Alcohol and Aldehyde Metabolizing Systems

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FOLATE DEFICIENCY AND METHANOL POISONING IN THE RAT

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Species differences in sensitivity to methanol toxicity is well known. Rodents do not develop metabolic acidosis or ocular toxicity after methanol treatment, whereas the monkey and man display these features. Since the administration of methanol results in formic acid accumulation in the monkey but not in the rat, and since both species metabolize formate primarily through a folate-dependent pathway, differences in the ability of the animal to metabolize formate could explain the relative sensitivity of the species to methanol. The rat metabolizes formate at rates approximately twice those observed in the monkey. Rats were placed on a folate-deficient diet and the maximal rate of formate oxidation was reduced to that of the monkey. Administration of methanol (4 g/kg, intraperitoneally) led to formate accumulation in the blood to levels comparable to those observed in the methanol-intoxicated monkey (18 mEq/l). This was accompanied by a marked decrease in blood pH (7.04). This represents the first demonstration of a metabolic acidosis in the rat after methanol and suggests that the species differences observed between the primate and the rat may be related to folate and its biological disposition.

II. INTRODUCTION

Unlike ethanol, which is rapidly metabolized in the animal organism to CO_2 and water, methyl alcohol displays a specific toxicity in man. It is slowly metabolized and produces metabolic acidoses, ocular toxicity and death. Until recently it has not been possible to completely characterize the toxicity except in man. But more recent studies show that the monkey may serve as an appropriate model for methanol toxicity in man since the syndrome in this animal has features common to those in man. McMartin *et al.*(1) and Clay *et al.*(2) have described an accumulation of formate in the blood of monkeys coincident with the development of metabolic acidosis. This is unlike the situation in ethanol ingestion where metabolic acidosis and

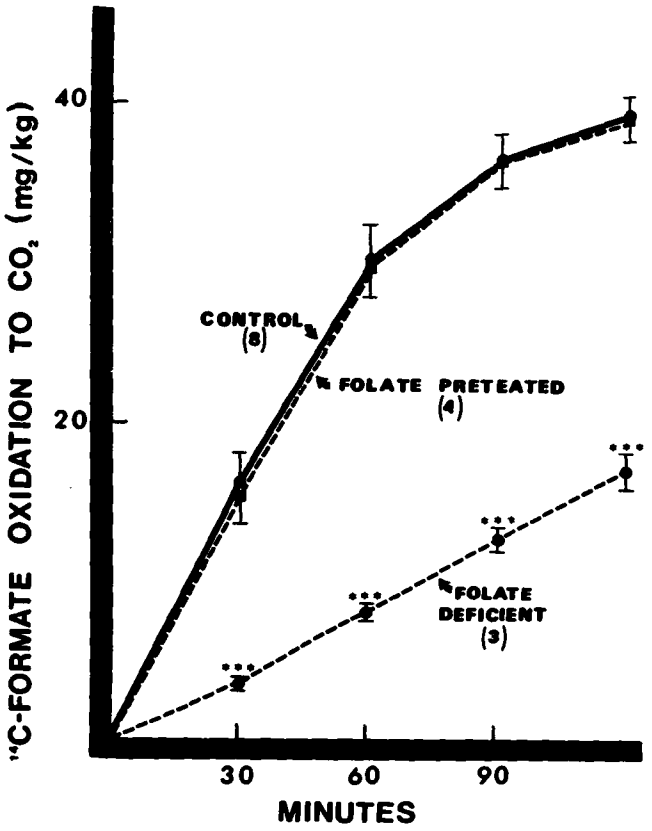

Fig. 1. In vivo rates of formate oxidation to CO_2 in control, folate-deficient and folate-pretreated rats. Procedures for production of folate deficiency and for folate pretreatment are described in the test. At zero time, rats were injected with ^{14}C-sodium formate (68 mg/kg intraperitoneally) and rates were determined as described by Palese and Tephly.

blindness do not develop. Although methanol is toxic in man and monkey it is not toxic to rat or other rodent species (3,4). An explanation for this may relate to formate disposition in the several species. Since formate accumulates in the monkey after methanol ingestion but does not accumulate in the rat it is possible that decreases in the ability to oxidize formate to CO_2 might lead to increases in sensitivity to methanol in a given species. Recent studies from our laboratory have shown

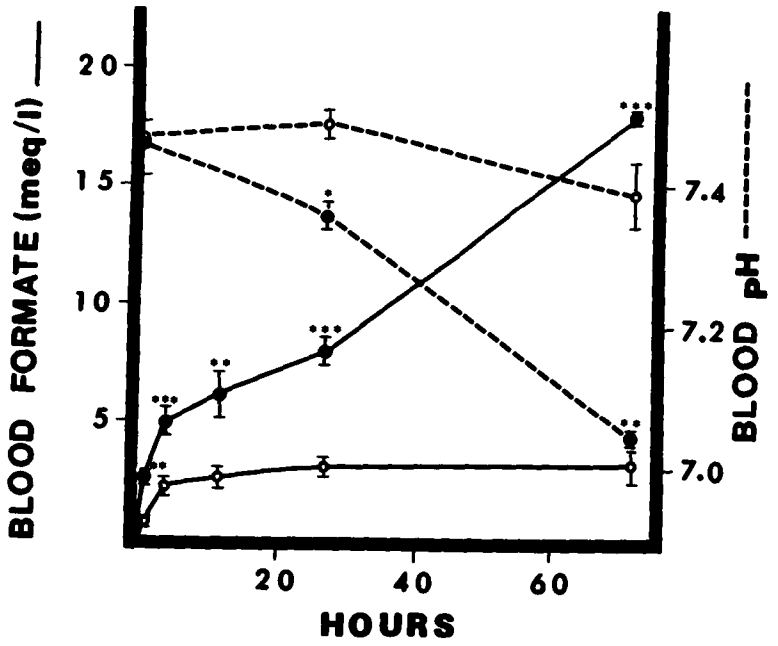

*Fig. 2. Blood formate levels and pH values of control (o) and folate-deficient rats (o). At zero time methanol was administered intraperitoneally at a dose of 4 g/kg. Each point represents the mean value obtained from 2-8 rats. Vertical bars represent ± S.E.M. *, **, and *** indicate statistically significant differences from corresponding control values at P levels of <0.05, <0.01, and <0.001, respectively. This work has been published previously (11).*

(5) that formate oxidation to CO_2 *in vivo* is dependent on a folate-related 1-carbon pool pathway. Others have held (6) that catalase played a major role in the metabolism of formate but studies using relatively selective inhibitors of the catalase pathway have been ineffective in decreasing formate oxidation in the rat (5) and in the monkey (7). The objective of the current work was to test whether folate deficiency and decreases in formate oxidation render the rat sensitive to methanol poisoning with the production of metabolic acidosis through increases in blood formic acid.

III. METHODS

Male Sprague-Dawley rats (about 200 g) were divided into groups fed either a folate-deficient diet or a control diet (Bio-Serve, Frenchtown, N.J.). A radioassay was employed to measure liver folate levels (8) using kits obtained from Diagnostic Biochemistry (San Diego, C.A.). Formate was determined by a method described previously (9) and formate meta-

TABLE 1
Hepatic Catalase Activity and Formate Oxidation in the Rat In Vivo

Treatment	Duration of Treatment	Hepatic Catalase Activity[c] (kat.f.units/g Liver)	In vivo[c] Formate Oxidation to CO_2 (mg/kg/hr)
Chow diet	-	863 ± 93	29.98 ± 2.12
Folate deficient diet	9 days	806 ± 88	31.63 ± 2.60
Folate deficient diet	10-12 weeks	590 ± 42	8.18 ± 0.40
Folate control diet	10-12 weeks	765 ± 57	28.81 ± 1.52
Chow diet + AT treatment[a]	-	46 ± 6	30.48 ± 2.29
Chow diet + MTX (1 mg/kg/day)[b]	9 days	228 ± 53	16.06 ± 1.10
Chow diet + MTX (0.5 mg/kg/day)	9 days	443 ± 71	23.01 ± 0.54
Folate deficient diet = MTX (1 mg/kg/day)	9 days	317 ± 66	9.67 ± 1.44
Folate deficient diet + MTX (0.5 mg/kg/day)	9 days	370 ± 29	18.89 ± 1.75

a. 3-Amino-1,2,4-triazole (AT) was ibjected 1 hour prior to the beginning of the experiment.

b. Methotrexate (MTX) was injected intraperitoneally once a day.

c. Values are the means obtained from 3 to 8 animals ± S.E.M.

A correlation coefficient of 0.37 between hepatic catalase activity and the rate of formate oxidation was obtained when data were submitted to linear regression analysis.

bolism was assessed as previously described (5). Blood pH was determined on samples obtained by cardiac puncture using a blood gas analyzer (Instrumentation Laboratories, Model No.713). In certain experiments folate was administered to animals receiving a diet adequate in folate in order to produce a folate hypervitaminosis. The treatment consisted of three i.p. injections of a preparation of sodium folate (Folvite R) obtained from Lederle Laboratories, American Company, Pearl River, N.Y. The dose was 50 mg/kg at 48, 24 and 1 hour prior to the injection of ^{14}C-sodium formate.

IV. RESULTS

Figure 1 shows results from experiments from rats fed a folate deficient diet for 10-12 weeks. The results are compared to those obtained in pair-fed animals on a control diet containing 2.25mg of sodium folate per kg of diet for a similar period of time. In another group, folate was injected into animals as described in Methods in order to produce a hypervitaminosis. There is marked decrease in formate oxidation in folate-deficient rats as has been reported previously (5). Injections of folate to animals on the control diet had no effect on formate oxidation to CO_2. The decrease in formate metabolism seen in rats or folate deficient diets brought the metabolic rate to those rates seen in the normal monkey.

Since the monkey metabolizes formate more slowly than the rat (7) it was of interest to determine whether the folate-deficient rat might be sensitive to methanol poisoning. Figure 2 compares blood formate after methanol levels and blood pH in control and folate-deficient rats. A marked elevation in formic acid levels and a marked decrease in blood pH was observed in folate-deficient rats whereas control rats did not accumulate formate in the blood and had no alteration of blood pH value. These results provide the first demonstration of metabolic acidosis in the rat after methanol treatment. The magnitude of changes seen in the folate-deficient rat are greater than those which have been reported for the rhesus and pigtail monkeys (1).

V. DISCUSSION

Results indicate the importance of the folate-dependent 1-carbon poll in formate metabolism and its role in methanol poisoning. It may be argued that folate-deficiency produces decreases in catalase activity and that decreases in catalase activity are responsible for the accumulation of formic acid in blood and the metabolic acidosis observed after methanol treatment in the folate-deficient rat. In order to demonstrate the relationship of catalase activity in the liver and the *in vivo* rate of formate oxidation to CO_2 in the rat results were obtained from animals which had been on a folate-deficient diet, rats fed control diets for 10-12 weeks, rats fed folate-deficient diets for 9 days and rats fed a Purina Chow diet which were treated daily with i.p. injections of methotrexate (1mg/kg) for 9 days. Other treatments of rats are included in this information in Table 1. There is a very poor correlation

between hepatic catalase activity and the ability of the rat to metabolize formate to CO_2. For example, aminotriazole-treated rats which have 5-10% of control catalase activity showed no inhibition of formate oxidation . A correlation coefficient of 0.37 was obtained when information was submitted to linear regression analysis. Thus, we suggest that formate is metabolized to CO_2 via a folate-dependent pathway in the rat and that by reducing the rate of formate metabolism it is possible to sensitize the rat to methanol poisoning. Recent studies in this laboratory have shown that the monkey is exquisitely sensitive to methanol poisoning and that formate accumulates in the blood coincident with the production of metabolic acidosis. Other studies have shown (10) that low level chronic formic acidemia leads to ocular toxicity in the rhesus monkey and that formate infusion alone at normal pH, produces this toxicity. Thus, formate is a major determinant in methanol poisoning and further studies are underway to determine which catalyst is responsible for formate oxidation to CO_2 in the rat and monkey.

VI. ACKNOWLEDGEMENTS

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VII. REFERENCES

(1). McMartin, K., Makar, A.B., Martin-Amat, G., Palese, M., and Tephly, T.R., Biochem.Med. 13, 319 (1975).
(2). Clay, K.L., Murphy, R.C., and Watkins, W.D., Toxicol.Appl. Pharamcol. 34, 49 (1975).
(3). Roe, O., Pharmacol. Rev. 7, 399 (1955).
(4). Gilger, A.P., and Potts, A.M. Amer.J.Ophthalmol. 39, 63 (1955).
(5). Palese, M., and Tephly, T.R. J. Toxicol. Environ. Hlth. 1, 13 (1975).
(6). Friedmann, B., Nakada, H.I., and Weinhouse, S., J. Biol. Chem. 210, 413 (1954).
(7). McMartin, K., Martin-Amat, G., Makar, A.B., and Tephly, T.R., Alcohol and Aldehyde Metabolizing Systems (R.G. Thurman, T. Yonetani, J.R. Williamson, H. Drott, and B. Chance, Eds.) Vol, II Acad. Press, New York, In Press (1977).
(8). Longo, D.L., and Herbert, V., J.Lab.Clin.Med. 87, 138 (1976).
(9). Makar, A.B., McMartin, E.Ke., Palese, M., and Tephly, T.R., Biochem. Med. 13, 117 (1975).
(10). Martin-Amat, G., McMartin, K.E., Makar, A.B., Baumbach, G., Cancilla, P., Hayreh, M.M., Hayren, S.S., and Tephly, T.R. Alcohol and Aldehyde Metabolizing Systems, (R.G. Thurman, J.R. Williamson, J.R. Drott and B. Chance, Eds.) Vol. II Acad. Press, New York, In press, (1977).
(11). Makar, A.B., and Tephly, T.R., Nature 261, 715 (1976).

THE MONKEY AS A MODEL IN METHANOL POISONING

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Methanol poisoning in man is characterized by a mild central nervous system depression, metabolic acidosis and ocular toxixity followed by coma and death. Previous work from this laboratory has described the production of metabolic acidosis, coma and death in rhesus and pigtail monkeys without the demonstration of definitive ocular lesions. Since animals used in those studies died rapidly after methanol administration a prolonged and less intense state of intoxication was deemed necessary for the production and recognition of ocular toxicity. Thus, methanol was administered at a dose of 2 g/kg followed by subsequent doses of 0.5 g/kg until signs of ocular toxicity were observed: usually at 48 hours, or later, after the first dose of methanol. Ocular toxicity was characterized as optic disc edema with dilated pupils and a slow reaction of the pupillary reflex to light. A rapid intraarterial perfusion of appropriate fixatives was used in order to minimize autolysis of tissues. Histopathologic changes included intracellular swelling and mitochondrial disruption in the area of the optic disc but otherwise retinal histology was normal. Clinical symptoms appeared to be similar to those described in man and may provide a basis for our understanding of the mechanism of methanol toxicity with respect to the ocular lesions observed in man.

I. INTRODUCTION

A marked species difference in susceptibility to methanol poisoning is well known and has been the subject of numerous reviews (1.2.3.4). It has been shown recently that the rat does not accumulate formate in the blood after methanol administration whereas the monkey rapidly accumulates formic acid coincident with the production of a metabolic acidosis (5,6). Potts and coworkers (2,7) observed metabolic acidosis in the rhesus monkey and certain signs of ocular toxicity, but Cooper and Felig (8) were unable to confirm these results. Recently, Clay et.al. (6) and McMartin et.al. (5) have reproduced certain of