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Macromolecular adduct formation by 4,4'-methylene-bis(2-chloroanaline) in adult male rat

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The compound 4,4'-methylene-bis(2-chloroanaline) (MOCA) is widely used as a curative extender in the production of isocyanate-based polymers and epoxy resins. A survey conducted by the National Institute for Occupational Safety and Health in the 1970s estimated that 55 000 workers were potentially exposed in the United States to MOCA through their employment (7). MOCA has been reported to produce tumors in dogs, rats, and mice (10) and is considered a suspect human carcinogen. Such chemical carcinogens or their reactive metabolites are thought to act by covalently binding to nucleophilic sites in target cell deoxyribonucleic acid (DNA) (5). Although traditional biomonitoring techniques have been developed to determine urinary MOCA or MOCA metabolite levels (3, 12), the use of protein or DNA adducts to detect long-term MOCA exposure has not previously been evaluated. Recent work on adduct formation of related aromatic amines with hemoglobin (2, 8) or tissue DNA (4) has suggested that the quantitation of MOCA macromolecular adducts may be of value in risk assessment by allowing a more accurate estimation of the cumulative target dose.

The objective of this study was to determine whether rat hemoglobin and liver DNA macromolecular adducts are formed after the oral administration of MOCA. Such adducts may serve as time-integral indices of exposure.

Materials and methods

Animals

Weanling male, Sprague-Dawley (Crl:CD(SD)BR outbred) rats, delivered by caesarean section, were obtained from Charles River Breeding Laboratories, Inc.

The animals were randomly assigned to one of six groups of five rats and were maintained on NIH-07 rat and mouse diet and tap water. The laboratory temperatures, 22 to 25°C, and relative humidity, 45 to 50 %, as well as a 12-h light-dark cycle, were controlled throughout the study.

Chemicals

The test compound MOCA (molecular weight 267) was synthesized by the Anderson Development Co. It was recrystallized from methanol-water and was subsequently found to be >99.5 % pure by high-performance liquid chromatography as described previously (3). (Methylene-^{14}C) MOCA (>99 %), obtained from Chemsyn Science Laboratories, and appropriate amounts of MOCA were dissolved in corn oil [concentration 56 µmol/ml, activity 42.4 µCi/ml ($156.88 \cdot 10^4$ Bq].

Experimental treatment

At 45 d of age, the rats (190—220 g) were administered ^{14}C-MOCA in a single oral dose of 281 µmol/kg of body weight (bw). Rats within a specified time group were anesthetized with pentobarbital and killed by exsanguination 1, 3, 7, 10, 14, and 29 d after the treatment.

Isolation of globin, hemin and deoxyribonucleic acid

The blood was cooled on ice in ethylenediaminetetraacetate-containing vacutainers® and centrifuged at 1 500 *g* for 25 min. Erythrocytes were washed with ice-cold phosphate-buffered saline and lysed by the addition of ice-cold distilled water prior to the elimination of cellular debris at 4 500 *g* for 25 min at 4°C. The concentration of hemoglobin in the supernatant was determined (9). Globin was precipitated by addition to cold 1 % hydrochloric acid in acetone and washed three times with cold acetone (8). Liver DNA was isolated by solvent extraction (6) and quantified with 3,5-diaminobenzoic acid (11). Protein levels (<1 %) were analyzed spectrophotometrically (1), and bound ^{14}C was quantified by liquid scintillation spectrometry (13).

Statistical analysis

The statistical significances of the differences between the group means were evaluated in an analysis of variance ($P<0.05$).

Results and discussion

The radioactivity present in blood and liver, 24 h after a single oral injection of ^{14}C-MOCA, was 3 417

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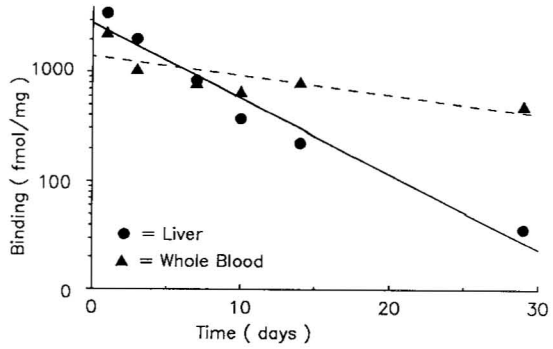

Figure 1. Comparison of ^{14}C linear regression plots for whole blood and liver of rats killed at 1, 3, 7, 10, 14, and 29 d after the oral administration of a single 281-μmol/kg dose of 4,4'-methylene-bis(2-chloroanaline) (MOCA) (mean ± SE, N = 5).

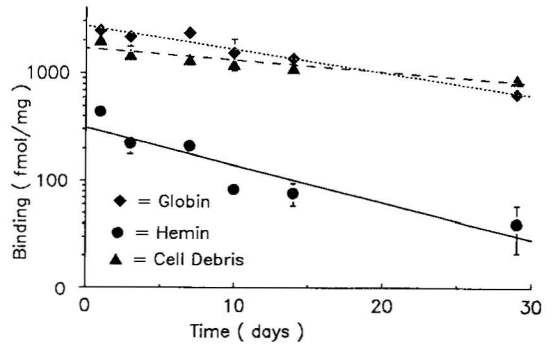

Figure 2. Comparison of ^{14}C linear regression plots for globin, hemin, and erythrocyte cell debris of rats killed at 1, 3, 7, 10, 14, and 29 d after the oral administration of a single 281-μmol/kg dose of 4,4'-methylene-bis(2-chloroanaline) (MOCA) (mean ± SE, N = 5).

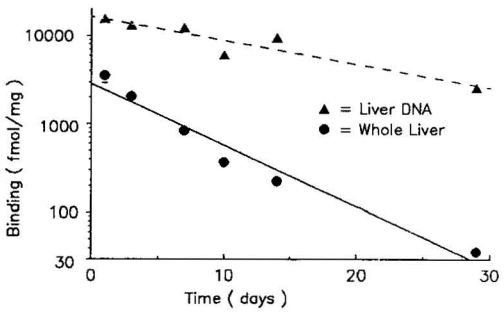

Figure 3. Comparison of ^{14}C linear regression plots for liver deoxyribonucleic acid (DNA) of rats killed at 1, 3, 7, 10, 14, and 29 d after the oral administration of a single 281-μmol/kg dose of 4,4'-methylene-bis(2-chloroanaline) (MOCA) (mean ± SE, N = 5).

fmol/mg of whole blood and 5 181 fmol/mg of liver (figure 1). These values, which may include unadducted MOCA or MOCA metabolites, decreased over the 29-d period to 725 fmol/mg for the blood and 53 fmol/mg for the liver. The ^{14}C binding was determined for globin, a potentially useful marker for adduct formation following MOCA exposure, and for hemin and

Table 1. Biological half-times for ^{14}C-4,4'-methylene-bis(2-chloroanaline (MOCA).

Tissue	Slope	Intercept	Half-time (days)	Elimination constant [b] (h^{-1})
Globin	−0.021	4.430	14.3	0.049
Deoxyribonuleic acid	−0.027	5.215	11.1	0.062
Hemin	−3.035	3.496	8.6	0.081
Liver	−0.069	4.455	4.4	0.158
Blood	−0.018	4.148	16.7	0.042
Debris	−0.011	4.229	27.4	0.025

[a] Tissues were taken at 1, 3, 7, 10, 14, and 29 d after the oral administration of a single 281-μmol/kg (body weight) dose of MOCA.
[b] Elimination constant $k = 0.693/t_{½}$.

erythrocyte cell debris. The amount of radioactivity present in the globin (figure 2) was not significantly different from that determined for the cell debris for the time points tested over the 29-d period. At 24 h, the hemin contained a ^{14}C-MOCA concentration of 646 fmol/mg of hemin, a level significantly lower than that found in the cell debris (2 912 fmol/mg cell debris) or the globin (3 595 fmol/mg globin). The adducted ^{14}C compound decreased to 57 fmol/mg of hemin, 933 fmol/mg of globin, and 1 281 fmol/mg of cell debris over the 29-d period. The presence of DNA adducts in the rat liver (figure 3) was indicated by the relatively high radioactivity in isolated liver DNA in relation to that of the unextracted liver. The ratio of liver DNA binding to whole liver ^{14}C activity increased from 4.3 after 1 d to 70.8 after 29 d. However, there was no significant change in the DNA to globin ^{14}C activity [5.4 (SE 0.5)] over the same period. The persistence of the MOCA adducts was evaluated by the calculation of the biological half-times of the radioactivity present in the tissues analyzed (table 1). The rat globin and liver DNA biological half-times for adducted ^{14}C-MOCA were similar. This observation, along with the relatively uniform ratio of DNA to globin ^{14}C over the 29-d experimental period, indicates that the quantitation of globin-MOCA adducts may be a useful monitor of MOCA exposure. Previous studies on hemoglobin binding by aromatic amines indicate that the cysteine, lysine, and terminal valine nucleophilic centers may be available for adduct formation (2, 8). Additional studies using nonradioactive MOCA are planned to determine the sensitivity of the quantification of the most abundant MOCA adduct by high-performance liquid chromatography for application as a method for determining industrial exposure.

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