TISSUE DISTRIBUTION OF MONOCHLOROACETIC ACID AND ITS BINDING TO ALBUMIN IN RATS

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Distribution of monochloroacetic acid (CA) was studied in rats given a single oral dose of 0.1 mmole/kg body weight [1-^{14}C] CA, by gavage. The animals were sacrificed at 4, 8, 12, 24 and 48 hr following the treatment. The distribution of ^{14}C-label, determined in different tissues, suggests that CA is rapidly absorbed and eliminated from the body. The elimination phase appears to be fast for intestine and kidney as compared to other tissues. Maximum radioactivity was detected in intestine and kidney at 4 and 8 hr following the treatment which was followed by liver, spleen, testes, lung, brain and heart in a decreasing order. A group of rats treated with a single oral dose of 1 mmole/kg [1-^{14}C]CA, by gavage, was also sacrificed at 24 hr following the exposure to study the effect of a higher dose on distribution of [1-^{14}C]CA. The distribution of ^{14}C-label at both dose levels indicates that toxicokinetic properties of CA are dose-dependent. Another group of rats administered 1 mmole/kg [1-^{14}C] CA daily for three days was also sacrificed at 24 hr following the last dose to evaluate the bioaccumulating properties of CA and/or its metabolites in the tissues. As compared to the number of doses given, the accumulation of ^{14}C-label was not as large as expected. ^{14}C-Label determined in the dialyzed plasma, suggests an in vivo binding of ^{14}C-label to plasma proteins where albumin accounted for about 65% as determined by affinity chromatography. Isolation and identification of the adducts of amino acids will help in understanding the mechanism of CA toxicity and development of a suitable biomarker of CA exposure.

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INTRODUCTION

Monochloroacetic acid (CA), a toxic chlorinated analog of acetic acid (NTP TR 1990) used as a herbicide and for the synthesis of various organic compounds (Windholz 1983), is one of the most commonly detected disinfectant by-product in drinking water supplies in United States (Krasner et al. 1989). Besides continuous exposure to CA via chlorinated drinking water, approximately 11,500 workers in the United States are occupationally exposed to this chemical. Several other widely used chemicals such as vinyl chloride, vinylidene chloride, 1,2-dichloroethane and 1,1,2-trichloroethane also produce CA as one of their metabolites (Bartsch et al. 1976; Hathway 1977; Yllner 1971; 1971a).

Absorption of CA through skin is rapid and it is highly corrosive to tissues and may even lead to death by systemic exposure (Mann 1969; Quick 1983). CA is initially metabolized via S-carboxymethyl glutathione to S-carboxymethyl cysteine in mammals (Yllner 1971). Alkylation of sulfhydryl groups *in vitro* and inhibition of acetate oxidation due to CA have been reported (Chaiken and Smith 1969; Hayes et al. 1973). Earlier, we studied the toxicity of CA in rats and compared the data with higher chloro analogs of acetic acid at equitoxic dose levels using histological and electron microscopic techniques (Bhat et al. 1991; Kanz et al. 1991). We have also demonstrated that CA conjugates with cholesterol under *in vivo* conditions (Bhat and Ansari 1989). Covalent binding of CA to cholesterol and phosphatidylethanolamine may account for the observed toxicity (Reichert et al. 1979; Bhat and Ansari 1988; 1989; Bhat et al. 1990). However, very little is known about the mechanism of CA toxicity. Therefore, in the present study we have investigated the distribution pattern of [1-^{14}C] CA in different tissues and its binding to plasma proteins *in vivo* in rats in order to understand the mechanism of toxicity of CA and to develop a biological marker of CA exposure.

MATERIALS AND METHODS

Chemicals

Monochloroacetic acid (99+%, Gold Label) from Aldrich Chemical Co., Milwaukee, WI and 1-^{14}C-labeled CA (>98% pure; specific activity 2.4 mCi/mmol) from Sigma Chemical Co., St. Louis, MO, were used.

Animals and Treatments

Male Sprague-Dawley rats (~175 g) obtained from Harlan, Sprague-Dawley Inc., Indianapolis, IN were acclimatized to 12 hr light/dark cycles for one week. The animals were given Purina Chow and tap water *ad libitum*. Fifteen rats were given a single oral dose of [1-^{14}C] CA (0.1 mmole/kg body weight containing 25 μCi/dose) by gavage and the animals were transferred to metabolic cages. Urine was collected at 4, 8, 12, 24 and 48 hr following the treatment. Three rats at

each time point were sacrificed under ether anesthesia. Blood was withdrawn from inferior vena cava in tubes containing EDTA and separated into plasma and red blood cells (RBCs) by centrifugation (1000 × g) for 10 min at 4°C. A known amount of plasma was dialyzed for 72 hr (molecular weight cut off < 14000 dalton) against ice-cold 10 mM phosphate buffer (pH 7.0). Protein was determined according to the procedure of Bradford (1976) using bovine serum albumin as standard. Major organs and tissues were excised, washed with ice-cold saline, blotted and stored at −80°C for further analysis.

To another group of animals, 1 mmole/kg body weight of [1-^{14}C] CA (containing 14.2 μCi/dose) was given, by gavage, daily for three days. The animals were sacrificed at 24 hr following the doses 1 and 3 and blood was withdrawn. Plasma and RBCs were separated as described above. Major organs and tissues were also excised and stored at −80°C.

Albumin bound ^{14}C-label

Albumin from the rat plasma was separated by using an affinity chromatography cartridge (Affinity Technology, Inc., Laonia, NJ). The cartridge was first washed and equilibrated in 0.05 M phosphate buffer (pH 7.0), the serum sample was then loaded on to the cartridge and the non-albumin proteins were removed by washing with the equilibration buffer. The albumin was then eluted using 0.05 M phosphate buffer containing 0.5 M NaCl (pH 7.0). The radioactivity in the albumin fraction was measured as described below.

Radioactivity Determination

Known aliquots (50 μl) of urine, plasma or dialyzed plasma were mixed with 15 ml Tru-Count scintillation fluid (Tru-Lab Supply Co., IL) and counted in Searle Mark III Scintillation Counter. A small portion of organs or tissues (50 to 150 mg each) was placed on combusting aid and wrapped in a combusting cone. The cone was combusted in the Packard B306 Sample Oxidizer. The CO_2 generated was trapped in Carbosorb II, eluted with Permafluor V and the radioactivity was measured as described above.

Data Analysis

Disintegrations per minute, obtained in our studies, are expressed as nmoles/g tissue or mg protein for distribution and covalent binding studies, respectively. Significance of the data was determined by two-tailed Student's t-test.

RESULTS

Distribution of CA in different tissues following a single oral dose of 0.1 mmole/kg body weight (low dose) is summarized in Table 1. Urinary excretion of CA and/or its metabolites was found to be ~90% of the dose in 24 hr. Maximum ^{14}C-label detected in intestine was similar to the levels found in kidney at 4 hr and disappeared more rapidly than in any other tissue analyzed in the present

TABLE 1
Distribution of ^{14}C-Label in Different Tissues of Rats Treated with a Single Oral Dose of 0.1 mmole/kg body weight [1-^{14}C] CA

	Hours after Treatment				
Tissues	4	8	12	24	48
Intestine	192.1 ± 8.8	154.0 ± 4.5	16.1 ± 0.8	8.5 ± 0.6	4.0 ± 0.1
Kidney	191.5 ± 13.8	156.3 ± 4.8	33.6 ± 1.3	16.1 ± 0.3	10.5 ± 0.6
Liver	79.0 ± 0.9	90.5 ± 3.0	35.5 ± 1.3	21.1 ± 0.8	15.5 ± 0.2
Spleen	53.7 ± 2.0	42.4 ± 5.3	19.3 ± 0.5	9.4 ± 0.4	6.2 ± 0.4
Testes	26.0 ± 1.1	21.0 ± 1.2	8.7 ± 0.7	4.2 ± 0.2	3.8 ± 0.1
Lung	24.0 ± 0.6	24.6 ± 0.9	14.8 ± 2.0	8.4 ± 0.5	7.6 ± 0.1
Brain	16.4 ± 0.9	18.9 ± 0.5	14.5 ± 0.5	7.8 ± 0.6	4.5 ± 0.4
Heart	12.3 ± 0.2	10.6 ± 0.5	6.1 ± 0.3	4.6 ± 0.5	4.3 ± 0.2

The values are mean ± SD of three animals expressed as nmole/g tissue.

study. The radioactivity in kidney also dropped sharply but not as fast as in the intestine. A similar pattern was also observed for spleen, testes and heart. However, the elimination phase was rather slow as compared to that found in kidney or intestine. ^{14}C-Label in liver increased from 79 nmole/g tissue at 4 hr to 90 nmole/g tissue at 8 hr, then decreased at a slower rate than that observed in the kidney or intestine. A similar pattern was also observed in the brain and to some extent in lung tissues. The ^{14}C-label detected in lung, brain and heart tissues at 48 hr of the treatment was 32, 27 and 35%, respectively of the labels at 4 hr.

^{14}C-Label in different tissues at 24 hr were 1.4 to 3.8 folds higher in rats following a single oral dose of 1 mmole/kg body weight (high dose) than those treated at low dose of [1-^{14}C] CA (Table 2). There was also a significant increase in ^{14}C-

TABLE 2
Distribution Pattern of ^{14}C-Label in Different Tissues after 24 Hr of Single Oral Dose of 0.1 and 1.0 mmole/kg Body Weight [1-^{14}C] CA to Rats

	Doses (mmole/kg body weight)	
Tissues	0.1	1.0
Kidney	16.1	57.7 (3.6)*
Liver	21.1	53.7 (2.5)*
Intestine	8.5	32.1 (3.8)***
Lung	8.4	18.9 (2.3)**
Spleen	9.4	17.8 (1.9)*
Heart	4.6	13.5 (2.9)**
Brain	7.8	10.7 (1.4)*
Testes	4.2	8.0 (1.9)

Values are mean of 3 animals in each group and expressed as nmole/g tissue. Values in parentheses are comparisons of ^{14}C-label at 0.1 and 1 mmole/kg body weight doses.
P values *≤0.05, **≤0.01, ***≤0.001.

label present in different tissues 24 hr following three high doses of [1-^{14}C] CA, as compared to those given same single high dose, except for liver and spleen. However, the overall increases of ^{14}C-label were not high enough, as compared to the number of doses given (Figure 1).

No significant binding of CA was observed in RBCs and hemoglobin. Figures 2 and 3 demonstrate the covalent binding of ^{14}C-label to the plasma proteins *in vivo*. The ^{14}C-labels were peaked around 8 hr after treatment in plasma of rats given a low dose of [1-^{14}C] CA, which rapidly depleted with increasing time of the exposure (Figure 2). The covalent binding of ^{14}C-label to the plasma proteins as determined in the dialyzed plasma were found to be proportional to the amount of radioactivity present in the undialyzed plasma. The binding was found to be 0.37, 0.62, 0.32, 0.16 and 0.12 nmoles/mg protein in dialyzed plasma at 4, 8, 12, 24, and 48 hr of the treatment, respectively (about 50% of the total ^{14}C-label in the plasma was present in dialyzed plasma). However, the binding was about 73 and 79% of the total ^{14}C-labels present in undialyzed plasma of rats treated with 1 and 3 high doses of [1-^{14}C] CA at 24 hr following the treatment, respectively. These values are significantly higher than those found at the same time point of 0.1 mmole/kg treated rats (Figure 3). Approximately 65% of the total

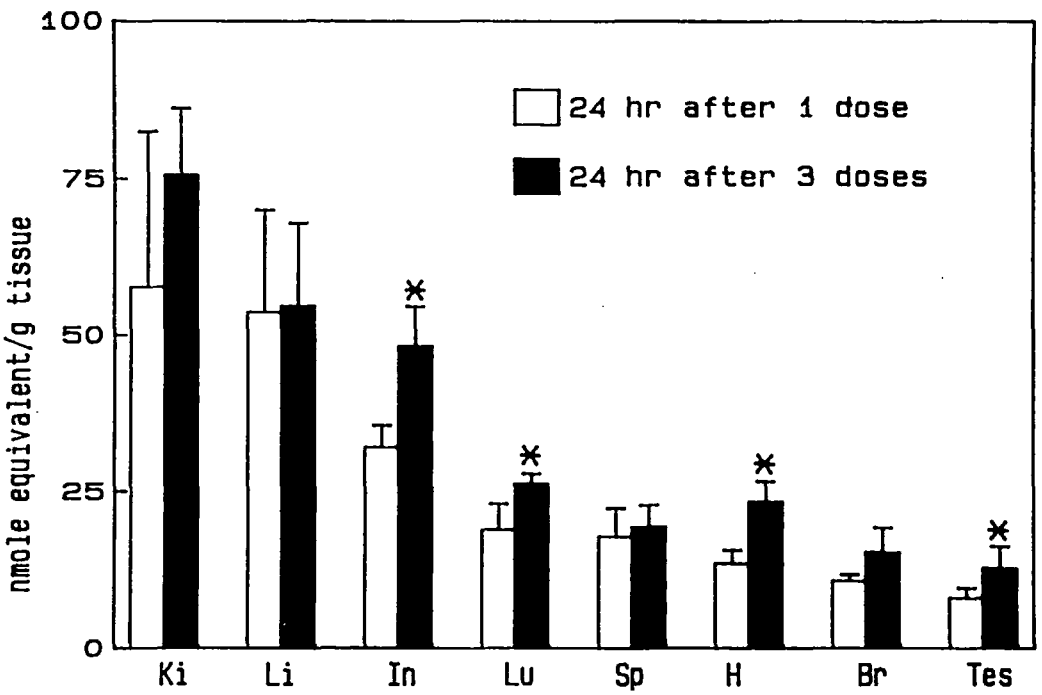

FIGURE 1. Relative concentration of nmole equivalent CA at 24 hr after single and three oral doses of CA at 1 mmole/kg body weight (Ki-kidney; Li-liver; In-intestine; Lu-lung; Sp-spleen; H-heart; Br-brain and Tes-testes). P values * ≤ 0.05.

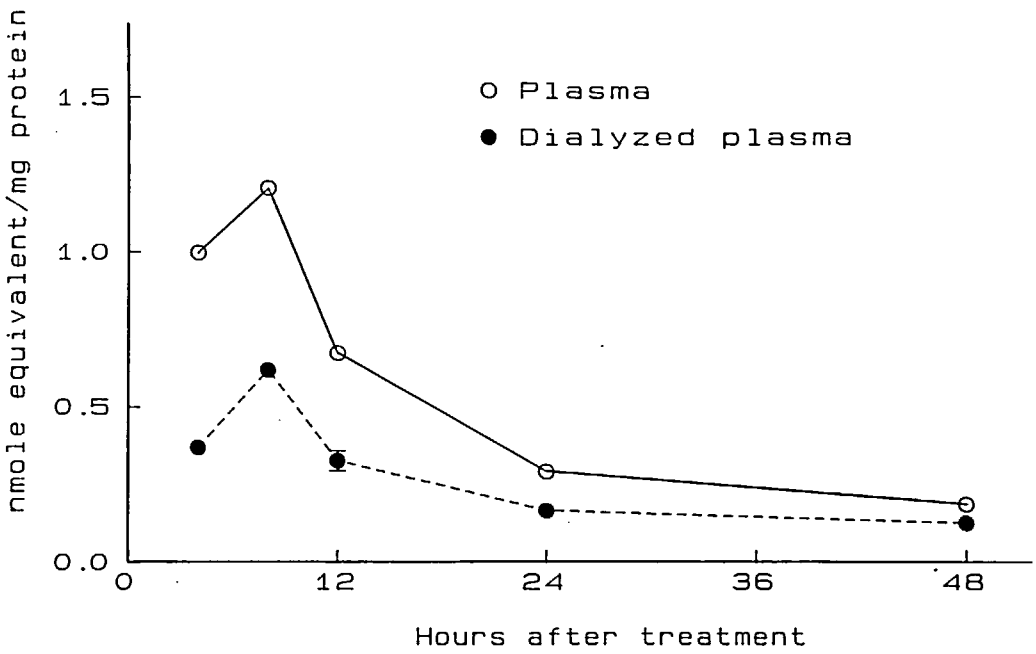

FIGURE 2. Binding of ^{14}C-label (nmole equivalent CA/mg protein) in the dialyzed plasma of rats treated with 0.1 mmole/kg body weight CA.

plasma ^{14}C-label was found to be associated to albumin as determined by affinity chromatography in rats treated with a high dose of [1-^{14}C] CA.

DISCUSSION

Because of a large production volume and presence in drinking water, CA is an important environmental contaminant. The studies conducted in our laboratory and by others have shown morphological changes in lung and liver of rats given CA via drinking water for 90 days (Bhat et al. 1991; Daniel et al. 1991). Autoradiographic studies of [1-^{14}C] CA (injected via tail vein) in rats indicated kidney, brain, thymus, liver, heart and intestine are the target tissues of ^{14}C-label accumulation (Bhat et al. 1990). Since the environmental exposure of CA is reported to be primarily via oral route, we studied the distribution of [1-^{14}C]-CA given by gavage at two different dose levels.

CA was absorbed and eliminated at a fast rate as evident from the distribution of ^{14}C-label in intestine and kidney and its urinary excretion. However, the absorption and elimination phases were augmented to some extent in other tissues, as significant amount of radioactivity was detected even at 48 hr following the exposure. We assume that, metabolism of CA leads to the formation of lipophilic metabolites which may bring about such changes in toxicokinetics.

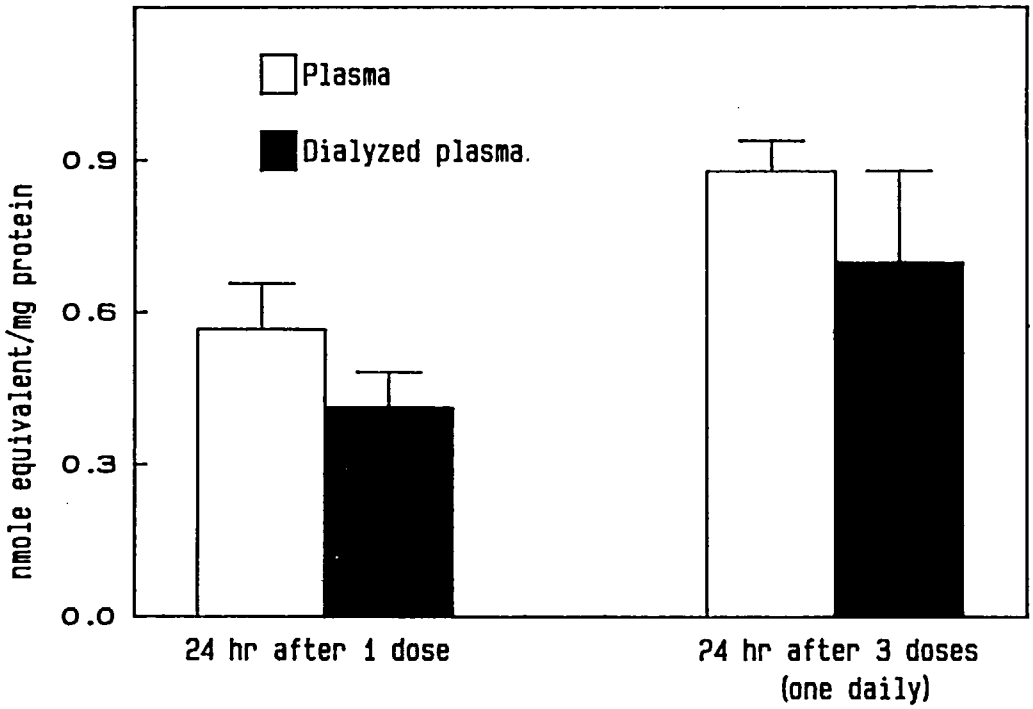

FIGURE 3. Binding of ^{14}C-label (nmole equivalent CA/mg protein) in the dialyzed plasma at 24 hr after the 1 and 3 oral doses of 1 mmole/kg body weight [1-^{14}C] CA.

Although the ^{14}C-label in different tissues at 24 hr following the exposure were several folds higher in rats treated with 1 mmole/kg [1-^{14}C] CA than those found in rats treated with 0.1 mmole/kg [1-^{14}C] CA, the distribution patterns were found to be comparable. Our data also indicate a dose-dependent accumulation of CA in the tissues. Similar to intestine and kidney, we also observed a comparable distribution pattern of ^{14}C-label in spleen, testes, heart and lung but, the rate of absorption and elimination of ^{14}C-label varied to a great extent in these tissues. Comparing the distribution of ^{14}C-label at 24 hr after the treatment of 1 mmole/kg and 0.1 mmole/kg [1-^{14}C] CA, we found a dose-dependent toxicokinetics of CA in kidney and intestine. However, these changes were not proportional to the magnitude of differences in doses. In other tissues, the differences in ^{14}C-label at both dose levels were relatively less than that of intestine and kidney. A slow elimination rate of ^{14}C-label, especially at higher dose, may be a possible cause of such a big change in the ^{14}C-activity of these tissues. The comparable ^{14}C-label following 1 and 3 doses in different tissues may be due to the rapid absorption and elimination of CA and/or its metabolites (Figure 1). In most of the tissues (except for the liver and spleen) an accumulation of ^{14}C-label at 24 hr following the treatment was observed in rats treated with three consecutive doses as compared to those treated with a single dose of

[1-^{14}C] CA. Some of the radioactivity retained in the tissues may be related to the lipid conjugation of CA to cholesterol or phosphatidylethanolamine, as supported by earlier findings (Reichert et al. 1979; Bhat and Ansari 1988; 1989; Bhat et al. 1990).

Binding of ^{14}C-label to plasma proteins, which was about 50% of the total present in the plasma at low dose at all time points studied (Figure 2), could be made useful for developing methods for surveillance of CA exposure in general and the occupationally exposed population. The percent binding of ^{14}C-label in the plasma was much higher at the high dose, as well as at multiple dose level which shows that this binding will increase as the exposure prolongs. Apparently CA alkylates free amino and thiol groups of amino acids (Chaiken and Smith 1969; Hayes et al. 1973). Glutathione also competes for this binding to form carboxymethyl glutathione (Yllner 1971). Once glutathione is depleted, there is a greater opportunity for protein to be alkylated. However, CA can also be metabolized through oxidation at α-carbon atom and leads to the formation of glycine which can be metabolically incorporated in the protein (Weinhouse and Friedman 1951), since we observed a constant binding of ^{14}C-label to plasma proteins from the early time points. The possibility of metabolism of CA to glycine cannot be ruled out from the present data, although the contribution appears to be small.

Detailed studies are needed to explain dose-dependency of the distribution pattern in different tissues and the role of metabolism of CA in covalent binding to plasma proteins *in vivo*. Slow excretion of radioactivity from plasma, liver, lung and heart may be important from the covalent binding point of view. Isolation and characterization of lipid conjugates of CA and/or its metabolites will explain the nature of retention of ^{14}C-label in the tissues mentioned above. However, identification of covalently bound amino acid residues in the plasma proteins of [1-^{14}C] CA treated experimental animal may lead to the development of biomonitoring procedures which could be used for the surveillance of general and occupationally exposed population.

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