Monitoring Exposure to 4,4′-Methylene-bis(2-chloroaniline) through the Gas Chromatography–Mass Spectrometry Measurement of Adducts to Hemoglobin

by Eric Bailey,¹ Alan G. Brooks,¹ Peter B. Farmer,¹ and Brian Street¹

4,4′-Methylene-bis(2-chloroaniline) (MOCA) is widely used as a curing agent in the plastics industry. The determination of the covalently bound reaction products to hemoglobin (Hb) has been investigated as a biomonitoring method for occupational exposure to this potential human carcinogen. Initial studies using the ¹⁴C-ring-labeled MOCA showed that 24 hr after a single IP dosage to rats (3.74 µmole/kg), 0.08% of the administered dose was adducted to the Hb, and base hydrolysis liberated 38% of the bound radioactivity. The only product released on hydrolysis was the parent diamine. A specific and sensitive assay procedure using capillary gas chromatography–mass spectrometry has been developed for determining the base-released MOCA adduct down to levels of 20 pmole/g Hb. This method has been used to establish a linear dose-response relationship in IP dosed rats between production of the adduct and dose of MOCA (3.74–44.94 µmole/kg). It is proposed to use analysis of the Hb adduct as a dosimeter for industrial workers exposed to MOCA.

Introduction

4,4′-Methylene-bis(2-chloroaniline) (MOCA) is commercially important aromatic amine used in the production of isocyanates, polyurethane foams, and epoxy resins. Of importance with regard to the widespread industrial exposure, which can readily occur through skin absorption, is its reported carcinogenicity in mice, rats, and dogs (1,2). Measurement of MOCA levels in urine (3,4) may be used for biological monitoring, but this only indicates recent exposure, and the results depend critically on the time of sampling. The use of hemoglobin (Hb) adducts as dosimeters for aromatic amines as well as other genotoxic agents allows a retrospective assessment of exposure and gives an indication of the extent of metabolic activation (5–7). The mechanism of adduct formation between aromatic amines and Hb is believed to involve the reaction of N-oxidized metabolites of the amine with cysteine residues to form a sulfonic acid amide. Evidence for such reactions in vitro has been obtained for MOCA by Chen et al. (8). Sulfinic acid amide adducts can be readily liberated from Hb by mild hydrolysis, yielding the parent amine, the determination of which has been used for assessing human exposure to 4-aminobiphenyl (9), aniline, and p-chloroaniline (10), and 4,4′-methyleneedianiline (MDA) (11).

Measurements of MOCA adducts in rat Hb have been made by radiochemical (12), high-performance liquid chromatography (HPLC) with electrochemical detection (13), gas chromatography–mass spectrometry (GC-MS) (13), and by GC with electron capture detection (8). We report here on an improved GC–MS method, using a stable isotope-labeled internal standard, for the determination of MOCA Hb adducts and report on its application to binding studies in the rat.

Materials and Methods

Ring ¹⁴C-labeled MOCA (specific activity 56 mCi/mmole) was obtained from Amersham International (Amersham, UK). Unlabeled MOCA was recrystallized from aqueous methanol. The purity of both compounds was > 98%. Pentafluoropropionic anhydride (PFPA) (Pierce, Rockford, IL) was used without further purification. All solvents were of Analar grade and were redistilled before use. The synthesis of ²H₆-MOCA used as the internal standard will be described in a forthcoming publication.

Animal Studies

Female LAC Porton-derived Wistar rats (body weight 170–200g, 8–10 weeks old) were administered either ¹⁴C-MOCA or unlabeled MOCA IP in dimethyl sulfoxide. The labeled compound was given at a dose level of 3.74 µmole/kg and the unlabeled MOCA at doses of 3.74, 11.23, 26.21, and 44.94 µmole/kg (two animals per dose). Twenty-four hours after dosing, blood sam-

¹MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK.
Address reprint requests to E. Bailey, MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK.
to calibration curves constructed from the analysis of 20 mg of control rat Hb spiked with $^4\text{He}$-MOCA (5 ng) and from 0 to 30 ng unlabeled MOCA. Peak height ratios m/z 523:m/z 529 were linearly related to the amount of MOCA added (e.g., $r = 0.9995$; $y = 0.1295; x = -0.0397$).

**Results and Discussion**

Twenty-four hours after a single IP dosage of rats with $^{14}$C-MOCA (3.74 $\mu$ mole/kg), 0.08% of the administered dose was covalently bound to the Hb. Base hydrolysis liberated 38% of the added radioactive activity. The only released product observed on thin-layer chromatography with radioisotope scanning was the parent diamin, which was identified by GC–MS after PFPA derivatization. Ethyl acetate extraction of MOCA from Hb hydrolysates gave recoveries of 87.06% ± 2.12% (mean ± SD, $n = 6$). The reaction of MOCA with PFPA was quantitative and the N,N'-di-PFPA derivative had excellent GC properties. Monitoring the intense fragment ion in the EI mass spectrum at m/z 523 (M+–Cl) allowed 10 pg of the derivatized compound to be detected by SIR. A typical SIR trace from the analysis of the MOCA derived adduct in the Hb of a rat administered a single IP dose of 3.74 $\mu$ mole/kg MOCA is illustrated in Figure 1. The accuracy of the method was assured by the use of a deuterated analogue of MOCA as an internal standard. The mean calculated recoveries of authentic MOCA spiked into control rat Hb at levels of 7 ng and 25 ng/20 mg Hb were 98.48% (SD ± 1.33%, $n = 6$) and 100.34% (SD ± 3.49%, $n = 6$), respectively.

The level of base-released MOCA adduct in rat Hb increased linearly with dose over the range 3.74–44.94 $\mu$ mole/kg MOCA (Fig. 2). The determined hemoglobin binding index (binding mmole/mole Hb: dose mmole/kg) of the adduct of 2.63 ± 0.204 (mean ± SEM) was constant over this dose range. In conclusion, the described method should be useful as a dose monitor for industrial workers exposed to MOCA. The detection limit of the method, which is 20 pmole adduct/g Hb may, however, have to be lowered to allow monitoring of low levels of exposure. This can be readily achieved by operating the MS in the negative ion chemical ionization mode, giving a 10-fold improvement in detection limit.

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