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Recent developments in methanol toxicity

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Abstract

The disposition of methanol and its putative toxic metabolite formate has been studied in humans, non-human primates, and rodents after exposure to high, neurotoxic doses. The rate at which rodents detoxify formate is more rapid than that of primates. Formate, an endogenous biological substrate, is detoxified by metabolism to CO_2 via a tetrahydrofolate-(THF) dependent pathway. Species with high hepatic THF levels, such as rodents, are less sensitive to the neurotoxic effects of large methanol doses compared with species with low THF levels, such as primates. Data on the capacity of primates to detoxify formate derived from inhalation of low levels of methanol are critical for assessing human risk from methanol fuels. Female cynomolgus monkeys exposed to low concentrations of [^{14}C]methanol (10–200 ppm) for 2 h have blood levels of methanol-derived formate that are 100- to 1000-fold lower than endogenous levels of formate. Healthy human volunteers exposed at rest or during exercise to 200 ppm methanol for 6 h or exposed to 20 mg/kg orally have elevated blood levels of methanol, but blood formate concentrations are not significantly increased above endogenous concentrations. Deficiencies in THF may prolong blood levels of formate and increase the likelihood of toxic effects. Limited studies in non-human primates with low THF levels exposed to 900 ppm methanol for 2 h have shown that concentrations of methanol-derived formate in blood remain below endogenous levels. Thus human populations may not be at added risk of neurotoxic effects resulting from exposure to low levels of methanol.

Keywords: Methanol; Formate; Folate; Non-human primates

1. Introduction

Methanol (wood alcohol) has the potential to become a major automotive fuel in the United States in the next century [1]. Emissions of methanol can arise from its release as uncombusted fuel in automobile exhaust or from its evaporation during refueling and after the engine is stopped. The United States Environmental Protection Agency has modeled methanol expo-

sure levels that might occur under specific conditions of use [1]. For example, if 100% of all automobiles were powered by methanol-based fuels, simulation models predict concentrations of methanol in urban streets, expressways, railroad tunnels or parking garages ranging from a low of 1 mg/m^3 (0.77 ppm) to a high of 60 mg/m^3 (46 ppm). Predicted methanol concentrations in a personal garage range from 2.9 mg/m^3 to 50 mg/m^3 while predicted concentrations during refueling of vehicles range from 30 to 50 mg/m^3 . For reference purposes, the threshold limit value (TLV) of the American

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Conference of Governmental Industrial Hygienists (ACGIH) for worker exposure to methanol over an 8-h work day is 260 mg/m^3 (200 ppm).

A large body of evidence on the acute toxicity of methanol has been accumulating since the early 1900s, when exposure of humans to relatively large acute doses of methanol occurred either through accidental or intentional ingestion, percutaneous absorption or inhalation. In the early part of the century, painters used methanol as a cleaning fluid or as a dilution agent for shellac, varnish and paint. Methanol was also used as a solvent by hatters, dyers, shoemakers, and brass finishers and in the manufacture of rubber tires [2]. During this time, about 100 cases of vision impairment or death from inhalation of methanol vapors were reported. These cases demonstrate that acute human exposure to methanol can result in blindness, metabolic acidosis, and death.

Formate is the metabolic product responsible for the acute toxic effects of methanol [3–5]. Formate is detoxified by a multistep pathway to carbon dioxide (CO_2) [6]. In all species studied, this is achieved through a tetrahydrofolate- (THF) dependent pathway. Folate is an essential vitamin found in fresh fruits and vegetables and is the building block of THF. Rodents have higher concentrations of liver THF compared with primates and therefore are more efficient in the metabolism of formate to CO_2 than humans and non-human primates. The faster rate of formate removal in rodents prevents accumulation of formate above endogenous levels at any methanol dose. Therefore rodents are not susceptible to methanol-induced metabolic acidosis or ocular toxicity from exposures that cause these effects in humans and non-human primates.

2. Low level methanol exposure

The issue of blood methanol and formate concentrations following inhalation exposure to methanol vapors has been addressed by Horton et al. [7], who exposed male rhesus monkeys to concentrations of methanol ranging from 200 ppm to 2000 ppm for 6 h. Although these levels

are higher than those expected during normal fuel use they still enable us to get a perspective on the issue. Concentrations of methanol and formate in the blood of the primates were measured for up to 18 h after the end of the 6-h inhalation exposure. The highest blood methanol concentrations occurred immediately following the 6-h inhalation exposures and declined steadily thereafter. The concentrations of formate in the blood of the monkeys exposed to these methanol vapors were not elevated above the endogenous blood formate concentrations determined at the start of the exposure (Fig. 1). Blood concentrations of formate varied considerably among the individual monkeys and at various times up to 18 h after the end of the exposure. However, these formate concentrations were not influenced by either time or methanol exposure concentration, suggesting that the formate levels, although variable, were not elevated above endogenous levels by exposure to inhaled methanol. The highest methanol exposure concentration used by Horton et al. [7], 2000 ppm, is 10 times higher than the current time weighted average (TWA)-TLV for methanol of 200 ppm.

Although these studies showed that blood formate concentrations were not increased above endogenous levels following exposure to 200 ppm methanol, the contribution of methanol-derived formate to the total body pool of formate could not be determined. This issue was addressed by Dorman et al. [8] using female cynomolgus monkeys which were exposed to a

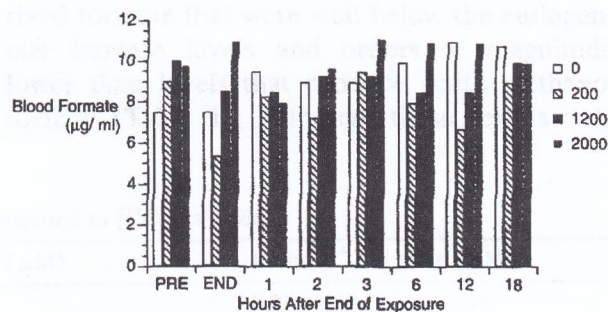


Fig. 1. Blood concentrations of formate in male rhesus monkeys prior to (pre) and after (end) exposure to 0 (control), 200, 1200, or 2000 ppm methanol for 6 h. Data represent average formate concentrations for three monkeys taken from Horton et al. [7].

range of inhalation exposure concentrations of methanol spanning both workplace and environmental exposures. Carbon-14 radiolabeled methanol was incorporated into the exposure atmosphere to aid in measuring concentrations of methanol and formate in blood and excretory products both during and after exposure to the inhaled methanol. The results summarized in Table 1 indicated that a 2-h exposure to [^{14}C]methanol concentrations up to 900 ppm produced a maximum blood [^{14}C]formate concentration that was only a fraction of the endogenous formate level (100–200 nmol/ml) and several orders of magnitude lower than levels of formate known to be toxic (>7000 nmol/ml). These studies suggest that the maximum blood concentration of formate resulting from exposure to low concentrations of methanol, such as those that might result from the use of methanol as an alternative fuel, is insignificant when compared with endogenous formate levels.

Recent experimental studies in humans exposed to concentrations of methanol at the TWA-TLV are consistent with results of experiments in non-human primates. For example, Lee et al. [9] exposed healthy volunteers to methanol concentrations of 200 ppm for 6 h. The subjects were either at rest or engaged in mild exercise. These investigators were able to observe a 3.5- to 4-fold increase in the peak methanol concentration in blood of the exposed subjects compared to their preexposure levels, indicating that they had absorbed some of the methanol vapor. However, when these investigators analyzed the concentrations of formate in the blood of these individuals, they were not able to detect a change in the blood formate concentration compared to the preexposure value. Similar results were ob-

served in humans exposed to methanol while resting or exercising. Most recently, d'Alessandro et al. [10] measured formate levels in urine and serum after controlled methanol exposures of healthy volunteers at the threshold limit value (200 ppm). These investigators also could not detect any increase in formate in urine or serum due to methanol exposure compared to the control values. Taken together, results of studies in humans and non-human primates exposed to concentrations of methanol ranging from 10 to 2000 ppm suggest that exposure to methanol vapors during the normal use of methanol fuel does not pose an unacceptable risk to healthy adults.

3. Sensitive subpopulations

As noted previously, the hepatic stores of folate in the liver are an important determinant for predicting whether or not a species is sensitive to methanol-induced acute toxicity [6,11,12]. Studies were conducted to determine how methanol is metabolized by non-human primates with reduced stores of folate [8]. The same female cynomolgus monkeys that were used in the previously described studies of Dorman et al. [8] were placed on a folate-devoid diet until reduced folate levels in red blood cells of these monkeys were observed. The monkeys were then exposed to 900 ppm of [^{14}C]methanol for 2 h. Even with a reduced folate status, monkeys exposed to 900 ppm of [^{14}C]methanol for 2 h still had peak concentrations of [^{14}C]methanol-derived formate that were well below the endogenous formate levels and orders of magnitude lower than levels that produce acute methanol toxicity (Table 1). Although these results only

Table 1
Measured peak methanol and formate concentrations in primates exposed to [^{14}C]methanol^a

| [^{14}C]Methanol exposure (ppm) | Blood [^{14}C]methanol (μM) | Blood [^{14}C]formate (μM) |
|--|---|--|
| 10 | 0.65 ± 0.3 | 0.07 ± 0.02 |
| 45 | 3.0 ± 0.8 | 0.25 ± 0.09 |
| 200 | 21 ± 16 | 2.3 ± 2.9 |
| 900 | 106 ± 84 | 2.8 ± 1.7 |
| 900 (folate-deficient) | 211 ± 71 | 9.5 ± 4.7 |

^a Data taken from Dorman et al. [8].

represent a single exposure and therefore preclude broad generalizations, they do suggest that the body contains sufficient folate stores to effectively detoxify small doses of methanol-derived formate from inhalation of methanol resulting from its normal use as an automotive fuel.

4. Developmental toxicity

Recent studies conducted in rodents [13–15] have demonstrated that methanol exposure impairs neural tube closure. The concentrations of methanol used in the teratogenicity studies greatly exceed estimates of likely exposure scenarios to methanol vapors relating to its use as an automotive fuel. Nonetheless, given what is known about the dramatic differences in the way rodents and primates metabolize methanol and formate, is the biochemical basis for the observed teratogenic effects in rodents relevant for humans?

Formate seems to play no apparent role in the development of methanol-induced exencephaly in mice [16]. Mice exposed to 15 000 ppm methanol for 6 h developed exencephaly and had high concentrations of methanol (223 ± 23 mM) in maternal plasma but did not accumulate formate in either plasma or conceptuses. Additionally, animals given a large single oral dose of sodium formate (750 mg/kg) did not develop exencephaly or decidual swelling in excess of 2.5 mm.

The relationship between the folate status and developmental neurotoxicity is well recognized. Sakanashi and coworkers (17) reported an increase in methanol-induced exencephaly and other terata in CD-1 mice on a folate-deficient diet and also reported that folate supplementation ameliorated those adverse developmental effects. Research on the relationship between methanol exposure and fetotoxicity should help answer further questions about the risk of exposure to low concentrations of methanol if it is used as an alternative fuel.

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