III. TOXICOKINETICS

Introduction

Studies relevant to the toxicokinetics of inorganic chloramines are severely limited. However, studies done with various chlorinated amino compounds (including organic chloramines) give information on the pharmacokinetics of chloramines.

Absorption

Scully et al. (1985) have shown that solutions of hypochlorite can react with amines and amino acids in stomach fluid to form the corresponding N-chloramines. In separate experiments piperidine (0.2 M solution) and glycine (0.5 M solution) were administered to Sprague-Dawley rats followed by a solution (either ~200 or 1000 mg/L) of aqueous hypochlorite. Stomach fluid was recovered from these animals and shown to contain the corresponding N-chloramino derivatives. The "chlorine demand" of stomach fluid was determined by chlorinating the stomach fluid to various levels, incubating the chlorinated fluid in the dark for 1 hour and measuring the residual chlorine - in essence developing a breakpoint curve for the fluid. The chlorine dosage at which a breakpoint was found was taken as the chlorine demand. The shape of the breakpoint curve was very similar to that of wastewater, exhibiting a significant irreducible minimum at the breakpoint and a high chlorine demand (400-800 mg/L) (Scully et al., 1986). Scully et al. (1985) also found that when N-chloropiperidine (1.3 mL of 1700 mg/L as chlorine containing 100 μCi tritium labeled N-chloropiperidine) was administered to

Sprague-Dawley rats, small amounts (1-3 μCi) of the added chloramine appeared in the plasma at 30, 60 and 120 minutes after the exposure.

Abdel-Rahman et al. (1983) administered single oral doses of 3 mL $NH_2{}^{36}Cl$ (370 mg/L) to four male Sprague-Dawley rats that had been fasted overnight. Each rat received 1.1 mg of test material (~4.6 mg/kg/day). A peak ^{36}Cl plasma level (10.3 μg/L) was reached 8 hours after administration, and the absorption rate constant was 0.278 mg/hour with an absorption half-life of 2.5 hours. The ^{36}Cl plasma level remained at a plateau from 8 to 48 hours after administration. After 48 hours the radiolabel was eliminated from the plasma with a half-life of 38.8 hours and a rate constant of 0.018 mg/hour.

Toxicokinetics data on other absorption rates with respect to various dosage media and different routes of exposure were not available.

Distribution

At 24 hours following the single oral treatment to rats of $NH_2{}^{36}Cl$ as described above, Abdel-Rahman et al. (1983) found the level of ^{36}Cl radioactivity in the plasma to be 0.87%/mL of the administered dose. During the first 24 hours the portion of the administered $NH_2{}^{36}Cl$ dose eliminated through the urine was 0.40% and 0.08% by the feces. After the addition of TCA, 0.14%/mL was precipitated from 1 mL of plasma, possibly indicating binding to the protein fraction. Packed cells had an activity of 0.20%/mL that decreased to 0.06%/mL after washing twice with saline, indicating that

most of the ^{36}Cl radioactivity in the packed cells was loosely bound to the erythrocyte membrane or was exchangeable with the chloride in saline.

Abdel-Rahman et al. (1983) also characterized the subcellular distribution of ^{36}Cl radioactivity in rat liver preparations 24 hours following $NH_2{}^{36}Cl$ administration. The major portion of the activity (75%) in the whole liver homogenate was recovered in the cytosol, 2.5% was recovered in the microsomal, 1.5% in the nuclear and <0.1% in the mitochondrial fractions. Only 4.0% of the total ^{36}Cl radioactivity of the whole homogenate was precipitated by TCA. In the control experiment using $^{36}Cl^-$ Suh and Abdel-Rahman (1983) found a similar liver subcellular distribution of ^{36}Cl radioactivity: 0.82 μg/g in the whole homogenate, 0.66 μg/g in the cytosol, 0.03 μg/g in the microsomes, 0.01 μg/g in nuclei, and 0.001 μg/g in mitochondria. ^{36}Cl was administered as 200 mg/L $Na^{36}Cl$ (0.6 mg/animal) solution orally.

The distribution of ^{36}Cl in various tissues was determined 120 hours following $NH_2{}^{36}Cl$ administration (Abdel-Rahman et al., 1983). Plasma contained the highest concentration of ^{36}Cl radioactivity (3.15 μg/g), followed by whole blood (2.66 μg/g), skin (2.13 μg/g), testes (2.09 μg/g), packed cells (1.90 μg/g), bone marrow (1.82 μg/g), kidney (1.62 μg/g), lung (1.58 μg/g), stomach (1.53 μg/g), thyroid (1.36 μg/g), thymus (1.36 μg/g), duodenum (1.20 μg/g), spleen (1.11 μg/g), carcass (0.77 μg/g), liver (0.74 μg/g), ileum (0.59 μg/g) and fat (0.18 μg/g) (Figure III-1). In the control experiment with ^{36}Cl-labeled chloride Suh and Abdel-Rahman (1983) using 47% as much labeled

material as in the $NH_2{}^{36}Cl$ study found an approximately proportional amount of ^{36}Cl distributed similarly

FIGURE III-1

$NH_2{}^{36}Cl$ Radioactivity Distribution in the Rat 120 Hours
After the Administration of $NH_2{}^{36}Cl$ (1.1 mg) Orally

Source: Abdel-Rahman et al., 1983

throughout the same organs, fluid and tissues (Figure III-2). The ^{36}Cl radioactivity was highest in plasma (1.4 μg/g) followed by whole blood (1.3 μg/g), testes (1.2 μg/g), packed cells (1.1 μg/g), skin (0.9 μg/g), kidney (0.8 μg/g), lung (0.8 μg), bone marrow (0.8 μg/g), stomach (0.7 μg/g), thymus (0.7 μg/g), spleen (0.6 μg/g), duodenum (0.5 μg/g), carcass (0.3 μg/g), liver (0.3 μg/g), ileum (0.4 μg/g) and fat (0.2 μg/g).

Metabolism

Abdel-Rahman et al. (1983) measured chloride, chlorite and chlorate in plasma 120 hours after administration of $NH_2{}^{36}Cl$ in rats for determination of its metabolites. Neither ^{36}Cl-labeled chlorite nor chlorate was detected in rat plasma. Most of the total ^{36}Cl was identified as ^{36}Cl-chloride, which (according to the investigators) indicated that the chlorine moiety was eliminated primarily in this form (Table III-1).

Scully et al. (1986) identified three organic N-chloramines (N-chloroglycine, N-chloroalanine and N-chlorophenylalanine) formed *in vitro* when stomach fluid from Sprague-Dawley rats was chlorinated with a concentration of 400 mg/L hypochlorite. After *in vitro* chlorination of stomach fluid from rats fasted for 8, 24 and 48 hours, Scully et al. (1990) identified and quantified N-chloroleucine or N-chloroisoleucine, N-chloroglycine and N-chlorophenylalanine by GC/MS. Scully et al. (1986) also found that because stomach fluid already contains high concentrations of amino nitrogen, the percent conversion of a single exogenous amine to its chloramino compound is low. They determined the actual yield of chloramine by administration of tritium-labeled piperidine followed by aqueous hypochlorite. The quantity of N-chloropiperidine formed

FIGURE III-2

$^{36}Cl^-$ distribution in the rat. Four fasted rats were each administered 200 mg/L $Na^{36}Cl$ in a 3 mL solution orally. The rats were sacrificed 120 hours after administration, and $^{36}Cl^-$ was determined in different organs. Values represent the mean ± SE as μg $^{36}Cl^-$/mL (or per gram tissue) from four rats.

Source: Suh and Abdel-Rahman, 1983

was determined by direct liquid chromatography of the compound. This quantity combined with the average chlorine demand determined for stomach fluid was used to calculate the yield of chloramine formed. For concentrations of hypochlorite ranging from 200-1000 mg/L, yields of organic chloramines varied from 50-75% of the theoretical amount expected for these compounds. However, Scully et al. (1986) showed that administration of hypochlorite to animals at low (<40 mg/L), medium (40-800 mg/L) and high (>800 mg/L) dosages can produce very different results. This may be due to different chemical reactions taking place at varying hypochlorite concentrations below the minimum chlorine demand. They cautioned that toxicologic studies conducted at high oxidant concentrations may not reflect chloramine reactions that would account for any toxicologic effects observed at lower oxidant concentrations or at actual drinking water concentrations.

Under conditions designed to simulate the GI tract, Bercz and Bawa (1986) found that monochloramine caused covalent binding of radioiodide to nutrient biochemicals. Saliva and gastric juice were obtained from rhesus monkeys under mild anesthesia. Depending on their solubilities, nutrient substrates were dissolved in solvents and added to a mixture of 600 ppm monochloramine, 0.02 N HCl and 0.1 M KI. Monochloramine was believed to oxidize iodide to iodine, which subsequently reacted with nutrient chemicals to form iodinated organic compounds. Tyrosine, 4-aminobenzoic acid, arachidonic acid, and folic acid were among the compounds that became iodinated under the conditions of the experiment. Some of the reactions occurred under basic pH conditions. The observed percent binding was generally lower for reactions occurring

at lower pHs. While complex mixtures of nutrients such as gastric juice and saliva appeared to bind iodine in dilute aqueous solution, it is important that these results be correctly extrapolated to physiologic pH before their significance is fully understood.

The biologic effects of chloramine, its persistence in biologic fluids and its possible conversion to more toxic products were examined by the U.S. EPA (1990). The persistence of monochloramine in saliva and gastric fluid was examined to determine the extent of formation of the products dichloramine, trichloramine and molecular chlorine. Pooled human saliva and gastric fluid samples were treated with monochloramine to produce samples with initial concentrations of monochloramine between 1.0 and 20 ppm. These samples were continuously analyzed by membrane introduction mass spectrometry and tandem mass spectrometry for monochloramine, dichloramine, trichloramine and chlorine. The continuous monitoring experiments for the saliva monochloramine mixtures were done using the technique of selected ion monitoring. Because of constituents present in the gastric fluid samples, selected ion monitoring experiments were shown not to give reliable results and the multiple reaction monitoring procedure was used.

Monochloramine was completely depleted in saliva in ~5 minutes at the 1 ppm level, the 5 ppm solution was incompletely depleted in 2 hours and the monochloramine in the higher concentration solutions was largely unaffected. Dichloramine, trichloramine and molecular chlorine were not produced in detectable levels from the chloramine-treated saliva samples.

Gastric fluid monochloramine disappeared completely in <30 seconds at all concentrations, and dichloramine, trichloramine and molecular chlorine were not observed.

Excretion

Abdel-Rahman et al. (1983) collected the urine, feces and expired air of Sprague-Dawley rats over a 5-day period after the administration of 370 mg/L (1.1 mg/animal or ~4.6 mg/kg/day) $NH_2{}^{36}Cl$ to four Sprague-Dawley rats. The amount of ^{36}Cl-radiolabeled material excreted by urinary and intestinal routes are summarized in Table III-2. During the first 24 hours after administration of $NH_2{}^{36}Cl$, only 0.40 and 0.08% of the total dose administered was eliminated in the urine and feces, respectively. The proportion of the dose eliminated through the urine and feces at the end of the 120-hour study period was 25.15 and 1.98%, respectively. By comparison, Suh and Abdel-Rahman (1983) found that over twice as much of the ^{36}Cl-radiolabel was eliminated over the 120-hour study period when ^{36}Cl-radiolabeled chloride ion (200 mg/L as $Na^{36}Cl$) was administered (Table III-3). They found that 57.2% of the administered ^{36}Cl-radiolabel was eliminated in urine and 3.0% was eliminated in feces. Unlike $NH_2{}^{36}Cl$, more of the ^{36}Cl-label was eliminated in the first 24 hours, 16.1% in urine and 0.92% in feces. After 48 hours ^{36}Cl was eliminated with a half-life similar to that found after 24 hours in the $NH_2{}^{36}Cl$ study ($t_{1/2}$ = 24 hours).

Discussion

In order to interpret the results of the toxicokinetics correctly, it is necessary to understand the complications of the synthesis of $NH_2{}^{36}Cl$ and possible interferences.

^{36}Cl-radiolabeled chloride ion is available commercially. $HO^{36}Cl$ is synthesized by acidic permanganate oxidation of the $^{36}Cl^-$ to $^{36}Cl_2$:

$$2KMnO_4 + 16H^{36}Cl = 2KCl + 2MnCl_2 + 5^{36}Cl_2 + 8H_2O.$$

The ^{36}Cl-labeled Cl_2 gas is passed from the generation flask to a receiving flask where it is dissolved in deionized water:

$$^{36}Cl_2 + H_2O = HO^{36}Cl + H^{36}Cl$$

The resulting hydrolysis converts half the labeled chlorine to $HO^{36}Cl$, the desired compound, and half to $^{36}Cl^-$.

Chlorine-36 radiolabeled inorganic chloramine is synthesized by reaction of the solution of $HO^{36}Cl$ (containing an equivalent amount of $^{36}Cl^-$) with aqueous ammonia in bicarbonate buffer:

$$NH_3 + {}^{36}ClO^- = NH_2{}^{36}Cl + HO^-$$

However, the observed kinetics of $NH_2{}^{36}Cl$ absorption and elimination are necessarily affected by the presence of an equivalent amount of $^{36}Cl^-$ formed during the preparation of the $HO^{36}Cl$.

In addition, the stomach contains high concentrations of Cl^-. There is a lack of information on the rate of chloramine-chloride exchange

$$NH_2{}^{36}Cl + Cl^- = NH_2Cl + {}^{36}Cl^-$$

in solutions of high chloride concentration. If this exchange rate is significant, the toxicokinetics of $NH_2{}^{36}Cl$ could appear to resemble chloride when, in fact, the compound has simply lost its radiolabel through exchange. This is the case for $HO^{36}Cl$ (Anbar et al., 1959) where the exchange reaction is so fast that at 25°C in the presence of 0.1 M chloride the exchange is 99% complete in <0.1 sec at any pH <10.8. In the absence of specific information on the isotopic exchange rates for $NH_2{}^{36}Cl$, the rate of hydrolysis of NH_2Cl as reported by Margerum et al. (1979) ($k = 1.9 \times 10^{-5}$ sec^{-1}) and by Anbar and Yagil (1962) ($k = 6.3 \times 10^{-5}$ M^{-1} sec^{-1}) would suggest that the rate of exchange is slow and that it would require between 4 and 10 hours for half the radiolabel to be lost at 25°C. Nevertheless, monochloramine exchanges its chlorine with organic amino nitrogen compounds at a rate faster than hydrolysis (Snyder and Margerum, 1982; Isaac and Morris, 1983, 1985). Since the stomach contains high concentrations of organic amino nitrogen, the chlorine from the $NH_2{}^{36}Cl$ can be transferred to these organic amino nitrogen compounds. How this affects the rate of isotope exchange is unknown.

The control reagent used in the above toxicokinetic study (Anbar et al., 1959) of $NH_2{}^{36}Cl$ was $^{36}Cl^-$. It is not likely that an oxidant as strong as NH_2Cl will survive intact absorption, distribution, and excretion from an animal. If the chloramine is rapidly detoxified in the stomach to ^{36}Cl-radiolabeled chloride and ammonia, the observed toxicokinetics will be identical to the control kinetics. However, if the chloramine acts as a chlorinating agent, other more stable chlorinated organic compounds may form. The observed kinetics may then be due to exposure to these chlorinated compounds.

Summary

Information on the absorption of inorganic chloramines is extremely limited. In one study Abdel-Rahman et al. (1983) calculated an absorption rate constant for ^{36}Cl at 0.278 mg/hour (after 8 hours) with an absorption half-life of 2.5 hours after male Sprague-Dawley rats were administered a single oral dose of ~4.6 mg/kg/day. After 48 hours the radiolabel was eliminated from the plasma with a half-life of 38.8 hours and a rate constant of 0.018 mg/hour. Absorption rates with respect to various dosage media and different routes of exposure were not available. However, studies done with various chlorinated amino compounds (including organic chloramines) give information on the pharmacokinetics of chloramines. Scully et al. (1985) found that when the organic chloramine, N-chloropiperidine, was administered to Sprague-Dawley rats, 1-3 μCi of the chloramine appeared in the plasma at 30, 60 and 120 minutes after the exposure.

The distribution of radiolabeled chlorine in the subfractions of rat liver homogenates, in organs, tissues and fluids was similar (120 hours) after oral administration of either

^{36}Cl (200 mg/L as $Na^{36}Cl$) or $NH_2^{36}Cl$ (370 mg/L $NH_2^{36}Cl$) (Abdel-Rahman et al. 1983; Suh and Abdel-Rahman (1983). Plasma contained the highest concentrations of ^{36}Cl radioactivity for $NH^{36}Cl$ followed by whole blood skin, testes, packed cells, bone marrow, kidney, lung, stomach, thyroid, thymus, duodenum, spleen, carcass, liver, ileum and fat (Abdel-Rahman et al., 1983).

Information on the metabolism of chloramines is also extremely limited. Abdel-Rahman et al. (1983) indicated that chloramine ($NH_2^{36}Cl$) administered to rats was metabolized primarily to ^{36}Cl-chloride, which indicated that the chlorine moiety was eliminated in this form.

When pooled saliva and gastric fluid were treated with monochloramine (U.S. EPA, 1990), saliva monochloramine was depleted in 5 minutes at 1 ppm, and in higher concentrations was not affected, while gastric monochloramine disappeared in <30 seconds at all concentrations tested. Dichloramine, trichloramine and molecular chlorine were not produced in detectable levels from the chloramine-treated saliva or gastric samples.

Chloramines or their metabolites (principally chloride) are eliminated primarily through the urine. Abdel-Rahman et al. (1983) found that during the first 24 hours after a single administration of $NH_2^{36}Cl$ (1.1 mg/animal) to Sprague-Dawley rats, only 0.40-0.08% of the total dose was eliminated in the urine and feces, respectively. At the end of 120 hours 25.15 and 1.98% of the dose was eliminated in the urine and feces,

respectively. By comparison, Suh and Abdel-Rahman (1983) found that over twice as much of the ^{36}Cl-radiolabel was eliminated over a 120-hour period when 200 mg/L of $Na^{36}Cl$ was administered orally to rats. Unlike $NH_2{}^{36}Cl$, more of the ^{36}Cl-label was eliminated in the first 24 hours, 16.1% in urine and 0.92% in feces. The major difference between the $NH_2{}^{36}Cl$ and $Na^{36}Cl$ studies (Abdel-Rahman et al., 1983; Suh and Abdel-Rahman 1983) was in the total amount of label excreted over the test period. Only half as much label was eliminated in the chloramine study as in the chloride study.

Interpretation of the toxicokinetics of inorganic chloramines is difficult since synthesis of $NH_2{}^{36}Cl$ is complicated by possible interferences. There is a lack of information on the rate of chloramine/chloride exchange in solutions of high chloride concentrations.

$$NH_2{}^{36}Cl + Cl^- = NH_2Cl + {}^{36}Cl^-$$

If this exchange rate is significant the toxicokinetics of $NH_2{}^{36}Cl^-$ could appear to resemble chloride when, in fact, the compound has simply lost its radiolabel through exchange. Since the stomach contains high dose concentrations of organic amino nitrogen, the chlorine from the $NH_2{}^{36}Cl$ can be transferred to these organic amino nitrogen compounds (Snyder and Margerum, 1982; Issac and Morris, 1983, 1985). How this affects the rate of exchange is unknown.

TABLE III-1

Metabolism of $NH_2{}^{36}Cl$ in Rat Plasma[a]

Treatment	Analyte (%/mL)[b,c]	
	Total ^{36}Cl	^{36}Cl
$NH_2{}^{36}Cl$ (1.1 mg)	0.41 ± 0.08	0.35 ± 0.08

[a]Source: Abdel-Rahman et al., 1983

[b]Neither $^{36}ClO_2$ nor $^{36}ClO_3$ was detected in rat plasma at the time period studied.

[c]Values represent mean ± SE from seven (fasted) rats after 120 hours following $NH_2{}^{36}Cl$ treatment expressed as percentage of total administered dose per mL of plasma.

TABLE III-2

Excretion of ^{36}Cl in Rat 120 Hours After Single Oral Administration of $NH_2{}^{36}Cl$[a]

Collection Period (hours)	Proportion of $NH_2{}^{36}Cl$ Excreted (%)[b]		
	Urine	Feces	Total
0-8	0.07 ± 0.07		
8-16	0.13 ± 0.02		
16-24	0.21 ± 0.08		
0-24	0.40 ± 0.02	0.08 ± 0.05	0.48 ± 0.02
24-48	6.28 ± 2.87	0.48 ± 0.24	6.75 ± 3.10
48-72	6.30 ± 5.52	0.31 ± 0.29	6.60 ± 5.80
72-96	5.03 ± 3.03	0.26 ± 0.21	5.29 ± 3.24
96-120	7.16 ± 1.93	0.87 ± 0.49	8.02 ± 1.44
0-120	25.15 ± 13.32	1.98 ± 0.29	27.13 ± 13.61

[a]Source: Abdel-Rahman et al., 1983

[b]Values represent the mean ± SE from four treated (fasted) rats expressed as the proportion of administered dose. ^{36}Cl was not detected in expired air throughout the 120 hours studied.

TABLE III-3

Excretion of $^{36}Cl^-$ in Rat 120 Hours After Single Oral Administration of $Na^{36}Cl$[a]

Collection Period (hours)	Proportion of $Na^{36}Cl$ Excreted (%)[b]		
	Urine	Feces	Total
0-12	8.2 ± 1.9		
12-24	9.7 ± 1.9		
0-24	16.1 ± 3.8	0.92 ± 0.43	17.0 ± 3.4
24-48	8.2 ± 1.8	0.43 ± 0.12	8.6 ± 1.8
48-72	14.8 ± 3.2	0.55 ± 0.50	15.4 ± 2.8
72-96	9.5 ± 1.9	0.93 ± 0.17	10.4 ± 2.0
96-120	8.6 ± 1.6	0.17 ± 0.07	8.8 ± 1.6
0-120	57.2 ± 10.6	3.0 ± 0.91	60.2 ± 9.7

[a]Source: Suh and Abdel-Rahman, 1983

[b]Values represent the mean ± SE from four treated rats, expressed as percentage of administered dose. $^{36}Cl^-$ was not detected in expired air through the 120-hour study.