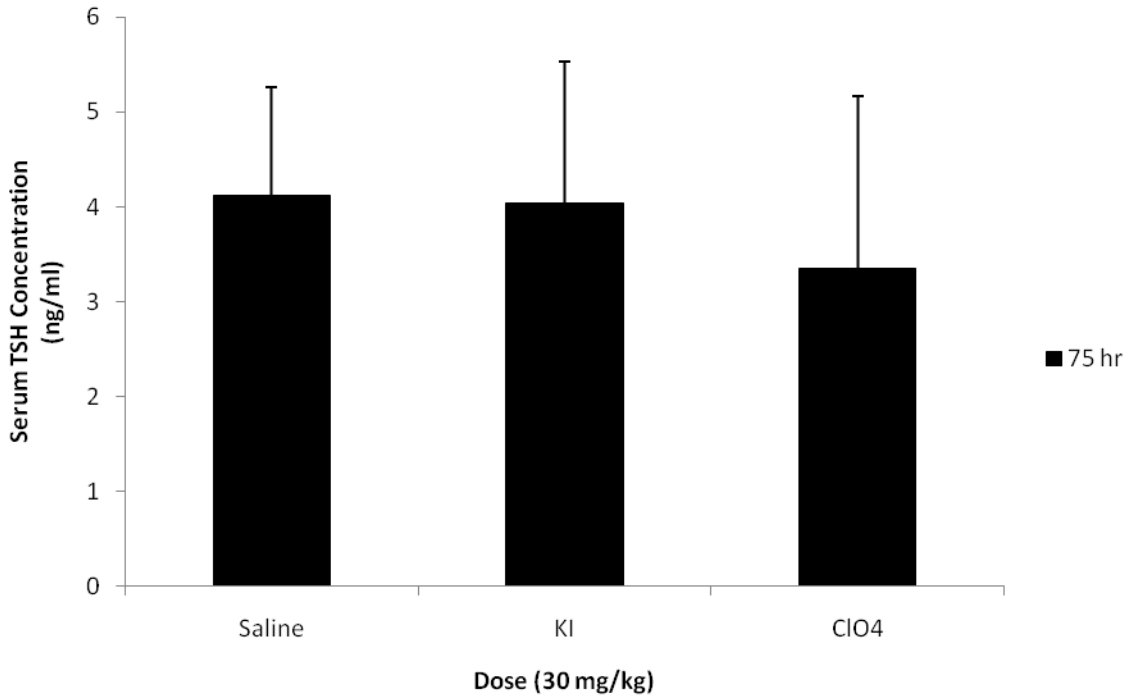

6
5
4
3
2
1
0
Serum TSH Concentration (ng/ml)
Saline
KI
ClO4
Dose (30 mg/kg)
75 hr

Figure 3.3

Figure 3.4. 75 hour $^{131}I^-$ concentrations in the thyroid of male rats dosed with $^{131}I^-$ via gavage followed by 0.9% saline, KI (30 mg/kg), or perchlorate (30 mg/kg) as described in Figure 1. Data are means ± SD (n=12). *Significantly different from control ($p<0.001$). †Significantly different from perchlorate dose ($p<0.01$).

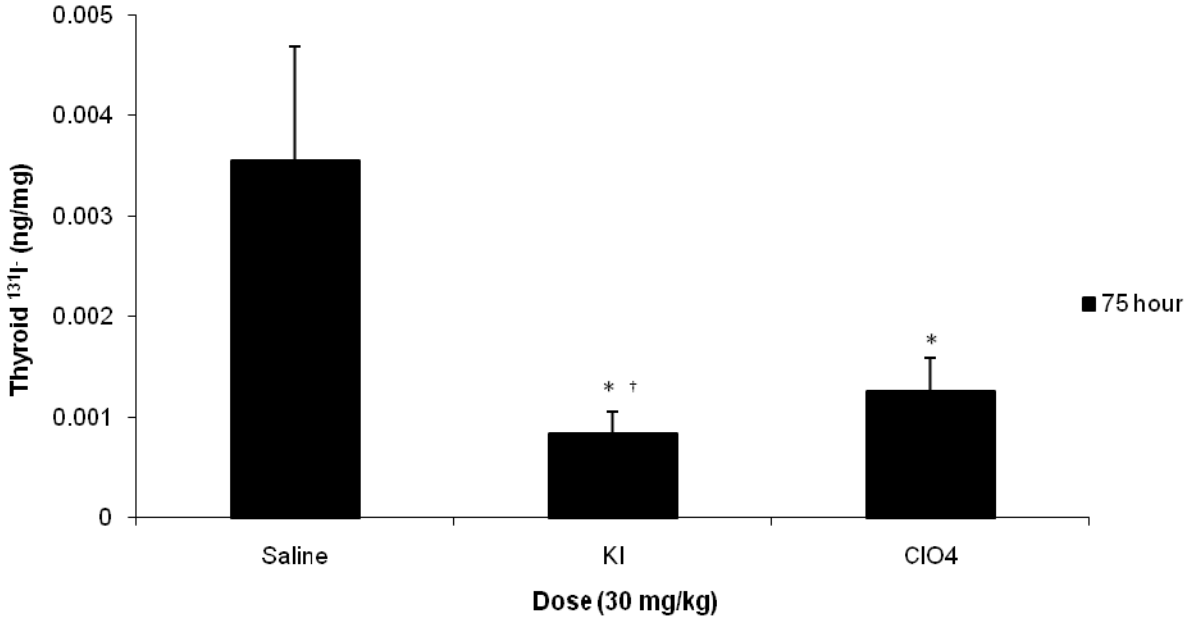

0.005
0.004
0.003
0.002
0.001
0
Thyroid $^{131}I^-$ (ng/mg)
* †
*
Saline
KI
ClO4
Dose (30 mg/kg)
75 hour

Figure 3.4

Figure 3.5. Cumulative $^{131}I^-$ in urine of male rats collected via metabolism cages dosed with $^{131}I^-$ followed by 0.9% saline, KI (30 mg/kg), or perchlorate (30 mg/kg) at +3 hours and replacement T_4 doses at +3, +27, and +51 hours with urine collections every 6 hours for 75 hours (n=6). *Significantly different from control ($p<0.05$).

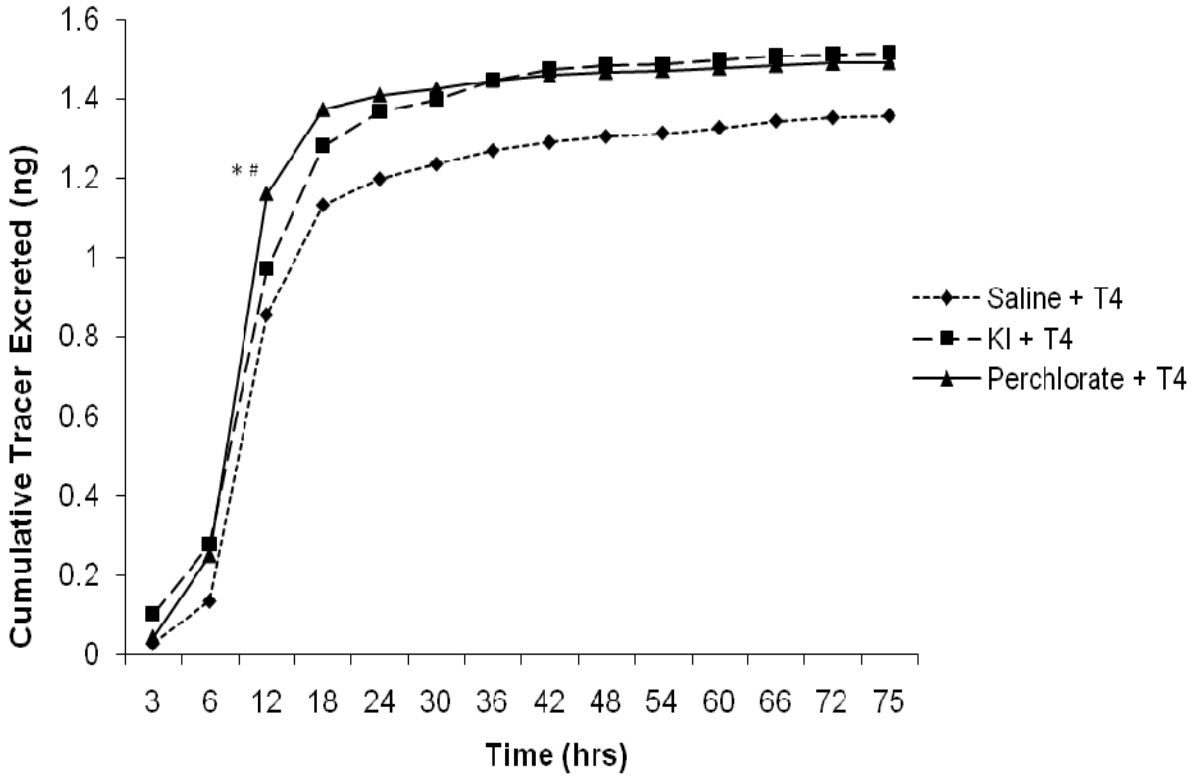

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Cumulative Tracer Excreted (ng)
* #
3
6
12
18
24
30
36
42
48
54
60
66
72
75
Time (hrs)
Saline + T4
KI + T4
Perchlorate + T4

Figure 3.5

Figure 3.6. $^{131}I^-$ concentrations in serum of male rats collected via tail vein bleed at +15 hours and via cardiac puncture at + 75 hours. Animals were dosed with $^{131}I^-$ followed by saline, KI (30 mg/kg), or perchlorate (30 mg/kg) with replacement T_4 doses as described in Figure 3.5. Data are means ± SD (n=6). *Significantly different from saline ($p<0.01$) and $^{\#}$is significantly different from KI ($p<0.05$).

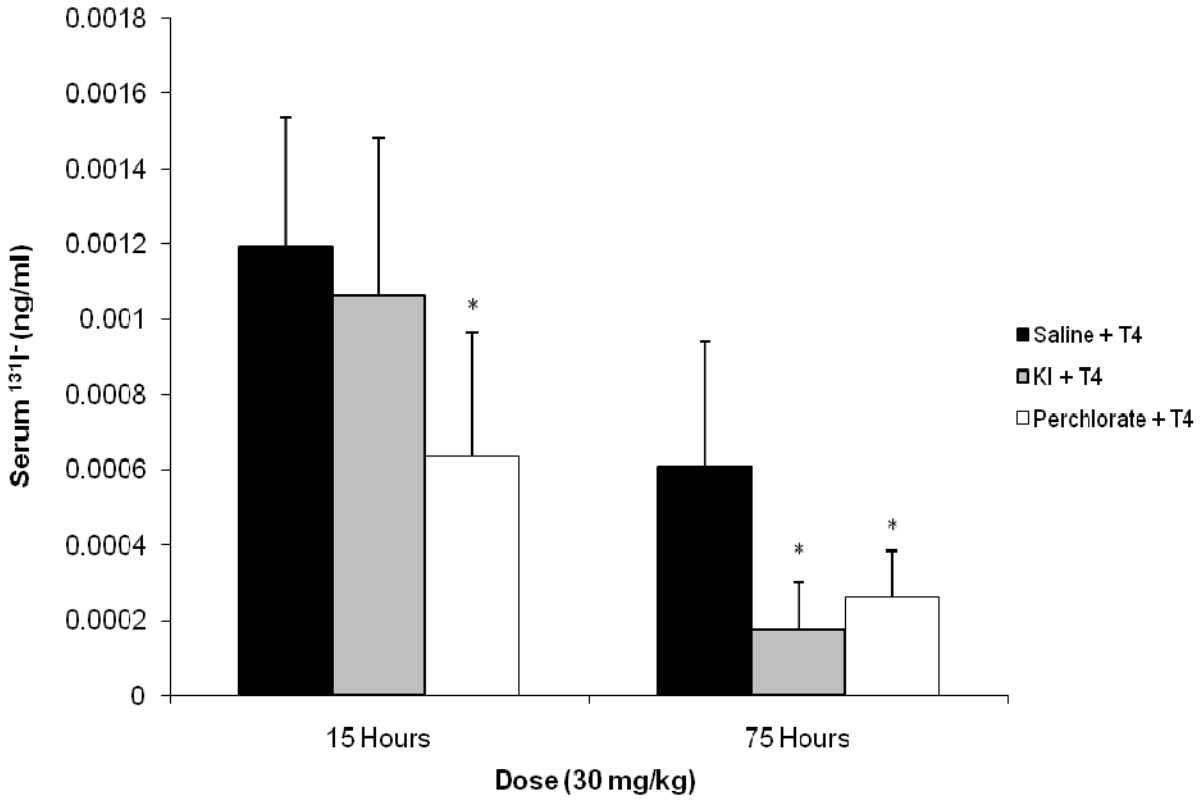

0.0018
0.0016
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
Serum $^{131}I^-$ (ng/ml)
*
*
*
15 Hours
75 Hours
Dose (30 mg/kg)
Saline + T4
KI + T4
Perchlorate + T4

Figure 3.6

Figure 3.7. 75 hour + T_4 $^{131}I^-$ concentrations in the thyroid of male rats dosed with $^{131}I^-$ via gavage followed by 0.9% saline, KI (30 mg/kg), or perchlorate (30 mg/kg) with T_4 replacement as described in Figure 5. Data are means ± SD (n=6). *Significantly different from control ($p<0.01$).

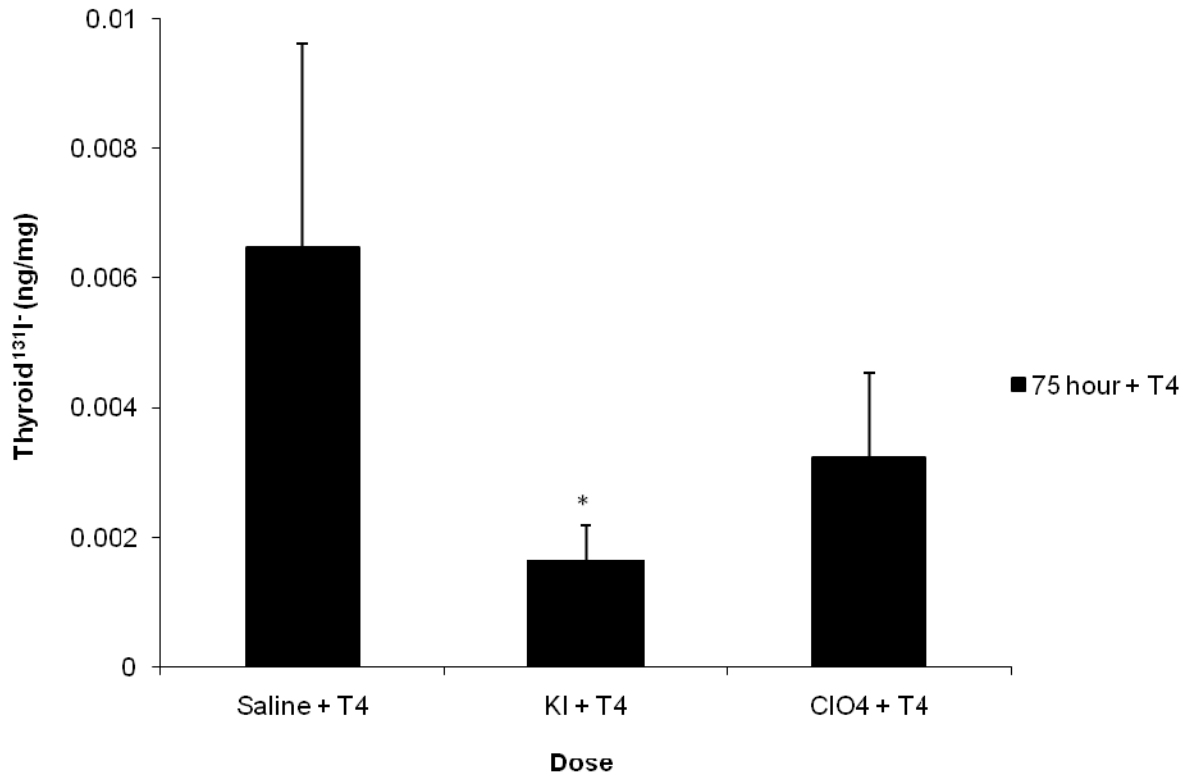

0.01
0.008
0.006
0.004
0.002
0
Thyroid $^{131}I^-$ (ng/mg)
*
Saline + T4
KI + T4
ClO4 + T4
Dose
75 hour + T4

Figure 3.7

Figure 3.8. Comparison of $^{131}I^-$ concentration following 0.9% saline, 30 mg/kg KI, or 30 mg/kg perchlorate after 75 hours in the thyroid of male rats administered T_4 versus rats not administered hormone replacement therapy (n=12 for non-T_4 and n=6 for T_4). * is $p < 0.05$ for saline comparisons and ‡ $p < 0.001$ for KI and perchlorate comparisons.

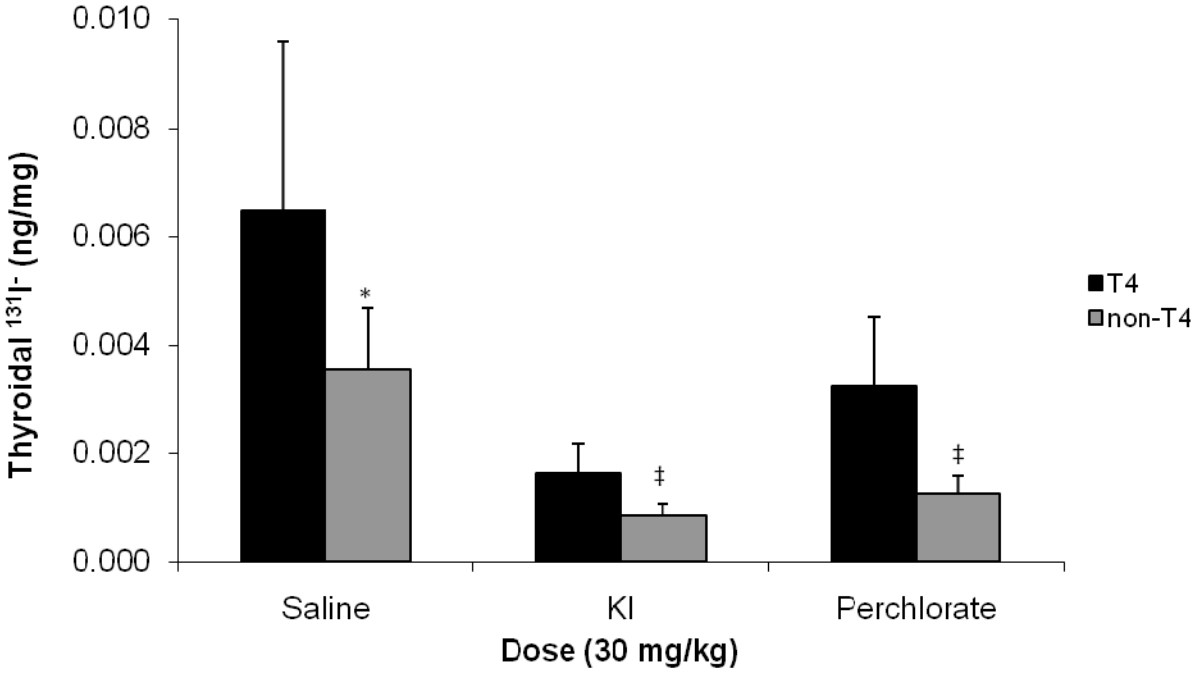

0.010
0.008
0.006
0.004
0.002
0.000
Thyroidal $^{131}I^-$ (ng/mg)
*
‡
‡
T4
non-T4
Saline
KI
Perchlorate
Dose (30 mg/kg)

Figure 3.8

CHAPTER 4

SUMMARY AND CONCLUSIONS

Radiation exposure has been a major source of concern for the past several decades from nuclear power plant releases and from the increasing of nuclear weapon use during times of war or asymmetric terrorist attack. Many different types of radioisotopes are released from power plant meltdowns and nuclear weapons, including radioiodide. Concerns over radioiodide emerged from the Chernobyl disaster of 1986 when the largest airborne release of radioactive materials in history occurred and dumped large quantities of radioiodide ($^{131}I^-$) across Russia, Belarus, and Ukraine. $^{131}I^-$ operates identically to stable iodide ($^{127}I^-$) when ingested into the body. Iodide is rapidly taken up by the sodium-iodide symporter (NIS) protein and transported to the thyroid where it is organified and incorporated into thyroid hormones. After being incorporated, the hormones are then liberated from the thyroid and go out into the systemic circulation and peripheral tissue to carry out their physiological functions. Pronounced pharmaceutical interventions are crucial for preventing uptake of the $^{131}I^-$ into the thyroid and subsequently preventing their incorporation into thyroid hormones. Of the various pharmaceutical interventions that have been evaluated over the years, two have emerged as the most viable candidates: potassium iodide (KI) and perchlorate (ClO_4^-). KI is currently the only drug approved for treatment of $^{131}I^-$ poisoning by the Food and Drug Administration (FDA), while perchlorate was used extensively during the 1950's through the 1960's for the treatment of

hyperthyroidism but lost its favor after seven reported cases of fatal aplastic anemia were diagnosed. Up until now most all of the radiation protection studies involving KI or perchlorate have focused on administering loading doses of the interventions followed by administration of $^{131}I^-$ and have yielded substantial blocking efficacies with minimal $^{131}I^-$ being up-regulated by the thyroid. However, taking loading doses of KI or perchlorate will not always be feasible, as was determined by the Chernobyl disaster (with over 11,000 reported cases of thyroid cancer) and the Nagasaki and Hiroshima atomic bomb survivors.

KI/ClO_4^- Ameliorating $^{131}I^-$ Exposure

To answer the question of what happens when pre-exposure loading doses are not possible, this dissertation employed single dose, single time-point experiments to see what effects KI and perchlorate would have if administered post $^{131}I^-$ contamination. We hypothesized that perchlorate would have a more pronounced blocking efficiency of up-regulation of $^{131}I^-$ relative KI treatment in rats since perchlorate has been demonstrated to have a higher affinity for the NIS protein and thyroid receptor sites.

Results from our studies suggest that perchlorate and KI have equal efficiencies in being able to block $^{131}I^-$ from entering the thyroid when administered post $^{131}I^-$ dose. Each intervention started to work almost immediately after ingestion and prevent any further accumulation of $^{131}I^-$ for the remainder of the experiment. However, nearly all the $^{131}I^-$ that had been taken up by the thyroid gland had already been organified and incorporated into thyroid hormones, rendering KI and perchlorate therapy of little consequence for the organified fraction of $^{131}I^-$. The major difference that occurred between the KI and perchlorate treatments was in the serum and urine. Urinary excretion data proved to be most definitive determinant of which therapeutic was the

most efficient and led to the relative difference of an increased serum concentration of $^{131}I^-$ in KI dosed animals relative to perchlorate dosings. The urinary excretion data of the perchlorate treatment group far exceeded that of animals administered KI for $^{131}I^-$ excretion and excretion of the therapeutic anions. Our hypothesis for these experiments was nullified based on perchlorate and KI possessing an equal affinity for blocking the uptake of $^{131}I^-$ into the thyroid gland. However, the initial experiments also presented findings for a need of further experimentation that focused on urinary excretion profiles of $^{131}I^-$ following KI and perchlorate prophylaxis.

Urine Time-Course Following KI and Perchlorate Prophylaxis

New studies were designed to look at urinary excretion over time, in which we hypothesized that over the experimental time-course, perchlorate treated animals would have an enhanced urinary output of $^{131}I^-$ relative to the KI dosings.

Results from these studies suggested that by the conclusion of the experiment perchlorate and KI dose groups excreted an almost equivalent cumulative amount of $^{131}I^-$. Again, our hypothesis was nullified, but under closer inspection of the early time points of the time-course, we discovered that perchlorate dosed animals had a significantly higher cumulative excretion of the $^{131}I^-$ during the first 30 hours of the experiment. This finding was significant because >90% of the total $^{131}I^-$ and therapeutic anions that were excreted occurred during the first 30 hour time period. This finding suggested that after 30 hours there was a similar response to that of a control study. With a higher concentration of $^{131}I^-$ residing in KI dosed animals, these dose groups essentially caught up in their excretion profiles.

No study of this nature would be complete without considering the target organ of $^{131}I^-$, KI, and perchlorate. The results of the thyroid uptake of $^{131}I^-$ from KI and perchlorate dosed

animals at the end of the time-course were somewhat unexpected based on the findings of the initial study. It was determined that KI dosed animals had a significantly lower thyroid $^{131}I^-$ concentration than did its perchlorate treated counterparts.

Urine Time-Course Following KI and Perchlorate Prophylaxis with Hormone Replacement

A final set of experiments were designed following a similar experimental protocol as the previous time-course study except all animals were administered thyroxine (T_4) every 24 hours, beginning three hours after $^{131}I^-$ administration. We hypothesized that the hormone replacement therapy would allow the thyroid to be in more of an inactive state and help to prevent any further up-regulation of $^{131}I^-$ into the thyroid.

Results from these studies indicate that urinary clearance of the $^{131}I^-$, as well as the therapeutic anions, were mostly unaffected by the addition of the hormone replacement therapy. The only change that occurred in the urinary excretion was that significant $^{131}I^-$ increases were only determined for the first 12 hours in perchlorate versus KI treated animals, rather than the first 30 hours determined in the previous time-course experiment.

The most significant of these changes occurred in the thyroid gland. No significance was determined between the KI and perchlorate dosed animals for thyroidal $^{131}I^-$ concentration. Therefore, our hypothesis was confirmed. However, when the previous time-course data was compared to the time-course with hormone replacement, we found that for similarly dosed animals (i.e. KI and KI + T_4 or perchlorate and perchlorate + T_4) there was a significantly higher concentration of $^{131}I^-$ in the thyroid of animals that received hormone replacement compared to animals that did not. We concluded from this study that hormone replacement therapy actually worked to the detriment of the thyroid. When $^{131}I^-$ is given a head start, as in these experiments,

it is continually being taken up by the thyroid gland and organified into hormones. After prophylaxis with KI or perchlorate and then hormone replacement, the hypothalamus-pituitary-thyroid axis determines that there is enough hormone in the systemic circulation and becomes virtually inactive. This means that all of the $^{131}I^-$ that has be taken up by the thyroid is essentially trapped and waiting until a need for more hormone arises. Therefore, hormone replacement therapy actually allows previously accumulated $^{131}I^-$ to unleash more of its harmful gamma and beta radiation into the thyroid gland instead of releasing it into the body where the hormones can be degradated or possible cleaved off during the conversion of T_4 to triiodothyronine (T_3) in the peripheral tissues.

Future Work

Several aspects of this work could be expanded on in the future. First, $^{131}I^-$ has more of a toxic effect in the thyroids of children and adolescence due to the same amount of activity ingested in a much smaller tissue mass. A study involving pups and juvenile rats would be appropriate to test the relative effects of administering KI and perchlorate following $^{131}I^-$ exposure in young animals. Second, perchlorate was effective during the first 36 hours of the time-course experiment without hormone replacement in excretion of $^{131}I^-$ relative to KI treatment. However, by the termination of the experiment an identical cumulative excretion of $^{131}I^-$ was determined for KI and perchlorate treated animals. Multiple dosings of each therapeutic would be appropriate to see if perchlorate dosings could maintain their lead in urinary excretion of the isotope and possibly remain identical in blocking the uptake of $^{131}I^-$ into the thyroid gland. Since giving replacement doses of T_4 cause the thyroid gland to store the previously accumulated $^{131}I^-$ once therapy was initiated, a third research approach would be to give replacement doses of

thyroid releasing hormone (TRH) instead of T_4. Administering TRH in conjunction with KI or perchlorate would cause the depletion of any stored iodide in the form of hormone that was trapped in the gland and also stimulate the production of NIS protein and up-regulation of iodide into the thyroid. Having the large doses of either KI or perchlorate administered with the TRH would allow for a much larger concentration of protective anions that could fill the NIS and thyroid receptor sites while also allowing the thyroid to rid itself of the toxic radionuclides.