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Induction of ornithine decarboxylase activity by 4,4′-methylene bis(2-chloroaniline) in the rat

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Summary

The effects of the curative extender 4,4′-methylene bis (2-chloraniline) (MOCA), an established experimental carcinogen that exhibits activity in rat liver, on hepatic ornithine decarboxylase (ODC) activity was investigated. Male Sprague – Dawley rats were injected i.p. with 75 mg/kg MOCA and killed 6, 12, 18, 24, 42 and 48 h later. Stimulation with MOCA of liver cytosolic ODC was first evident at 6 h, peaked at 12 h and returned to control levels by 42 h. The liver enzyme was refractory to stimulation by a second treatment of MOCA within the dosing intervals examined. The magnitude of stimulation of the enzyme by this aromatic amine was dependent on dose and route of administration.

Keywords: MOCA; ornithine decarboxylase

Introduction

The polyurethane curative extender 4,4′-methylene bis (2-chloraniline) (MOCA) has been demonstrated to be carcinogenic in a number of experimental animals [8,13,16,17]. In 1987 MOCA was classified as a probable human carcinogen by IARC [7]. Recently, ACGIH published a notice in which they stated the intent to classify MOCA as a confirmed human carcinogen [1]. While the synthesis of MOCA in the United States ceased in 1979, estimated occupational exposures during the manufacture of MOCA cured products ranged from 1400 to 33 000 as late as 1987 [19]. Presently, approximately 200 castable polyurethane processors, most of which constitute small businesses, annually use 2.5 million pounds of imported MOCA on a regular and continuing basis. While this represents a dramatic reduction in the current estimates of exposure when compared to 1987 (E. Ward, personal communication) NIOSH has remained interested in the scientific utilization of this compound as a model occupational aromatic amine carcinogen. These investigations include the development of biological monitoring assays for exposure [4] and studies designed to elucidate underlying biochemical mechanisms associated with chemical carcinogenesis. These latter studies are aimed at developing biomarkers for effects of occupational carcinogens and initially examined the effects of MOCA on selected molecular events which have been linked to chemical carcinogenesis. One such event; the induction of

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the enzyme ornithine decarboxylase (ODC), with subsequent increases in polyamine synthesis, has remained a research focal point since earlier studies that correlated increases in both with tumor cell multiplication [2].

ODC, the rate-limiting enzyme in polyamine synthesis, is induced by hormones, drugs, mitogens, tumor promoters and carcinogens. Induction of the enzyme occurs prior to the stimulation of cell proliferation and tissue growth which characterizes the spectrum of the biological effects of these types of compounds [12]. While ODC induction appears to be an obligatory step in chemical carcinogenesis [10], several characteristics of the enzyme complicate what might normally be simply a requirement for elevation of the enzyme activity. These complications include: (a) existence of multiple forms of ODC, (b) occurrence of ODC-Antizyme and (c) documented but unexplained refractory states which often lead to supersensitive states.

The purpose of this study was to determine if MOCA induced rat hepatic ODC, whether or not the enzyme exhibited the refractory characteristic reported with other chemical carcinogens and to establish dose-response and route of administration criteria.

Materials and Methods

Materials

4,4′-Methylene bis (2-chloroaniline) (MOCA) was purchased from Anderson Development Corporation (Adrian, MI) and DL-[1-^{14}C]-ornithine monohydrochloride from New England Nuclear (Boston, MA). Chemicals routinely employed in the ODC assay included dithiothreitol, Tris base, pyridoxal-5-phosphate and EDTA, among others, and were obtained from Sigma Chemical Company (St. Louis, MO).

Animals and treatment

Male Sprague–Dawley CD-1 rats (180–230 g; Charles River Breeding Laboratories, Wilmington, MA) were employed in this study. For the time course study, rats were injected intraperitoneally (i.p.) with 75 mg/kg body wt. MOCA dissolved in corn oil and killed at 6, 12, 18, 24, 42 and 48 h following treatment. In the refractory study, animals received two doses (75 mg/kg body wt. i.p.) of MOCA at dosing intervals of 24 and 48 h and were killed 18 h following the second dose. In the last study, route, dose and time study, animals received various doses of MOCA dissolved in corn oil for varying times and by different routes of administration. In all instances control animals received only the corn oil vehicle.

Ornithine decarboxylase assay

Regardless of treatment schedule or study design, all animals were killed by decapitation between 0800 and 0900 h. The livers were removed and homogenized in 15 vols. of ODC homogenizing buffer as previously described [15]. The crude homogenates were sequentially centrifuged at 8000 × *g* for 15 min and that supernatant at 100 000 × *g* for 60 min. Prior to the ODC assay the supernatant from the ultracentrifugation was subjected to chromatography on Pharmacia PD-10 disposable columns preequilibrated with ODC assay buffer [15]. ODC activity was determined in the post PD-10 cytoplasm and expressed as pmoles $^{14}CO_2$ evolved per 30 min incubation per mg cytoplasmic protein. Cytoplasmic protein was determined by the Pierce protein assay procedure [3].

Statistics

Analysis of variance was used to compare groups receiving MOCA to their respective control groups, and to compare doses, routes of administration, and dosing schedule.

Results and Discussion

Independent of route of administration or treatment time, the corn oil vehicle treated animals exhibited no change in hepatic ODC activity. They were thus grouped by route of administration (i.p. or p.o.) and incorporated into the appropriate figure or table(s) as controls.

Figure 1 clearly illustrates the time course of MOCA (75 mg/kg i.p.) induction of rat hepatic ODC activity. Increased activity is detectable but not statistically significant as early as 6 h following MOCA administration. From the selected time points it appears that maximal stimulation occurs at 12 h following MOCA treatment and begins to decline at 18 h. The decrease in activity continues through 24 h until at 42 and 48 h, ODC activity is comparable to controls. These data strongly complement those of Gottlicher and Cikryt [5] who reported the effect of a number of aromatic amines on rat liver ODC. Their studies featured the rat liver carcinogen 2-acetylaminofluorene (2AAF), the strong liver tumor initiator *trans*-4-acetylaminostilbene (AAS) and the non-carcinogen 4-acetylaminofluorene (4AAF). They demonstrated induction of the enzyme with all test chemicals within similar time frames; for 2AAF a 3.6-fold increase over 2.5 h, for 4AAF a 3.9-fold increase over 4 h and with AAS a 5.7-fold increase in 9 h). However, since these chemicals were not all carcinogens there was no correlation between induction of ODC activity and the known carcinogenic properties of the aromatic amines included in their study. From their study and others it is clear that while induction of ODC may be an obligatory step in experimental carcinogenesis, induction of ODC may occur in response to other tissue insults which do not culminate in carcinogenesis [12]. Olson and Russell [10] proposed that while induction of ODC is necessary, an additional component is that the induction must be sustained for a critical period. Our time course study demonstrates that there is no prolonged induction with a single MOCA treatment. However, in studies where MOCA was designated a carcinogen in experimental animals, chronic dosing was employed. We thus examined the effect of multiple MOCA treatments on hepatic ODC activity. Table 1 presents data that suggest that this second dose of MOCA is incapable of stimulating the enzyme. Even an alteration in the dosing interval could not elicit a stimulation equivalent to that seen with a

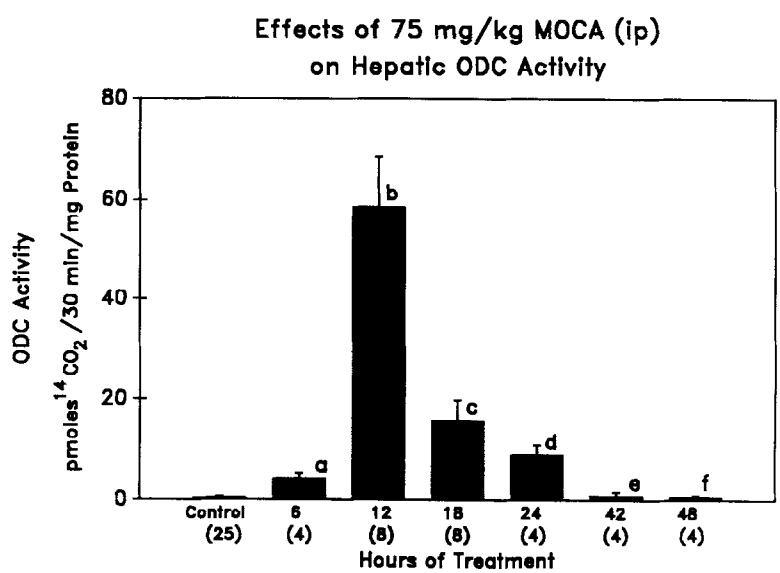

Fig. 1. Control rats received corn oil at 5 ml/kg i.p. Treated rats were dosed at 75 mg MOCA/kg i.p. Animals were fasted 18 h before being killed. Statistical differences were determined by the *F*-test ($\alpha = 0.05$). (a, e and f, not significantly different from control values; b, c and d, significantly different from control values).

Table I. The effect of two treatments of MOCA, at different dosing intervals, on rat hepatic ODC activity.

Dosing regimen	*n*	ODC activity[a] (mean ± S.E.)
Control (corn oil)[b]	25	0.51 ± 0.05
MOCA (single dose)[c]	8	15.85 ± 4.00*
MOCA (two doses)		
24 h dosing interval	4	1.74 ± 1.74
48 h dosing interval	4	1.67 ± 0.74**

[a]ODC activity is expressed as pmol $^{14}CO_2$ per 30 min per mg protein.
[b]Killing of animals with subsequent ODC assay always occurred 18 h after last treatment.
[c]MOCA dose at 75 mg/kg i.p. in corn oil.
*Significantly different from the control values by *F*-test at $\alpha = 0.05$.
**Not significantly different from the 24 h dosing interval.

single dose. This phenomenon of refractoriness has been demonstrated in other systems with other ODC inducers: TPA in skin [18], chrysarobin in mouse epidermis [9] and chloroform in rat liver [14]. The critical difference in these studies is that with tumor promoter TPA, after a discrete period of time with regard to dosing interval, the enzyme actually became supersensitive to stimulation. This response, which was dramatically greater than the already significant response to a single dose, only occurred after a finite refractory period. In studies with chloroform, this supersensitivity never occurred and the refractory period was evident with dosing intervals up to 31 days [14]. This two stage response to multiple dosing, i.e. initial refractory period followed by a period of superstimulation may be of value in distinguishing carcinogens from noncarcinogens. While the study presented here demonstrates an initial refractory period with MOCA, additional studies are warranted to further characterize the refractory period or unmask the period of supersensitivity.

Table 2 presents preliminary information on the role of dose and route of administration at selected time points on MOCA induction of rat hepatic ODC. While further studies are required to substantiate observed effects or trends, several items relating to this table are worthy of note. The low dose (15 mg/kg per i.p. injection) fails to stimulate ODC above controls at either of the two investigated time points, 12 and 18 h. This is especially significant at 12 h when the peak of MOCA mediated stimulation with the high dose is evident. Given the magnitude of the response at 12 h with 75 mg/kg, it seems peculiar that a related stimulation at 15 mg/kg is not demonstrable. This apparent lack of a dose related response (albeit with only two doses examined) suggests that the induction of rat hepatic

Table II. Effect of route and dose at various times on MOCA induction of rat hepatic ODC activity.

Time *n*	Route	Dose mg/kg	ODC activity[a] (mean ± S.E.)
Controls 25	i.p.		0.51 ± 0.05
12 h MOCA 8	i.p.	75	58.57 ± 9.89[b,c]
12 h MOCA 4	i.p.	15	0.88 ± 0.14
18 h MOCA 8	i.p.	75	15.85 ± 4.00[b,d]
18 h MOCA 4	i.p.	15	0.80 ± 0.15
18 h MOCA 4	p.o.	75	6.43 ± 4.23[b,e]
24 h MOCA 4	i.p.	75	8.96 ± 1.84[b,f]
24 h MOCA 4	p.o.	75	2.52 ± 0.74

[a]ODC Activity is expressed as pmol $^{14}CO_2$ per 30 min per mg protein.
[b]Significantly different from the control values by *F*-test (alpha = 0.05)
[c]Significantly different from 12 h MOCA at 15 mg/kg i.p.
[d]Significantly different from 18 h MOCA at 15 mg/kg i.p.
[e]Significantly different from 18 h MOCA at 75 mg/kg i.p.
[f]Significantly different from 24 h MOCA at 75 mg/kg p.o.

ODC requires a critical concentration (i.e. threshold) of MOCA. While this aromatic amine is an established mutagen [6,11], the carcinogenic potential in vivo with a single low dose (15 mg/kg) treatment has yet to be evaluated. Thus, the relationship between ODC induction (or lack thereof) and low dose MOCA carcinogenicity cannot be determined without additional appropriate experiments. Another observation worthy of note, but clearly more defensible, is the lack of significant stimulation observed when MOCA (75 mg/kg) is administered orally. While some elevation is detectable at 18 h, the change is not as dramatic as with i.p. administration. This is not surprising in that in the rat 75 – 90% of administered MOCA is excreted in the feces. It is not clear if this is because MOCA is not absorbed from the GI tract and thus does not reach the liver in appreciable quantities or whether it is subject to the phenomena of enterohepatic circulation. This issue requires further investigation in light of the fact that the reported rodent carcinogenic effects of MOCA are derived from chronic, oral studies [13,16]. Further studies employing a similar exposure duration and dose design as those previously reported [13,16] to evaluate the role of route of administration and the relationship of ODC to MOCA mediated rodent carcinogenesis are warranted.

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