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Toxicokinetics of monochloroacetic acid: a whole-body autoradiography study

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Summary

Monochloroacetic acid (MCA) is a toxic chemical used as a herbicide and in the synthesis of various organic compounds. MCA has also been shown to be present in chlorinated drinking waters. In order to understand the mechanism of MCA toxicity, we studied the tissue distribution of [1-^{14}C]MCA in rats, by whole-body autoradiographic technique. Male Sprague—Dawley rats were given a tracer dose of [1-^{14}C]MCA [6.8 μg/100 g (40 μCi) body weight] by tail vein and euthanized at different time intervals (5 min, 1, 4, 12, 24 and 48 h). The animals were embedded in carboxymethyl cellulose and frozen immediately. Frozen animals were sectioned and processed using whole-body autoradiographic techniques. Analysis of developed sections showed that at 5 min, there was a rapid accumulation of ^{14}C-activity in the kidney cortex and stomach walls. The radioactivity was rapidly removed from the circulation. There was high accumulation of ^{14}C-activity in the myocardial tissues. The liver was also loaded with MCA and/or its metabolites. After 1 h following administration of [^{14}C]MCA, radioactivity was extensively excreted into the small intestinal lumen. The accumulation of ^{14}C-activity in the brain, thymus, salivary glands and tongue was prominent at 1 h. After 4 h the liver and other tissues started to eliminate most of the radioactivity. Contrary to other tissues, however, the central nervous system, thymus and pancreas started to accumulate the radioactivity at later time periods. These observations suggest the accumulation of MCA and/or its metabolites into hydrophilic tissues at earlier time periods and into lipophilic tissues at later times.

Key words: Monochloroacetic acid; Distribution; Whole-body Autoradiography

Introduction

Monochloroacetic acid (MCA) is a toxic chlorinated analog of acetic acid which is used as a herbicide and in the synthesis of various organic compounds [1—3]. MCA is also one of the by-products of drinking water disinfection [4]. Therefore, a portion of the human population may be exposed to this chemical. Chloroacetate is a known metabolite of chlorinated hydrocarbons such as 1,1,2-

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trichloroethane, 1,2-dichloroethane, vinyl chloride and vinylidene chloride [5—10]. MCA is rapidly absorbed through skin and may cause death by systemic exposure [11,12]. MCA has been shown to damage the blood-brain barrier in mice [13]. MCA is neurotoxic in several animal species. In an oral toxicity study in geese, death occurred 3.5—6 h after the administration of 75—100 mg/kg of sodium salt of MCA [14]. After 3 h of the administration of MCA (sodium salt), the geese developed paralysis of the neck, convulsions, comma and death. Cattle which accidently drank sodium salt of MCA used as a herbicide, developed paralysis of the limbs, tremors and convulsions [12]. Mice exposed to acute oral dose (380 mg/kg) of MCA showed loss of purkenjee cells in the cerebellum and fewer pyknotic nuclei [13].

The metabolism of MCA proceeds, via the formation of *S*-carboxymethyl glutathione which is converted to *S*-carboxymethylcysteine and finally to thiodiacetic acid [15]. Hydrolysis of carbon-chlorine bond of MCA results in the formation of glycolic acid [15]. MCA has been shown to conjugate with lipids [10,16,17]. We have shown that MCA conjugates with cholesterol through esterification reaction [16] while it binds to phosphatidylethanolamine through acylation or alkylation reaction [17]. MCA resulting through the metabolism of vinylidene chloride (1,1-dichloroethene) has been shown to acylate phosphatidylethanolamine [10]. MCA is an uncompetitive inhibitor of acetate oxidation [18] and has been shown to reduce total sulfhydryl contents in the rat liver [18,19]. Tissue distribution study using MCA has shown that MCA is primarily accumulated in the liver and kidney [18]. Direct inhibition of sulfhydryls in the kidney has been suggested to account for the anuria present in the rats that received toxic levels of MCA [18]. In order to get an insight into the mechanism by which MCA exerts its toxicity, we studied the tissue distribution of [^{14}C]MCA as a function of time in rats, using whole-body autoradiography technique.

Materials and methods

Chemicals

[1-^{14}C]Monochloroacetic acid (Spec. act. 16.1 mCi/mmol, radiochemical purity > 98%) was purchased from Sigma Chemical Co., (St. Louis, MO). Other chemicals and materials used were of the highest quality available.

Animals

Male Sprague—Dawley rats (70—75 g, Harlan, Indianapolis, IN) were acclimatized in our animal facility for 1 week after arrival with free access to water and food (Purina Rat Chow) and housed in a 12 h light/dark cycle. Three rats were used for each time point.

Administration of radiolabeled MCA

A tracer dose of [1-^{14}C]MCA [6.8 μg/100 g (40 μCi) body weight, injection volume 25 μl in 10% Na_2CO_3] was given to each rat intravenously through the tail vein. Following treatment, the animals were transferred to metabolism cages and euthanized by carbon dioxide anoxia at various time intervals (5 min, 1, 4,

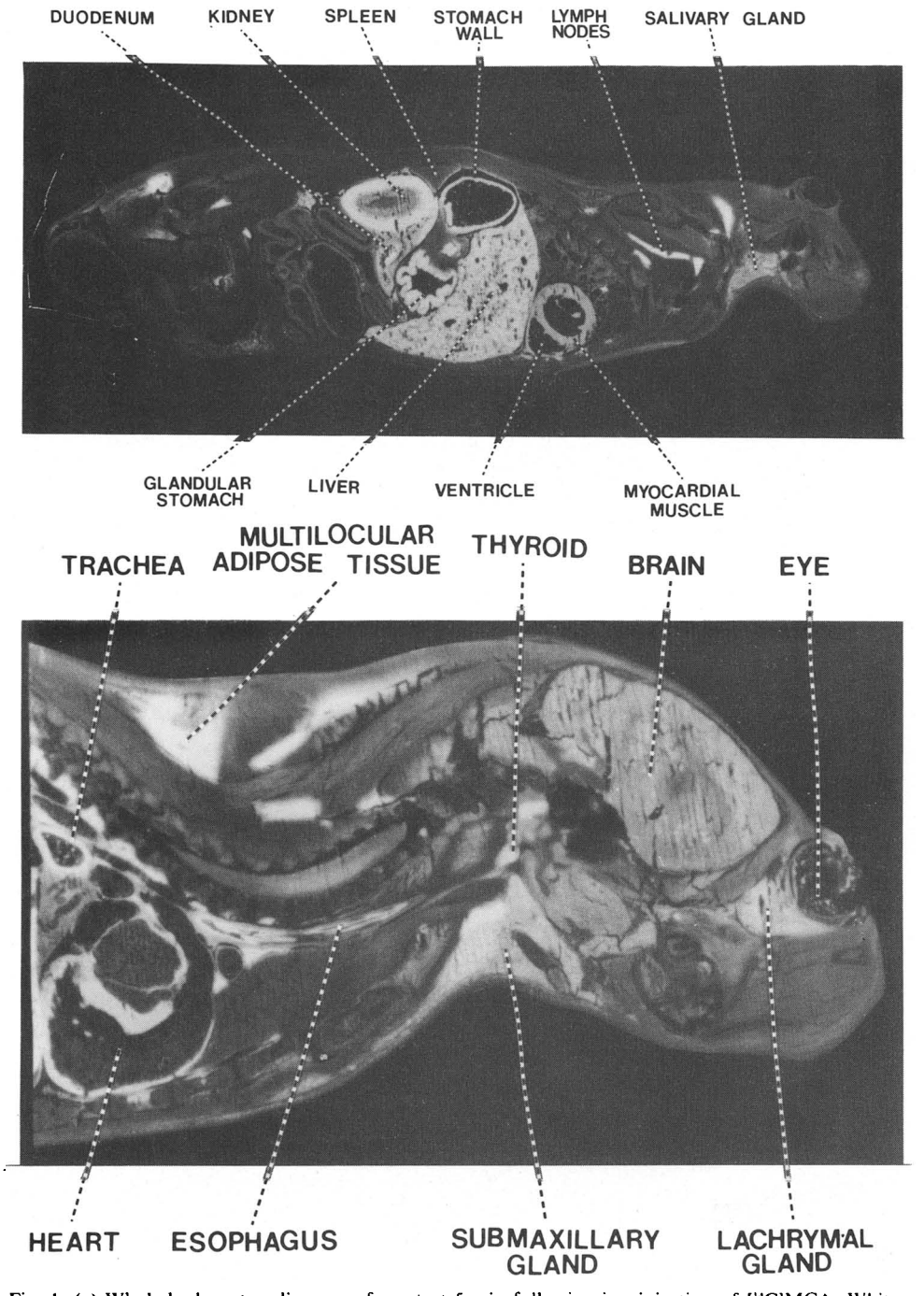

Fig. 1. (a) Whole-body autoradiogram of a rat at 5 min following i.v. injection of [^{14}C]MCA. White areas correspond to high concentration of radioactive substance. The accumulation of MCA and/or its metabolites can be seen in the myocardium, liver, stomach walls, kidney cortex, duodenum and salivary gland. (b) Details of an autoradiogram of the head and neck of a rat 5 min after injection of [^{14}C]MCA. White areas represent accumulation of radioactivity. Note the accumulation in the adipose tissue, thyroid, lachrymal gland, myocardium, brain and salivary gland.

12, 24 and 48 h). At each time interval animals were embedded in carboxymethylcellulose molds and frozen in hexane cooled with solid carbon dioxide (−75 °C). Frozen animals were stored at −20 °C until sectioned.

Whole-body autoradiography. Frozen animals were processed by the procedure described by Ullberg [20]. Briefly, frozen animals were sectioned using LKB PMV 2250 cryomicrotome at −20 °C. Sections were taken in Sagittal plane at a thickness of 20 and 60 μm on scotch tape 810 (3M Co., Minneapolis, MN). The sections were then freeze-dried and dehydrated at −20 °C. Autoradiography was performed by exposing the freeze-dried sections to Industrex AA (Kodak) X-ray film. After exposure (6—10 weeks, −20 °C) films and sections were separated and the films developed and printed by standard photographic techniques.

Results

The distribution of radioactivity in different tissues and organs, 5 min following administration of [^{14}C]MCA is shown in Fig. 1a. A rapid accumulation of radioactivity in the liver and the excretory systems was observed. MCA and/or its metabolites were present in the excretory organ walls, such as kidney cortex and stomach walls. There was an observable accumulation of ^{14}C-activity in certain areas of the brown fat such as in the upper dorsal areas of the neck. The distribution and uptake in the liver was not homogenous. The liver excreted the radioactivity in the bile as indicated by the presence of the radioactivity in the duodenal cavity. At this time interval (5 min) the presence of [^{14}C]MCA and/or

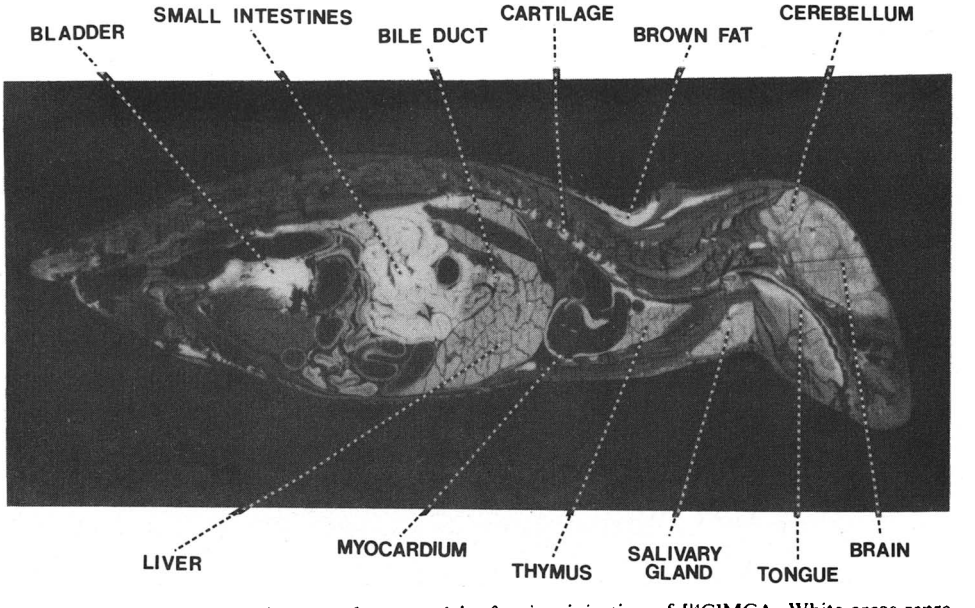

Fig. 2. Whole-body autoradiogram of a rat at 1 h after i.v. injection of [^{14}C]MCA. White areas represent radioactivity. Note the accumulation in myocardium, brown fat and cartilage.

its metabolites in the circulatory system was less prominent than its accumulation in the tissues as indicated by greater uptake in the myocardial muscle when compared to the blood. There was a rapid accumulation of MCA and/or its metabolites in the excretory organs such as salivary and lachrymal glands (Figs. 1a,b). At 5 min following administration, [^{14}C]MCA or its metabolites had started to accumulate into the brain. Similarly, accumulation of ^{14}C-activity in the pancreas was observed. Of specific interest was the accumulation of high levels of radioactivity in the esophagus and tracheal tissues, as well as, in certain ganglionic fibers within the peripheral nervous system (Fig. 1b).

The distribution of ^{14}C-activity 1 h following treatment of MCA is shown in Fig. 2. The radioactivity was extensively excreted in the small intestinal lumen and into the kidney contents and urinary bladder. In addition, the accumulation of the chemical in the salivary gland was associated with the deposition of radioactivity in the mouth cavity and tongue. The ^{14}C-activity was more prominent on the surface of the tongue. The radioactivity contents of the liver had started to move into the bile and eliminated in the upper intestinal tract. At this time period (1 h) the lachrymal glands had accumulated high concentration of ^{14}C-activity (data not shown). Accumulation of the ^{14}C-activity in the brain and spinal cord was greater at 1 h than that observed at 5 min. The increased accumulation of ^{14}C-activity in certain regions of the brown fat of the neck was also observed at 1 h. The cardiac tissues accumulated higher levels of radioactivity compared to that

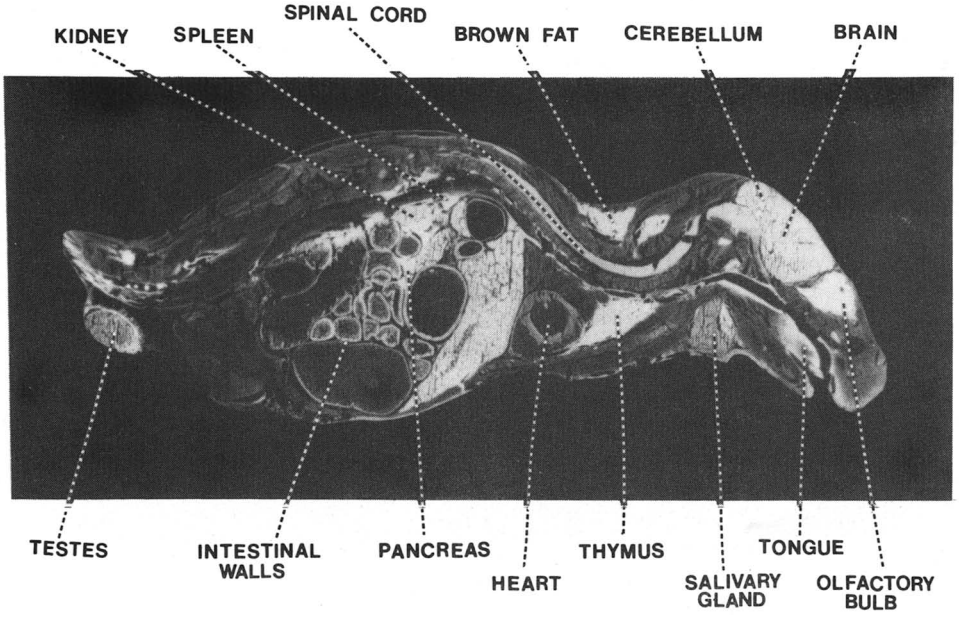

Fig. 3. Whole-body autoradiogram of a rat at 4 h after i.v. injection of [^{14}C]MCA. High levels of radioactivity are observed in brain, spinal cord, thymus, testes, pancreas, liver, kidney and intestinal walls.

found in the liver (data not shown). Thymus gland contained high concentrations of ^{14}C-activity at this time point (1 h). Accumulation in the cartilage was also observed.

The distribution of MCA and/or its metabolites in rats, 4 h after the treatment with [^{14}C]MCA is shown in Fig. 3. At this time period radioactivity concentration in the brain was high and almost equal to that of the liver. Both brain and spinal cord showed extensive uptake of ^{14}C-activity at this time. The cerebellum showed higher accumulation of radioactivity as compared to other areas of the brain. The thymus contained high concentrations of ^{14}C-activity and its contents matched with that of the brain. Radioactivity also accumulated in the spleen. Of interest was the deposition of radioactivity in the pancreatic tissues which was more demonstrable at 4 h than earlier time periods. The deposition of ^{14}C-activity in the testicular tissues was prominent at 4 h following treatment as compared to earlier time periods. A distinct accumulation of ^{14}C-activity in the intestinal walls was also observed. This accumulation of radioactivity in the intestinal walls at 4 h was in contrast to the radioactivity profile at 1 h, when the ^{14}C-activity was mostly present in the small intestinal lumen.

At 12 h following treatment with [^{14}C]MCA, persistent accumulation of radioactivity in the central nervous system was observed (Fig. 4). The brain and spinal cord contained a considerably higher level of radioactivity. The cerebellum showed the highest accumulation of ^{14}C-activity relative to other areas of the central nervous system. At this time period, the accumulation of radioactivity in the thymus and pancreas was higher than any other tissue. A pattern of distribution and accumulation of [^{14}C]MCA in rats, similar to that observed at 12 h was

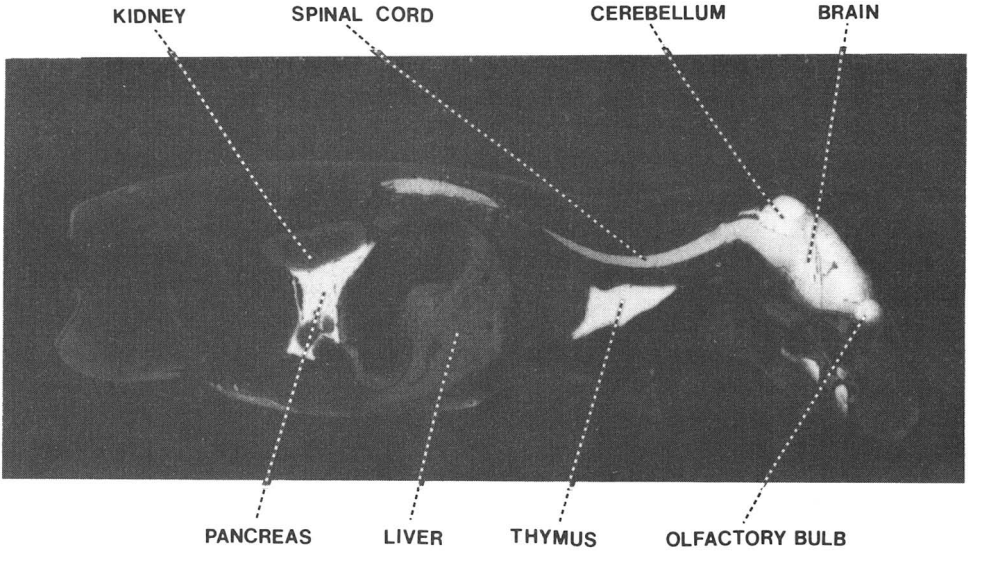

Fig. 4. Whole-body autoradiogram of a rat 12 h after i.v. injection of [^{14}C]MCA. High concentration of radioactivity are clearly present in brain, cerebellum, spinal cord, thymus and pancreas.

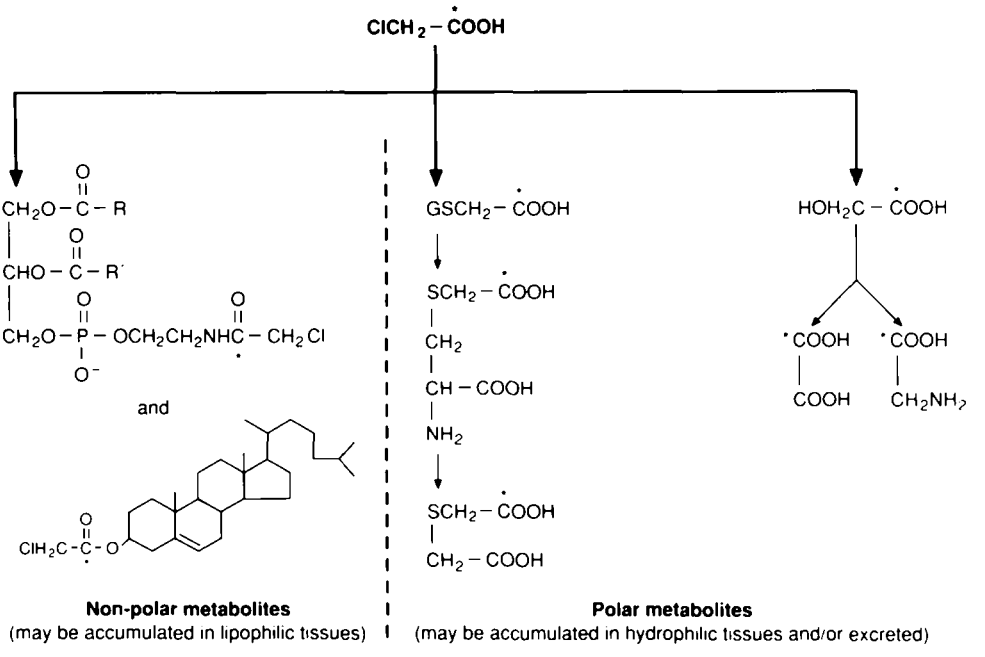

Fig. 5. Composite metabolic pathway of MCA. *^{14}C-carbon

detected at 24 and 48 h. After the ^{14}C-activity was significantly removed from the fat, the gastrointestinal tract and other visceral tissues; a high concentration of radioactivity was observed in the pancreatic tissues, brain and thymus, 24 h and 48 h after the treatment.

Discussion

MCA can be metabolized to three types of metabolic products as shown in Fig. 5 [10,15—17,21,22]:

(a) conjugated metabolites via the substitution of the chloride ion mainly by glutathione [15,21]. These metabolites and its breakdown products are water soluble and are excreted via the bile to the small intestine, and via the kidney to the urinary bladder. (b) oxidized products via the oxidation of the α-carbon atom. The products of this metabolic pathway may undergo further conjugation and excretion or utilized in the de novo synthesis of proteins [22—23]. (c) The interaction of MCA with cholesterol and other lipid components [10,16,17] will lead to the formation of lipophilic metabolites. These products will be mainly deposited into the lipophilic areas and in adipose tissues.

At early time intervals, 5 min following MCA treatment, rapid equilibrium of MCA between the blood and tissues occurred. Blood concentration of ^{14}C-activity was lower than that present in tissues as indicated by the lower levels of radioactivity in blood versus heart muscle and liver. This indicates a large volume of dis-

tribution for MCA or its metabolites which correlates well with various possible routes by which MCA can be metabolized (Fig. 5). The different types of metabolic products with different physicochemical properties will increase distribution sites with both lipophilic and hydrophilic characteristics. As can be seen from Figs. 1 and 2, MCA and/or its metabolites are distributed to both types of tissues such as aqueous secretory glands, e.g. lachrymal and salivary as well as highly lipophilic tissues such as brown fat, brain and thymus. In 1 h radioactivity was extensively excreted in the small intestinal lumen via biliary excretion. The accumulation of radioactivity at the surface of the tongue indicates that the compound is excreted in the saliva and into the mouth. At 4 h following administration of [^{14}C]MCA, it is clear that most of the radioactivity present in circulating fluids is removed. The remainder of radioactivity seems to be associated with macromolecules of specific organs. The finding from our laboratory that MCA binds covalently to lipid components such as cholesterol and phospholipid [16,17], adds more possibilities for MCA and its breakdown products to be found in lipophilic tissues. The increased uptake of ^{14}C-activity by the brain observed in the present study may be because of the covalent interaction of MCA or its break down products with lipids. The brain uptake of radioactivity was not transient but was slow and steady, and remained for the rest of the experimental period. This extensive uptake of MCA and/or its metabolites in brain tissues indicate the penetration of the blood-brain barrier. In our studies we used tracer amounts of MCA which were far below the toxic doses used to study the blood-brain barrier damage [13]. These tracer doses suggest that MCA penetration into the brain may not be dose dependent, and even the small amounts are also able to cross the blood-brain barrier. The role of this uptake to neurotoxicity of MCA needs to be investigated.

The delayed and extensive uptake of MCA by the thymus and spleen indicate a strong interaction between blood cells such as red blood cells and lymphocytes, and MCA and/or its metabolites. The impact of these interactions on the cellular damage of blood cells is not yet known. Furthermore our studies indicate an active uptake and accumulation of ^{14}C-derived MCA activity in the pancreas. The pancreas is one of the most active organs for protein synthesis. The incorporation or covalent interaction of MCA metabolites into amino acids is also possible. Thus the accumulation of [^{14}C]MCA may be a function of uptake in protein synthesis as a precursor amino acid. This is suggested by the delayed uptake and lag period of accumulation of radioactivity into the pancreatic tissue.

The chemical characterization of radioactive species within each organ, e.g. liver, stomach, gastrointestinal tract and bile at early times, and brain, spinal cord, thymus and pancreas at later time intervals is needed for the identification of the chemical species responsible for MCA toxicity.

Acknowledgments

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