Methanol Toxicity: Treatment With Folic Acid and 5-Formyl Tetrahydrofolic Acid

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After methanol administration to monkeys, an accumulation of formate in blood occurs coincident with the development of metabolic acidosis and a depletion of blood bicarbonate. Formate metabolism in monkeys depends upon and is regulated by a folate-dependent system; therefore, the effect of folic acid pretreatment and 5-formyl tetrahydrofolic acid administration on methanol toxicity was investigated. Treatment of monkeys with repetitive doses of either sodium folate (48, 24, 12, and 4 hr prior to methanol) or 5-formyl tetrahydrofolic acid (2 mg/kg at 0, 4, 8, 12, and 18 hr after methanol) resulted in a marked decrease in the levels of blood formate and an absence of both metabolic acidosis and depletion of blood bicarbonate following methanol administration. Also, 5-formyl tetrahydrofolic acid reversed methanol toxicity once it was established in the monkey. The results indicate that folate compounds decrease formate accumulation after methanol by stimulating formate oxidation or utilization and suggest a possible use for folates in the treatment of certain cases of human methanol poisoning.

THE TOXICOLOGY of methanol is a unique and important problem which, in humans and other primates, has as key features the syndrome of metabolic acidosis and ocular toxicity.[1] Common rodent species such as the rat and mouse display neither of these symptoms. The toxicity of methanol occurs as a result of its metabolism to either formaldehyde or formate and is not due to the alcohol per se.[1,2] Recent investigations in the monkey have shown that following methanol adminstration a metabolic acidosis and depletion of blood bicarbonate occur coincident with an accumulation of formate in blood.[3-5] Further, after the administration of sodium formate, an ocular toxicity similar to that seen with methanol has been produced in monkeys.[6] In contrast, a role for formaldehyde in the toxicity has been difficult to establish because it has been detected only at very low levels in blood and tissues of sensitive species after methanol administration.[2,7,8] The inability to detect formaldehyde following methanol metabolism has been attributed to its rapid oxidation to formate and high degree of reactivity with body constituents.[9,10]

Whereas formate accumulates in species sensitive to methanol, it does not accumulate in the rat and other insensitive species.[3,4] The species difference in formate accumulation is not accounted for by different rates of methanol metabolism since similar rates of metabolism are seen in the rat and monkey.[3,11] However, the rate of formate oxidation to CO_2 in the rat is significantly faster (about double) than the rate observed in the monkey.[12] Experiments in vivo have shown that a folate dependent pathway is involved in the oxidation of formate to CO_2 in both the rat and monkey.[12,13] Thus, the rate of formate oxidation is at least partially dependent on the folate status of these species. In both the rat and monkey the rate of formate metabolism is decreased during states of folate deficiency[12,13] and, alternatively, in the monkey but not in the rat, treatment with folic acid increases formate oxidation to CO_2.[12,14] Further, it has been possible to produce metabolic acidosis and blood formate accumulation in folate deficient rats treated with methanol[15] and to intensify the methanol poisoning syndrome in monkeys made folate deficient.[12]

This information suggested that certain folate derivatives could alter the rate of formate metabolism and the pattern of formate accumulation and metabolic acidosis seen in monkeys after methanol administration. We have observed that the administration of repetitive doses of either 5-formyl tetrahydrofolic acid (5-fTHF) or folic acid, either before or after methanol, results in a significant decrease in blood formate levels and an absence of both the metabolic and

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blood bicarbonate depletion which usually occurs during methanol intoxication. Treatment with 5-fTHF is effective when initiated at the same time methanol is given or when initiated after toxicity has developed. The results indicate that methanol toxicity in the primate may be modified by stimulating formate oxidation and suggest a possible use of folate compounds in the treatment of certain cases of human methanol poisoning.

MATERIALS AND METHODS

Methanol-^{14}C (2–5 mCi/mole) was purchased from New England Nuclear (Boston, Mass.). Formyl-^{5}N-tetrahydrofolic acid (calcium salt) was obtained from Grand Island Biological Co. (Grand Island, N.Y.) and folic acid was purchased from Sigma Chemical Co. (St. Louis, Mo.). Preblend 3a70B scintillation cocktail was purchased from Research Products International Corp. (Elk Grove, Ill.). Metabolism chambers and primate restraining chairs were obtained from Plas-Labs (Lansing, Mich.). All other reagents employed were of the highest available purity.

Metabolic Experiments

Male and female cynomolgus (*Macaca fascicularis*) monkeys (2.0–3.0 kg) were employed and prepared for experimentation as previously described.[4] An indwelling catheter was implanted in the femoral vein and/or artery of each animal.

Methanol-^{14}C 20% (w/v) methanol in water; specific activity, 1500 dpm/mg methanol, was administered via a nasogastric tube at a dose of 2 g/kg (62.5 mmoles/kg) body weight and the monkey was immediately placed in a metabolic chamber. Blood samples were obtained and expired ^{14}C-methanol,$^{14}CO_2$, and urine were collected at timed intervals during the experiments. The methods of collection and analysis have been previously described[4] except that a 3a70B scintillation cocktail was used in the analysis of radioactivity. When given, the folate compounds were administered as 1% solutions in either saline (5-fTHF) or 2.5% sodium bicarbonate (folic acid).

Assays

Blood levels of methanol were determined using the gas chromatographic assay reported by Baker et al.[16] and formate levels were measured by the specific assay described by Makar et al.[17] Blood gases and blood pH were measured using a blood gas analyzer (Instrumentation Laboratories, Model 713). Bicarbonate values were calculated from pH and pCO_2 values using the Henderson-Hasselbalch equation.

Statistical Evaluation

Statistical evaluations were made using the Student's t test for unpaired data. A p value of <0.05 was considered statistically significant.

RESULTS

Pattern of Methanol Toxicity in Monkeys Treated With Folates

The interrelationships observed, in both the rat and monkey, between hepatic folate levels and rates of formate oxidation[12,13] suggested that folate compounds might be effective in altering the pattern of formate accumulation and metabolic acidosis following methanol administration to the monkey. In an initial experiment, the effect of folate treatment prior to methanol administration was investigated. One monkey was administered methanol (62.5 mmoles/kg) and the toxic syndrome produced by methanol was monitored. Several weeks later the same animal was treated with sodium folate and then methanol. Sodium folate (2 mg/kg) was administered intravenously 48, 24, 12, and 4 hr prior to methanol. Supplemental folate doses were given i.v. at the same time methanol was administered and 8 and 22 hr thereafter. As shown in Fig. 1, sodium folate treatment resulted in a susbtantial reduction in the level of formate that accumulated in the blood of the monkey after methanol administration. Peak blood formate levels were decreased from values of about 6 mEq/liter to less than 3 mEq/liter following folate treatment. Additionally, metabolic acidosis was not ob-

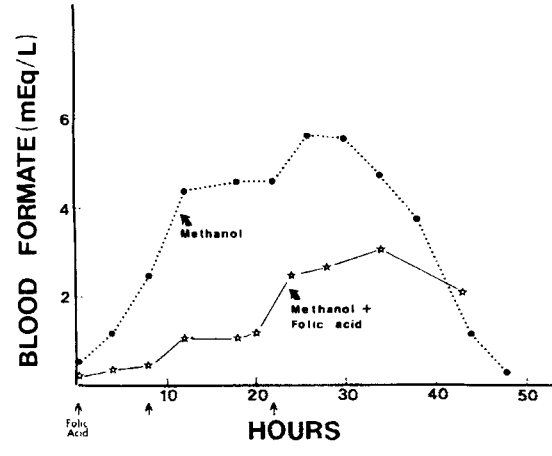

Fig. 1. Blood formate levels in a methanol poisoned monkey following pretreatment with folic acid. Methanol (62.5 mmole/kg) was administered orally as a 20% (w/v) solution before and after treatment with sodium folate (2 mg/kg at 48, 24, 12, and 4 hr prior to methanol and where indicated by an arrow).

served in the monkey after folate supplementation (data not shown).

Since pretreatment with sodium folate appeared to alleviate methanol toxicity in the monkey, the effect of folate compounds on the development of the toxic syndrome was extended to studies in which 5-fTHF was given after methanol administration. The 5-fTHF (folinic acid, leucovorin), a chemically stable and reduced form of folic acid, is commonly used following methotrexate chemotherapy since it is effective in rescuing normal cells from cytotoxicity by increasing the intracellular level of reduced folates.[18]

In a series of experiments, the course of methanol toxicity was followed in monkeys administered radiolabeled ^{14}C-methanol (62.5 mmole/kg) or ^{14}C-methanol plus repetitive doses of 5-fTHF. Figure 2 shows that, similar to that observed with sodium folate, treatment with 5-fTHF (2 mg/kg at 0, 4, 8, 12, and 18 hr after methanol) resulted in a marked decrease in blood formate levels. In the 5-fTHF treated monkeys, blood formate levels increased at a slower rate immediately after methanol administration and reached a plateau within 12 hr of initiation of 5-fTHF therapy; thereafter, formate levels remained at least 50% lower than

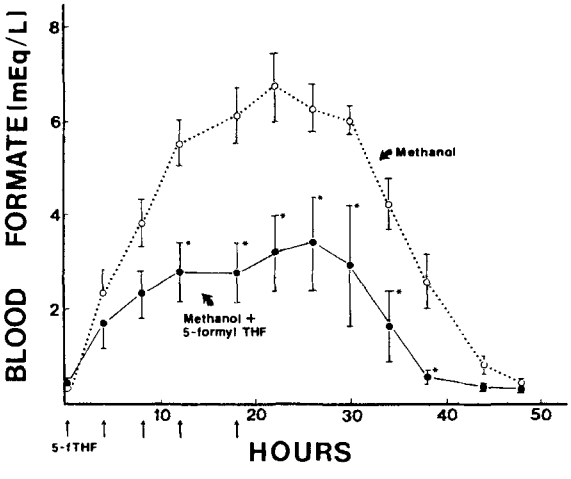

Fig. 2. Blood formate levels in methanol poisoned monkeys treated with 5-fTHF. C^{14}-methanol (62.5 mmole/kg) was administered orally as a 20% methanol (w/v) solution in H_2O. Each value represents the mean ± SEM for 4 control and 3 5-fTHF treated animals. 5-fTHF (2 mg/kg) was administered i.v. where indicated by the arrows. Asterisk indicates significant difference from the value obtained for animals administered methanol alone ($p < 0.05$).

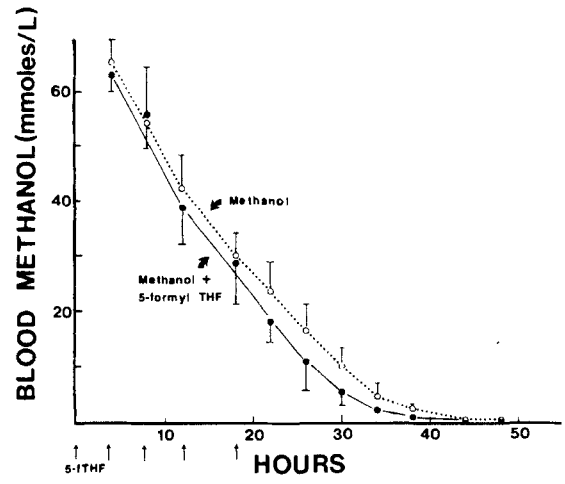

Fig. 3. Blood methanol levels in methanol poisoned monkeys treated with 5-fTHF. Data were obtained from the monkeys represented in Fig. 2.

those observed in the untreated animals. These results indicated that either the rate of formate formation or the rate of formate oxidation was altered by folate administration.

As shown in Fig. 3, in both the treated and untreated animals, the elimination of methanol from blood followed zero-order kinetics and occurred at a rate of 2.5 μmole/ml/hr in the 5-fTHF treated animals and 2.2 μmole/ml/hr in the untreated animals, data which demonstrate that, under the experimental conditions employed, the oxidation of methanol to its metabolites was not altered by 5-fTHF. Recovery determinations of ^{14}C-label further indicated that the metabolism and excretion of ^{14}C-methanol were not altered by 5-fTHF administration. As shown in Table 1, similar amounts of ^{14}C-label were recovered from urine or as either expired ^{14}C-methanol or $^{14}CO_2$ from both the treated and

Table 1. Recovery of ^{14}C-Level From Methanol Intoxicated Monkeys*

		mmole/kg				
Treatment	*n*	Expired ^{14}C-methanol	$^{14}CO_2$	^{14}C-Urine	Total Recovered	% Recovery
Methanol	4	6.1	31.6	12.1	49.8	79.7
Methanol + 5-fTHF†	3	4.5	34.6	11.4	50.5	80.8

*Data were obtained from same monkeys represented in Fig. 2. Samples were collected during a 48-hr period after ^{14}C-methanol (62.5 mmole/kg) administration.

†5-fTHF (2.0 mg/kg) was administered i.v. at 0, 4, 8, 12, and 18 hr after methanol.

untreated monkeys. Although the total amount of $^{14}CO_2$ produced by the 5-fTHF treated animals was the same as that produced by the untreated animals, the rate of $^{14}CO_2$ formation was significantly faster in the treated animals throughout the time period 5-fTHF was administered and for several hours thereafter. During the initial 30 hr after methanol administration, the mean rate of CO_2 production (±SEM) was 656.8 (±13.6) μmole/kg/hr in those monkeys which received 5-fTHF and 504.5 (±9.1) μmole/kg/hr in the untreated animals. The result in Table 1 and Fig. 4 indicates that 5-fTHF treatment did not decrease formate formation but was effective, at least in part, in reducing blood formate levels by increasing the rate of formate metabolism to CO_2.

Data in Fig. 5 show that metabolic acidosis did not develop in those monkeys treated with 5-fTHF. Further, throughout the course of methanol intoxication, blood bicarbonate levels remained within the normal range in these animals. These results are in contrast to the severe bicarbonate depletion, high formate blood levels and metabolic acidosis observed in animals not given either 5-fTHF or sodium folate.

Reversal of Methanol Toxicity With 5-fTHF

Since repetitive treatment of monkeys with 5-fTHF was effective in reducing the severity of methanol toxicity when treatment was initiated at the same time methanol was administered, further experiments were conducted to determine the effectiveness of 5-fTHF in reversing the toxic syndrome once it had developed. Two monkeys were used as their own controls and were administered methanol. In a separate experiment, both methanol and 5-fTHF were given to each animal but treatment with 5-fTHF was delayed until significant levels of formate had accumulated in blood. The data in Fig. 6 show that the pattern of formate accumulation was markedly altered in the animals shortly after the onstart of 5-fTHF administration. Within a few hours of initiation of 5-fTHF treatment, at a time when blood formate levels were still increasing in the untreated monkeys, a rapid decline in blood formate levels was observed. In both monkeys, the decline in formate levels following 5-fTHF treatment was paralleled with a concomitant increase in the rate of CO_2 formation during this time (Fig. 7).

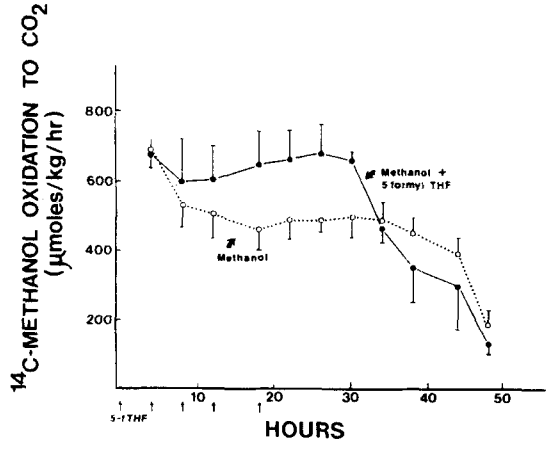

Fig. 4. Rate of methanol oxidation to CO_2 in methanol poisoned monkeys treated with 5-fTHF. Data were obtained from the monkeys represented in Fig. 2.

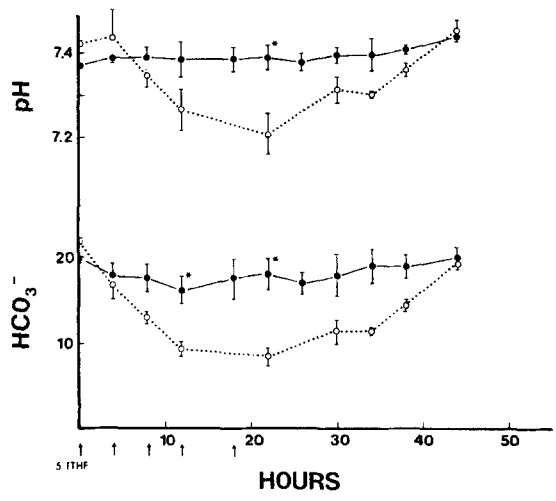

Fig. 5. Arterial blood pH and bicarbonate levels in methanol poisoned monkeys treated with 5-fTHF. Data were obtained from the monkeys represented in Fig. 2.

DISCUSSION

Recent investigations[3-5] have shown that an accumulation of formate in blood and other tissues correlates well with the development of metabolic acidosis and ocular toxicity produced by methanol. In species that metabolize formate through folate-dependent systems, a direct relationship has been established between the formate oxidation rate, sensitivity to methanol and functional integrity of the folate pathway. Rietbrock et al.[19] observed that in dogs

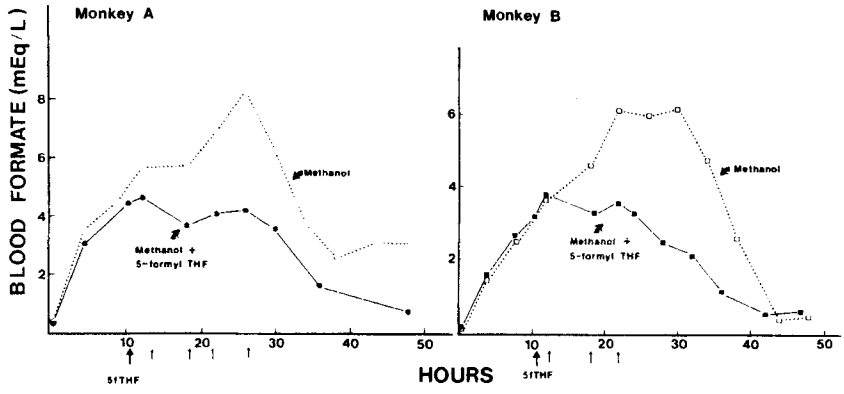

Fig. 6. Reversal of blood formate accumulation in methanol poisoned monkeys treated with 5-fTHF. At zero time, monkeys A and B were administered ^{14}C-methanol (62.5 mmole/kg) orally as a 20% methanol (w/v) solution. In a separate experiment, the same monkeys were given methanol at zero time followed by 5-fTHF (5 mg/kg), i.v., where indicated by the arrows.

pretreated with the folic acid antagonist, methotrexate, the administration of methanol resulted in plasma formate levels that were 3.5-fold higher than those seen in untreated dogs. Previous work in our laboratory has shown that, in contrast to rats that are fed a chow diet and are insensitive to methanol, folic acid deficient rats not only metabolize formate at a reduced rate but also accumulate formate and develop metabolic acidosis following methanol administration.[13,15] Further, McMartin et al.[12] have demonstrated that folic acid deficient monkeys are hypersensitive to methanol and that this hypersensitivity can be prevented by supplementation of diets with folic acid. The results reported here demonstrate that the severity of methanol toxicity and the accumulation of formate in blood can be significantly reduced in monkeys by the administration of either folic acid or its reduced derivative, 5-fTHF. Monkeys pretreated with sodium folate or administered repetitive doses of 5-fTHF after methanol intoxication maintained blood levels of formate that were 50% lower than those observed in untreated animals. In addition, folate treated monkeys did not develop metabolic acidosis. Further, 5-fTHF was effective in alleviating methanol toxicity even when therapy was initiated after signs of toxicity were apparent. When 5-fTHF was administered, the rate of methanol elimination from blood was not changed; however, the rate of CO_2 production was increased. These results indicate that the decreased blood formate levels observed with folate treatment were not the result of a decreased rate of formate formation from methanol but the result, at least in part, of an increased rate of formate oxidation to CO_2. This is substantiated by the finding that prior treatment of monkeys with either 5-fTHF or folic acid stimulates the oxidation of ^{14}C-formate

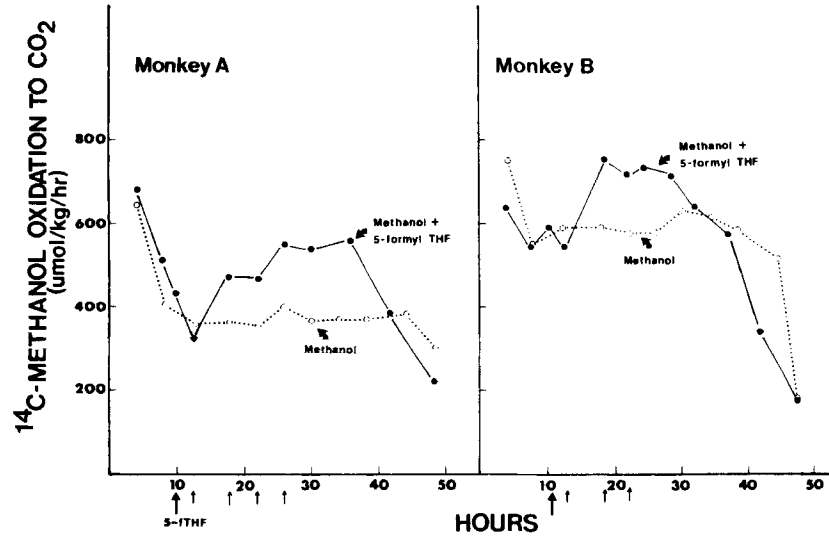

Fig. 7. Stimulation of methanol oxidation to CO_2 following initiation of 5-fTHF treatment. Data were obtained from the same animals represented in Fig. 6.

to CO_2.[12,20] That formate metabolism in monkeys can be stimulated by the administration of certain folate compounds suggests that the capacity of the folate pathway is normally limited or not functioning at full efficiency in this species. Species differences in the regulation or functioning of this system may explain species differences in formate metabolism and sensitivity to methanol. Preliminary data obtained in our laboratory indicate that formate metabolism in monkeys may be limited due to inadequate levels of free tetrahydrofolic acid, the coenzyme to which formate binds upon entrance into the one carbon pool. Studies are currently in progress to identify and quantify the various coenzymes and enzymes of this pathway in an attempt to discern the exact mechanism by which formate is metabolized.

The results presented here suggest that folate administration may be an effective therapeutic modality in the treatment of certain cases of human methanol toxicity. Formate plays a major role in the production of metabolic acidosis and ocular toxicity.[4,6] Thus, procedures that either decrease the rate of methanol metabolism to formate or increase the rate of formate oxidation are useful in treating methanol poisoning. Currently, hemodialysis is used where available and has been reported to be successful in lowering blood methanol levels.[21] In addition, ethanol and 4-methylpyrazole are effective inhibitors of methanol metabolism in vivo.[4,22] Treatment with these agents is usually accompanied by the simultaneous administration of sodium bicarbonate to correct the metabolic acidosis.[1] Since folates decrease formate accumulation by stimulating formate oxidation or utilization, the administration of 5-fTHF or folic acid in conjunction with an agent that blocks methanol metabolism should be effective in decreasing the toxicity produced by methanol. Folate treatment also may be useful in those instances when alternate forms of therapy, such as hemodialysis, are unavailable or inappropriate.

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