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MINIREVIEW

THE TOXICITY OF METHANOL

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Summary

Methanol toxicity in humans and monkeys is characterized by a latent period of many hours followed by a metabolic acidosis and ocular toxicity. This is not observed in most lower animals. The metabolic acidosis and blindness is apparently due to formic acid accumulation in humans and monkeys, a feature not seen in lower animals. The accumulation of formate is due to a deficiency in formate metabolism which is, in turn, related, in part, to low hepatic tetrahydrofolate (H_4folate). An excellent correlation between hepatic H_4folate and formate oxidation rates has been shown within and across species. Thus, humans and monkeys possess low hepatic H_4folate levels, low rates of formate oxidation and accumulation of formate after methanol. Formate, itself, produces blindness in monkeys in the absence of metabolic acidosis. In addition to low hepatic H_4folate concentrations, monkeys and humans also have low hepatic 10-formyl H_4folate dehydrogenase levels, the enzyme which is the ultimate catalyst for conversion of formate to carbon dioxide. This review presents the basis for the role of folic acid-dependent reactions in the regulation of methanol toxicity.

Methanol (methyl alcohol, wood alcohol) is a commonly employed organic solvent and reactant in organic synthetic procedures. It is employed in a variety of consumer products, such as solid fuels (Sterno®), and as a solvent in photocopying machine solutions. Automobile manufacturers have stated that methanol would be suitable in replacing gasoline for use in motor vehicles (1,2). Thus, it is possible that it will be a major fuel for automobiles in the near future.

Methanol has long been recognized as a human intoxicant (3-5) due to its abuse as a substitute for ethyl alcohol in alcoholic beverages. Although humans are susceptible to methanol poisoning, lower species, such as rats or mice, are not. An understanding of factors which play an important role in determining susceptibility or non-susceptibility to methanol is important and this review will consider issues related to the mechanism of toxicity of methanol.

In 1855, MacFarlan (6) made the unhappy suggestion that a mixture of one part of the impure methanol (wood naphtha) to 9 parts of ethanol would

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constitute a cheap substitute for the use of ethyl alcohol in manufacturing processes. Purification processes developed late in the 19th century improved the quality of methanol and, with these, an increase in the quantity of methanol used followed. In 1904, Wood and Buller (7) reported on 235 cases of blindness and death due to the exposure to methanol. The best characterization of methanol poisoning in humans is summarized in reviews and papers by Röe (3), Bennett et al. (4) and Kane et al. (5). The signs and symptoms are:

1. A central nervous system depression of short duration but milder than that seen following ethanol ingestion.
2. An asymptomatic latent period of 12-24 hr following ingestion of the alcohol where no signs or symptoms are noted.
3. After the latent period, severe metabolic acidosis occurs.
4. Complaints characteristic of ocular toxicity are described followed by blindness, coma, other CNS signs and death.

Death may occur if patients are not treated for metabolic acidosis, and blindness may result even if treatment for metabolic acidosis is performed. Thus, a patient may be left partially or totally blind even though they may recover from the metabolic acidosis (3). The time-toxicity relationship is a key to an understanding of some aspects of the mechanism of methanol poisoning. The latent period following the initial mild inebriation suggests that there is little relationship betheen the concentration of methanol in the body and the toxicity produced. Methanol, like ethanol, is rapidly absorbed and distributed uniformly to body water (8). The lack of relationship of methanol concentration and the production of ocular toxicity and metabolic acidosis implies the role of methanol metabolites in the intoxication. This is important to our understanding of the mechanism and treatment of methanol poisoning. The fact that inhibitors of methanol oxidation, such as ethanol (3) and 4-methylpyrazole (9), can alleviate the syndrome of methanol poisoning substantiates the role of metabolites of methanol in the poisoning and provides treatment potentials.

The ocular toxicity has been described at length in many publications (3,4,10,11). Initially, when symptoms of visual disturbance are reported, hyperemia of the optic disc is seen followed by peripapillary edema. Ultimately, the optic nerve head will be swollen along with the retinal vasculature. The edema may persist for a substantial period of time, and if damage is sufficiently severe, optic nerve atrophy will ultimately result. It is clear that the ocular toxicity is also produced by a methanol metabolite for the same reasons as given for the metabolic acidosis.

Doses of methanol reported to result in toxicity and lethality vary widely. Bennett et al. (4) suggested that ingestion of as little as 15 ml of a 40% (v/v) methanol solution resulted in the death of one person; and yet, another person was reported to have survived following the consumption of about 500 ml of the same solution. The apparent variability and sensitivity of humans to methanol poisoning may have several explanations. One variable may be the problem of obtaining exact information from patients who have been intoxicated. Röe (3,12) suggests that the variability in reaction to methanol could be explained by concomitant ingestion of ethanol with methanol. Thus, those patients who had consumed ethanol at or about the same time that methanol was taken had longer latent periods prior to the onset of intoxication. It is even possible that individuals who have consumed ethanol with methanol will demonstrate no toxic effects at all. Röe concludes that, in the absence of ethanol, methanol dose levels of 1 g/kg or higher are required for severe intoxication leading to death.

Recent work suggests another possible explanation for the individual variation. It is conceivable that nutritional differences exist among

individuals, such as folate deficiency. An inadequate folate metabolic system may compromise an individual in its ability to metabolize one carbon units. This may be the case for the debilitated person or in an individual who is a chronic alcoholic.

Methanol Poisoning in Non-Primates

The effects of methanol in lower animals are quite different from those seen in humans and primates in that metabolic acidosis and ocular toxicity are normally not seen (13). This makes it impossible to extrapolate results obtained from experiments using non-primate animals to humans. The major effect of methanol in non-primates is only a central nervous system depression similar to but milder than that seen with other alcohols. Monkeys are susceptible to methanol toxicity and serves as the only model of intoxication (14). Despite this, it is important to understand why lower animals do not exhibit methanol poisoning.

Methanol Poisoning in Primates

A number of laboratories have established that several species of monkeys are susceptible to methanol poisoning. Gilger and Potts (10) were the first to demonstrate the ocular toxicity and metabolic acidosis produced in monkeys following the administration of methanol. These workers reported a minimum lethal dose of 3 g/kg body weight for Rhesus monkeys and, clinically, the signs observed in the monkeys were similar to those observed in humans. They reported a slight initial central nervous system depression followed by a latent period. After the latent period, they found a progressive weakness, coma and death resulting about 20-30 hr following administration. Four monkeys given a lethal dose became severely acidotic within 24 hr, and two of the monkeys showed signs of ocular toxicity similar to those described in humans. These included dilated, unresponsive pupils and changes of the retina on ophthalmoscopic examination. One monkey showed optic disc hyperemia and retinal edema.

More recently, we and others have confirmed and extended this work (9,11,15-17). The administration of methanol (3 g/kg) to monkeys produced a syndrome similar to that described in humans; an initial slight central nervous system depression followed by a latent period of 12-16 hr, during which time the monkeys showed no sign of toxicity. This was followed by a progressive deterioration characterized by anorexia, vomiting, weakness, hyperpnea and tachypnea. Animals died in respiratory failure 20-30 hr following methanol administration. A somewhat similar study was performed to provide a model for ocular toxicity (11). In this work an attenuated and prolonged intoxication was produced by repetitive administration. These animals developed a prolonged formic acidemia, metabolic acidosis and ocular toxicity 40-60 hr following the initial dose.

The time sequence relating to the ingestion of methanol, the latent period, the alteration of blood pH, the accumulation of formate in the blood and lowered blood bicarbonate levels following the administration of methanol is consistent with human intoxication. Although the latent period may represent a time during which no symptoms are reported, formate levels in blood and tissues increase and bicarbonate concentrations fall. Clay et al. (15) and Tephly (18) demonstrated that the depletion of bicarbonate in methanol poisoned monkeys occurred in a mole-for-mole fashion with the accumulation of formate. Apparently, the buffering capacity of blood and tissues is maintained during the latent period so that the blood pH is not significantly decreased, i.e. the latent period represents compensated metabolic acidosis. The ocular toxicity and a metabolic acidosis with prolonged elevation of blood formate occurs with the uncompensated metabolic acidosis due to formic acid accumulation.

The elevation of formate in the blood of monkeys following methanol administration appears to be due to the fact that monkeys metabolize formate to carbon dioxide slower than rats. McMartin et al. (19) showed that formate at any dose is metabolized to carbon dioxide in monkeys at rates much slower than those seen in rats.

Methanol Disposition

Following oral ingestion of methanol, there is a rapid uptake from the gut and distribution to body water (8). Although human exposure to methanol vapor is less prevalent, methanol is rapidly absorbed through the skin or by inhalation and, in certain cases, has resulted in methanol poisoning in humans (7,20). However, no differences exist in the rate of absorption of methanol between various animal species, and blood levels are predictable once the dose of methanol is known.

The rate of elimination of methanol from the blood is relatively slow compared to ethanol. Furthermore, the rate of methanol disappearance from the blood is dependent upon the blood level. At low levels of methanol in the blood, a first-order rate of disappearance occurs (21-23) with a half-life of 2-3 hr. At high blood methanol levels (300 mg/dl), disappearance from the blood obeys first-order kinetics (9) with a half-life of about 27 hr. This is due to the saturation of metabolic systems which, when exceeded, bring into play routes of elimination other than metabolism, i.e. pulmonary and renal. Somewhere between high blood levels of methanol (> 300 mg/dl) and low levels (about 2-10 mg/dl), kinetics that approach zero-order disappearance of methanol from the blood are observed. Eells et al. (24) showed in monkeys that, at doses of 1 g/kg, methanol disappears from blood via zero-order kinetic and at a rate of about 4.4 mg/dl/hr. In rats, the rate of disappearance of methanol from the blood is about 3.7 mg/dl/hr (unpublishd results). Thus, the first step in the overall pathway of methanol oxidation appears to be similar in both monkeys and rats.

Knowing which metabolic system is responsible for the oxidation of methanol is important for the selection of appropriate inhibitors which would prevent or delay the onset of toxic symptoms produced by this alcohol. Two systems have been investigated. The role of the catalase-peroxidative system in methanol metabolism has been studied extensively and it is the major system functioning in methanol oxidation in rats. Catalase in the presence of hydrogen peroxide forms a catalase-H_2O_2 complex which mediates the oxidation of a variety of alcohols to their corresponding aldehydes (25). Methanol and ethanol react with similar velocities; whereas propanol and 1-butanol display lower substrate reactivity. The use of 3-amino-1,2,4-triazole (AT) has been very useful in demonstrating the role of catalase in biological systems. Heim et al. (26) discovered that AT inhibits hepatic and renal catalase activity in rats when injected intraperitoneally. AT was demonstrated to have no effect on ethanol elimination (27), although hepatic catalase activity was markedly reduced. Mannering and Parks (28) showed that AT inhibited hepatic catalase activity in rats in vivo and that, in liver homogenates from rats whose catalase had been reduced to 90% of control values, a marked inhibition of methanol oxidation to formaldehyde was observed in vitro. Tephly et al. (29) demonstrated that, in rats, AT markedly inhibited the oxidation of ^{14}C-methanol to $^{14}CO_2$ in vivo. Consistent with the role of a catalase-peroxidative system functioning primarily for methanol oxidation in the rat, equimolar doses of methanol and ethanol led to a 50% inhibition of methanol oxidation by ethanol. This was expected since these alcohols are equireactive with the catalase-peroxidative system.

Although a major role of the catalase-peroxidative system for the metabolism of methanol in rats was shown, results obtained in monkeys led to

the conclusion that a different system operates in this species. Makar et al. (30) showed that pretreatment of monkeys with high doses of AT 1 hr prior to methanol injection did not inhibit methanol oxidation. Alternate substrates for monkey liver alcohol dehydrogenase, such as ethanol or 1-butanol (which have greater affinities for this enzyme) produced marked inhibition of methanol oxidation in monkeys (> 90%). These results support the conclusion that the catalase-peroxidative system is not functional in methanol oxidation in monkeys and that metabolism of methanol in the monkey is catalyzed by alcohol dehydrogenase.

Watkins et al. (31) demonstrated that the alcohol dehydrogenase inhibitor, pyrazole, markedly inhibited methanol oxidation in Rhesus monkeys. A derivative, 4-methylpyrazole, also inhibited methanol oxidation and prevented the development of methanol poisoning in the monkey (9). The inability of monkey liver catalase to oxidize methanol in vivo has been attributed to low levels of peroxide-generating enzymes in monkey liver (32) or different distributions of peroxide-generating enzymes in this species (33). Although different catalysts are involved in the oxidation of methanol in rats and monkeys, this does not explain the species difference in susceptibility to methanol poisoning since the rate of methanol elimination from the blood is similar in these species.

Formaldehyde Metabolism

In the past, formaldehyde has often been suggested as the methanol metabolite which produces toxicity (34,35). Today, a great deal of information is available concerning its lack of such a role. The presence of elevated formaldehyde levels in body fluids or tissues following methanol administration has not been observed. No formaldehyde has been detected in blood, urine or tissues obtained from methanol-treated animals (36,37) and, in methanol-poisoned humans (38), formaldehyde increases have not been observed. McMartin et al. (39) could not demonstrate elevated formaldehyde levels in methanol-poisoned monkeys which exhibited marked metabolic acidosis and blindness using techniques designed to trap and analyze formaldehyde.

There are several enzyme systems which are very reactive with formaldehyde and which rapidly convert it to metabolic products on the route to formate and carbon dioxide. Strittmatter and Ball (40) isolated a formaldehyde-specific, NAD-dependent formaldehyde dehydrogenase from beef liver and showed that this enzyme required reduced glutathione (GSH). The protein is quite specific for formaldehyde and is associated with a thiolase (41,42). In this reaction, formaldehyde reacts with GSH to form S-hydroxymethylglutathione, which is converted to S-formylglutathione. The thiolase catalyzes the hydrolysis of S-formylglutathione to formic acid and reduced glutathione. GSH is, therefore, a key agent in the generation of formate from formaldehyde. The first reaction is reversible, but the second reaction is not, a feature which explains the apparent irreversibility of the overall two-step reaction. Formaldehyde dehydrogenase activity is present at high levels in rat liver, human brain and a number of other species and tissues, including the retina (43). However, these tissues have not yet been examined for the presence of S-formylglutathione hydrolase. Goodman and Tephly (44) showed that formaldehyde dehydrogenase activity of human liver is higher than that of rat liver. Thus, the rate of conversion of formaldehyde to formate may be even higher in monkeys and humans than that of rats.

Formaldehyde oxidation can also proceed in liver mitochondria or cytosol through the mediation of various aldehyde dehydrogenase activities (45-47). Thus, the overall formaldehyde metabolizing capabilities of liver and other tissues appear to be high.

Formaldehyde metabolism has been studied *in vivo*. Studies show rapid metabolism. The half-life of formaldehyde disappearance from the blood following its intravenous infusion is about 1 min in dogs, cats, rabbits, guinea pigs, rats (48) and monkeys (39). About 85% of a low dose of ^{14}C-formaldehyde is excreted as pulmonary $^{14}CO_2$ (49,50). Malorny *et al.* (51) demonstrated that, when equimolar doses of formaldehyde, formic acid or sodium formate were infused intravenously into dogs, equivalent peak concentrations of formate in the blood were obtained. McMartin *et al.* (39) showed that, following the intravenous infusion of formaldehyde into monkeys, formate accumulated rapidly in the blood.

Humans also rapidly metabolize formaldehyde to formate. Eells *et al.* (52) reported on a case of human formaldehyde poisoning where an individual committed suicide by drinking 4 oz. of a solution containing 37% (v/v) formaldehyde and 12.5% (v/v) methanol. This individual was studied a short time after ingestion and the level of formate in the blood was high (about 7 mM) consistent with the marked metabolic acidosis observed. The blood level of methanol could be completely accounted for based on the known amount of methanol ingested and its distribution to total body water. Thus, the formate found in blood was derived from the formaldehyde. Eells *et al.* (52) concluded that formaldehyde poisoning was due, in large part, to its conversion to formic acid and that dialysis procedures could be useful in the treatment of fomaldehyde poisoning. Although it is possible that formaldehyde may be responsible for certain features of methanol poisoning, it does not account for the metabolic acidosis.

Formic Acid Metabolism and Toxicity

A substantial amount of evidence indicates a strong and direct relationship between formate and methanol toxicity. Formate does not accumulate in methanol-treated rats and rats do not demonstrate methanol toxicity, whereas formate accumulates to high levels in the body fluids and tissues of methanol-treated monkeys and humans intoxicated with methanol (9,53-55). Clay *et al.* (15) showed that the accumulation of formate in the blood of monkeys treated with methanol completely accounted for the depletion of plasma bicarbonate in these animals. Sejersted *et al.* (55) showed that in 11 patients who were victims of an epidemic of methanol poisoning, the accumulation of formate in the plasma completely accounted for the fall in bicarbonate. Treatment of animals with 4-methylpyrazole prevented the accumulation of formate and the development of metabolic acidosis in monkeys administered methanol (9). Jacobsen *et al.* (56) reported on one case in which ethanol therapy was initiated soon after the ingestion of a toxic amount of methanol. In this individual, no accumulation of formate in the plasma was seen.

Formate has also been demonstrated to produce ocular toxicity in monkeys. Direct evidence demonstrating the role of formate in the pathogenesis of ocular toxicity was shown by Martin-Amat *et al.* (57). Intravenous infusion of formate into monkeys was performed under conditions where blood formate levels similar to those observed in methanol-poisoned monkeys were maintained for 25-50 hr. In addition, in these experiments, the pH of the blood was maintained within normal limits. In all monkeys, ocular toxicity developed early (about 24 hr) and was essentially identical clinically to that produced by methanol (11). These results strongly suggest that the ocular toxicity observed in methanol poisoning was the result of a direct effect of formate which occurred independent of the production of metabolic acidosis. Numerous clinical reports which indicate that the correction of metabolic acidosis in methanol-poisoned patients does not necessarily prevent or reverse the development of ocular toxicity (12) lend further support for this concept.

Important clues concerning the mechanism of methanol toxicity, or formate toxicity, have come from studies on the regulation of formate metabolism in species which are either insensitive or sensitive to methanol poisoning. Important issues are: how is formate metabolized; what regulates the rate at which it is metabolized; and how do species differ in their disposition of formate?

The major factor dealing with formate elimination from the body of rats and monkeys appears to be the oxidation of formate to carbon dioxide. The liver appears to be the primary site of formate oxidation since the maximum rate of formate oxidation in isolated rat hepatocytes is similar to the rate observed in intact rats (58). McMartin et al. (53) demonstrated that the rate of formate oxidation to CO_2 in monkeys was markedly lower than that observed in rats. The rate of formate oxidation was dose-dependent in both species; but at each dose, the rate in monkeys was one-half or less than that observed in rats. The maximal rate of formate oxidation was 35 mg/kg/hr in monkeys and 75 mg/kg/hr in rats.

Are different enzymatic systems involved in the oxidation of formate in different species, or is there a relative deficit in the capability of a given system in susceptible species (i.e. monkeys and humans) in the ability to oxidize formate? Two enzymatic systems are known to catalyze the oxidation of formate to carbon dioxide, the catalase-peroxidative pathway (59) and a folate-dependent pathway which consists of a number of folate-dependent enzymes whereby formate is led to 10-formyl-H_4folate and subsequently to carbon dioxide and H_4folate (14,60). It appears that, in all species studied thus far, formate oxidation in vivo occurs primarily via the folate-dependent pathway. In rats, formate oxidation in vivo is insensitive to inhibitors of the catalase system (61). On the other hand, folate deficiency leads to an inhibition of formate oxidation in rats (60,61); administration of exogenous folate reversed the effect of folate deficiency and restored formate oxidation (61). Makar and Tephly (62) showed that folate-deficient rats which were treated with methanol developed formic acidemia and metabolic acidosis, whereas untreated animals did not accumulate formate following methanol administration.

In monkeys, the folate-dependent pathway is also the major route of formate oxidation. The rate of formate oxidation or the rate of formate disappearance from the blood in monkeys was not altered by AT (53). However, the rate of formate oxidation in folate-deficient monkeys was approximately 50% of that observed in control monkeys (53). The administration of either folic acid or 5-formyl-THF (63) was capable of increasing formate oxidation in monkeys which received a normal diet and which were considered to be adequate with respect to their folate nutriture. Monkeys which were fed a folate-deficient diet were found to be very sensitive to methanol (53).

Noker and Tephly (63) showed that methanol toxicity can be significantly ameliorated by administration of either folic acid or 5-formyl-H_4folate. Although monkeys treated with methanol (2 g/kg) developed a moderate metabolic acidosis and a significant elevation of blood formate, the administration of folate prevented the elevation of blood formate and the development of metabolic acidosis in these animals. The clearance of methanol was not altered by folate administration; thus, the effect of exogenous folate was due to its effect on the rate of formate oxidation.

If most species metabolize formate to carbon dioxide via the folate system, what is the regulation of the rate of metabolism? Recent studies have explored the relationship between hepatic H_4folate and the rate of formate oxidation within a given species and between species. Studies in rats have been performed on the regulation of H_4folate levels in liver as perturbed by nitrous oxide. The conversion of 5-methyl H_4folate to H_4folate is catalyzed by

5-methyl H_4folate homocysteine methyltransferase (methionine synthetase), an enzyme that is dependent upon vitamin B_{12} (methylcobalamin) and catalytic amounts of S-adenosylmethionine. This enzyme is present in liver and other tissues and is inhibited in all tissues by the administration of nitrous oxide (64). Eells et al. (65,66) showed that, following nitrous oxide treatment of rats, there was a significant decrease in hepatic H_4folate levels with a concomitant increase in hepatic 5-methyl H_4folate. Rats treated with nitrous oxide also exhibited a marked decrease in the rate of formate oxidation. When methanol was administered to rats which were exposed to nitrous oxide for 2 hr, there was a marked accumulation of blood formate, a decrease in blood pH to 7.2 and a depletion of blood bicarbonate. An excellent correlation between the rate of formate oxidation in rats and hepatic H_4folate levels was shown.

Treatment of monkeys with nitrous oxide also leads to a marked sensitization of these animals to methanol (24). Following nitrous oxide treatment and a dose of 1 g/kg of methanol (a dose which produces only a slight increase in blood formate in untreated monkeys), there was a marked accumulation of formate 12 hr following methanol administration. Values observed were greater than blood formate levels observed when 2 g/kg of methanol were given to untreated monkeys. Thus, the perturbation of the folate system with either folate-deficient diets or nitrous oxide treatment markedly decreases formate oxidation and permits severe methanol intoxication at doses which would generally only lead to mild effects.

Further studies carried out across species shows the importance of hepatic H_4folate in the oxidation of formate. Three species were examined with respect to their maximal rate of formate oxidation and their hepatic H_4folate levels (67). Mice have an extraordinarily high level of hepatic H_4folate (42.9 nmol/g liver) and a very high rate of formate oxidation (300 mg/kg/hr). Rats have hepatic H_4folate levels of 11.4 nmol/g liver and oxidize formate at a maximal rate of 78/mg/kg/hr. Monkeys which have 7.4 nmol/g liver of H_4folate oxidize formate at about 40 mg/kg/hr. Thus, an almost linear relationship can be drawn between formate oxidation and hepatic H_4folate levels across species. Although human formate oxidation rates cannot be determined, one might predict from human hepatic H_4folate levels (6.5 nmol/g liver) that humans should oxidize formate at rates similar to those observed in monkeys. This suggests that, in part, hepatic H_4folate concentrations govern a species' ability to metabolize formate.

To extend this further, a recent study by Makar et al. (68) in young swine must be noted. These investigators showed that pigs have the lowest level of hepatic H_4folate of any species yet studied (3.3 nmol/g liver). Although formate oxidation rates were not determined in this work, formate disappearance from the blood was followed and found to be extremely slow. The half-life for formate disappearance was 87 min compared to 36 min observed in rats. Formate oxidation rates have recently been determined for microswine in our laboratory (preliminary data). The maximal rate of formate oxidation was 20 mg/kg/hr, a rate half of that found in monkeys. As expected, hepatic H_4folate concentrations were extremely low (1.6 nmol/g liver). Thus, this species may prove to be extremely useful in further studies on methanol poisoning and in studies on the regulation of folate metabolism.

Studies on 10-Formyl H_4folate Dehydrogenase

In the conversion of formate to carbon dioxide, formate is converted first to 10-formyl H_4folate in a reaction catalyzed by formyl H_4folate synthetase followed by oxidation to carbon dioxide in a reaction catalyzed by 10-formyl H_4folate dehydrogenase (14). Johlin et al. (67) have recently shown that 10-formyl H_4folate dehydrogenase activity in monkey and human liver is much lower than that of rat liver. Western blot analysis using a monospecific antibody

(69) showed that there is reduced immunoreactivity in the hepatic cytosol obtained from humans. Thus, in methanol-susceptible species, there is a reduced level of hepatic H_4folate and in the enzyme required for the oxidation of formate to carbon dioxide. Makar et al. (68) have also seen reduced 10-formyltetrahydrofolate dehydrogenase activity in pig liver where hepatic H_4folate levels are very low. Thus, there may be several factors operating in animals that metabolize formate poorly; low hepatic H_4folate levels and low levels of 10-formyl H_4folate dehydrogenase. One might also speculate that these factors may be inter-related since low 10-formyl H_4folate dehydrogenase activity is low only in those species where hepatic H_4folate levels are low. Future studies are needed to explore this possible relationship.

Conclusion

Methanol toxicity is governed by the metabolism of this alcohol to formate which accounts for the acidosis and blindness seen clinically. The regulation of formate metabolism depends on the folate biochemical pathway where H_4folate is central to the disposition of formate. The sensitivity of a species or, perhaps, a given individual is determined by the level of hepatic H_4folate and 10-formyl H_4folate, each of which is low in those species where methanol toxicity is seen. The major question still unresolved is why H_4folate levels are low in higher species and in pigs. This question may have significance beyond that of the problem of methanol poisoning.

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