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Metabolism Clinical and Experimental 57 (2008) 920–926
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Formate pharmacokinetics during formate administration in folate-deficient young swine

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Received 11 May 2007; accepted 28 February 2008

Abstract

The objective of the study was to investigate the effect of folate deficiency on formate pharmacokinetics during formate administration in folate-deficient young swine. Methanol is a one of the congeners found in alcoholic beverages. Methanol toxicity is mediated through formic acid and thus plays a significant role in the pathophysiology of alcoholism. Folate is a required cofactor in the metabolism of formate to CO_2 and H_2O. We investigate the effect of folate deficiency on the pharmacokinetics of formate. Twelve young pigs were pair-matched and randomly placed into 2 groups on acquisition (~5 weeks). One group was made folate deficient (FFD) by feeding with a folic acid–deficient diet; the other group (FFC) was fed a diet supplemented with folate. Four animals (31-38 kg) from each group were infused (intravenous) with 351 mg/kg of sodium formate. The remaining 2 animals were infused with isotonic sodium chloride solution. Blood samples were collected before and at 10, 20, 30, 45, 60, 90, 120, 180, 240, and 480 minutes post dose and analyzed for formate levels by gas chromatography. Pharmacokinetic parameters were estimated using a noncompartmental approach. Formate (mean ± SE) accumulation was higher in the FFD group than the FFC group ($AUC_{0-\infty}$ of 72.37 ± 8.29 vs 30.08 ± 2.58 g min/L, respectively). Elimination was also slower in the FFD group (FFD systemic clearance = 0.12 ± 0.01 L/min compared with FFC systemic clearance = 0.27 ± 0.02 L/min). Half-life of elimination was 2.5 times longer in FFD group (113 ± 1 minute) than in FFC group (45 ± 6 minutes). Folate deficiency had no influence on the volume of distribution of formate (18.84 ± 1.05 L in FFD vs 17.21 ± 1.35 L in FFC). Adequate folate status is important in the elimination of formate. A folate-deficiency state results in a reduction in formate elimination kinetics, which may increase the risk of formate toxicity.

1. Introduction

Formic acid and its conjugate base formate are essential metabolites that participate in one-carbon pool of intermediary metabolism. It is produced in the body as a result of catabolism of several amino acids including serine, glycine, histidine, and tryptophan as well as by the recycling of methylthioadenosine from the polyamine biosynthesis pathway [1,2] through the oxidation of formaldehyde generated during cytochrome P450–catalyzed *N*- and *O*-demethylation reactions [3]. Formate is also produced during the hydrolysis of certain prodrugs containing acyloxymethyl substituents as protecting groups, such as fosphenytoin and its analogs [4]. Exogenous sources of formate include methanol-containing alcoholic beverages, fragrances, roasted coffee, food flavors (especially fruit and honey mixtures), and industrial products such as decalcifiers, acidulating agents in textile dying and finishing, and mold inhibitors in grain and silage. Formic acid, a metabolite of methanol, is responsible for the toxicity observed in methanol poisoning, although methanol itself has direct toxic effects such as central nervous system depression, weakness, vomiting, and headaches [5-7,29].

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doi:10.1016/j.metabol.2008.02.006

Table 1
Levels of hepatic folate intermediates in various species and subspecies

Folate intermediate	Species/Subspecies concentration (nmol/g)						
	Mouse [a]	Rat [a]	Monkey [a]	Human [a]	Outbred swine [b]	Micropigs [c]	Yucatan minipigs [d]
Tetrahydrofolate	42.9 ± 1.2	11.4 ± 0.8	7.4 ± 0.8	6.5 ± 0.3	3.3 ± 1.1	1.6 ± 0.2	
5-Methyltetrahydrofolate	11.6 ± 0.4	9.3 ± 0.6	7.6 ± 0.6	6.0 ± 0.7	1.0 ± 0.2	5.4 ± 0.5	
Formyltetrahydrofolate	6.4 ± 0.6	4.6 ± 1.3	10.5 ± 0.8	3.3 ± 0.5	0.7 ± 0.1	1.3 ± 0.2	
Total	60.9 ± 2.1	25.3 ± 0.9	25.5 ± 1.2	15.8 ± 0.8	5.1 ± 1.2	8.2 ± 0.6	17.5 ± 2.2

Values represent mean ± SE.
[a] Data from Johlin et al [17].
[b] Data from Makar et al [14].
[c] Data from Tephly et al [19].
[d] Data from Dorman et al [23].

During methanol intoxication, methanol is first metabolized by alcohol dehydrogenase to formaldehyde and then to formic acid by aldehyde dehydrogenase. Excess formate production may then lead to formate accumulation and toxicity such as optic nerve damage, increased anion gap, and metabolic acidosis [8,9]. Urinary excretion of formic acid is low (about 2%-7%) [10-12].

The vitamin folic acid plays a critical direct role in formate metabolism because it is a critical cofactor in the elimination of formate, thus indirectly altering formate elimination. Folate coenzymes function to accept or donate one-carbon units in folate-requiring metabolic pathways [13]. During formate metabolism, folate coenzymes transfer a formyl group (CHO) to tetrahydrofolate, resulting in the formation of CO_2 and H_2O [8,14]. Thus, adequate tetrahydrofolate levels are important for efficient formate metabolism. The importance of maintaining optimal folate levels in reducing the detrimental effects of formate in the body cannot be ignored given the ubiquitous nature of methanol and formate, formate's role in free radical damage [15], and the dependency of its metabolism on folate status.

Species differences exist in the metabolism of formate (Table 1). This is due to differences in the activities of formyltetrahydrofolate synthetase, the enzyme catalyzing the transfer of CHO group to tetrahydrofolate; methylenetetrahydrofolate dehydrogenase, which catalyzes the removal of H^+ from 5,10-methylenetetrahydrofolate to form 5,10-methylenetetrahydrofolate; and the tetrahydrofolate substrate [16-18]. Species susceptible to methanol/formate poisoning have lower hepatic tetrahydrofolate levels and thus slower formate metabolism than resistant species such as rats. In such susceptible species, folate status may have a marked impact on formate levels and on the predisposition to formate toxicity. Pigs have lower hepatic tetrahydrofolate levels (3.3 ± 1.1 nmol/g, mean ± SE) compared with rats (11.4 ± 0.8 nmol/g, mean ± SE) [19]. Hence, mice and rats have higher rates of formate metabolism and are less susceptible to formate toxicity, whereas humans and pigs tend to be more susceptible. This suggests that pigs may be an appropriate animal model for investigations into the effects of formate toxicity and its relationship to folate status. The purpose of this experiment was to determine the influence of folate deficiency on the pharmacokinetics of exogenously administered formate in a pig model.

2. Experimental

2.1. Chemicals

Pure formic acid standard (88% vol/vol) was obtained from Fisher Scientific (Fair Lawn, NJ); and sodium formate, from Sigma-Aldrich (Oakville, Ontario, Canada). Isotonic sodium chloride solution was obtained from Fluka (Oakville, Ontario, Canada). Sulfuric acid and anhydrous ethyl alcohol were purchased from JT Baker (Phillipsburg, NJ) and Commercial Alcohols (Brampton, Ontario, Canada), respectively. Gastight syringes (1.25 mL, 22/2"/2) were purchased from Hamilton (Reno, NV). All other chemicals used were analytical grade.

2.2. Animals and husbandry

The diets consisted of a semipurified diet formulated to meet the nutrient requirements of the 5-to 20-kg pig [20]. The basal diet (folate control diet, FFC) consisted of 48.6% corn starch, 20% lactose, 15% vitamin-free casein, 5% corn oil, 5% cellulose, 2.2% monocalcium phosphate, 1.5% limestone, 0.5% salt, and 0.2% cysteine (Sigma Chemical, Oakville, Ontario, Canada) and a vitamin/mineral premix that provided 150% of the nutrient requirements of swine for micronutrients (Sigma Chemical). Additional ingredients were procured from a commercial animal feed supplier. Diets were prepared at the University of Manitoba, and primary macronutrient-containing ingredients were purchased from a commercial research diet supplier (Harlan Teklad, Madison, WI). Folic acid (Sigma Chemical) was provided at a rate of 0.6 mg/kg. The folate-deficient diet (FFD) consisted exactly of the FFC diet with the exception that folic acid was not supplemented. Vitamin-free casein contains approximately 0.50 mg/kg of folate (Harlan Teklad) and, as the sole source of protein at 15% inclusion, would yield a folate level of 0.08 mg total folate per kilogram diet in the FFD (25% of requirement). In addition, 1% succinylsulfathiazole was added to both diets as an antibiotic to prevent the synthesis and absorption of folate

produced by commensal microorganisms in the gastrointestinal tract. Diets were offered to piglets in the form of powder for 6 weeks before administration of formate. Folate deficiency was confirmed with plasma and red blood cell folate level determinations.

Twelve pigs (all male, ~5 kg) were acquired from the main swine research herd at the University of Manitoba (Cotswold Canada genetics; maternal line = Landrace, Yorkshire; paternal line = Hampshire, Duroc). Piglets were weaned at 18 days of age, weighing approximately 5 kg, and were randomly assigned to 1 of 2 treatment groups (n = 6 per group). Pigs receiving the FFC diet were matched (by body weight) to corresponding folate-deficient pigs (FFD) and received equivalent diet allotment using a pair-feeding protocol. Feed intake was measured daily, with the FFD diet consumption (ad libitum) serving as the allotment for the corresponding FFC-matched piglet. Folate status was monitored weekly for 6 weeks. When plasma folate concentrations had reached a nadir (after 6 weeks), the pigs were subjected to the formate experiments.

Pigs were individually housed on raised, plastic-coated mesh flooring in an environmentally controlled room with a 12-hour light/12-hour dark cycle. Each pen was equipped with a self-feeder and a nipple waterer. Temperature was initially set at 30°C and was gradually reduced to 25°C over the 6-week experimental period. Metal chains and plastic balls were provided in each pen for pigs to play with. All the animals in the experiment were cared for in accordance with the guidelines of the Canadian Council on Animal Care [21].

2.3. Formate formulation and administration

Four pigs (31-38 kg) in each group were administered sodium formate (351 mg/kg; 237 mg/kg formate base) while awake via a catheter surgically inserted previously in the left jugular vein. This level of formate was chosen based on 2 considerations: firstly, to attain plasma formate levels that will not cause overt toxicity and, secondly, to work with formate doses that do not exceed that used by previous workers [14,19]. The remaining 2 pigs in each group were administered sterile saline via the same vein. Formate infusion over a 10-minute period (109-133 mL solution in each animal depending on the weight of the animal) was carried out according to good practice guidelines [20-22]. Saline infusion (111-130 mL depending on body weight) was also performed in a similar manner. Sodium formate was prepared in isotonic sodium chloride solution and sterilized by autoclaving (AMSCO isothermal sterilizer, AMSCO, Erie, PA, operated at 17.1-19.5 psi at 121°C for 15 minutes) before use. Blood samples (~10 mL) were collected using sterile syringes coated with sodium heparin before and at 10, 20, 30, 45, 60, 90, 120, 180, 240, and 480 minutes post infusion. For plasma, samples were kept on ice during the collection period and centrifuged soon after sample collection was over for that period to remove the cells. The resultant plasma was then stored at −80°C until analysis. For red blood cell folate, whole blood samples were stored at 4°C till analysis. The animals were then recovered for other unrelated experimental work.

2.4. Formate analysis

A stock solution of 100 mmol/L formic acid was prepared in deionized water. Aqueous serial dilutions were made from the stock to produce solutions at 20, 10, 4, 2, 1, 0.5, 0.25, 0.125, and 0 mmol/L. A 10% (vol/vol) ethanol in deionized water was prepared as an internal standard. The working standards and ethanol were prepared fresh each time. Two linear calibration curves were constructed from the above solutions. One standard curve ranged from 0 to 4 mmol/L, for low-formate samples; and the other ranged from 0 to 20 mmol/L, for high-formate samples. Plasma samples (200 μL) and aqueous standards were each placed in a 1-mL glass vial. Aqueous ethanol (50 μL, 10% vol/vol) was added as internal standard and as a derivatizing agent. The formation of ethylformate was enhanced by addition to the reaction mixture of 200 μL concentrated sulfuric acid as a catalyst. The vials were then sealed immediately and placed in a water bath for 15 minutes at 60°C to allow the ethylformate formed to equilibrate in the headspace above the reaction mixture in the vial. One milliliter of this headspace gas was siphoned using a gastight syringe and injected into the gas chromatograph.

2.5. Instrumentation

Analysis of ethylformate (headspace analysis) was performed on a gas chromatograph fitted with a flame ionization detector (Series II 5890; Hewlett-Packard, Palo Alto, CA) and a DB-ALC1 capillary column (30 m × 0.53 mm internal diameter, 3.0 μm; J & W Scientific, Chromatographic Specialities, Brockville, Ontario, Canada). Separation was achieved by running the oven at 40°C isothermally, injector at 250°C, detector (flame ionization detector) at 300°C, carrier gas (helium measured at 40°C) at 80 cm/s, and detector gas (hydrogen) at 23 mL/min. Ethylformate peak eluted at 4.5 minutes, whereas ethanol (internal standard) eluted at 2.9 minutes.

2.6. Data analysis

Integration of the internal standard peak (ethanol) and ethylformate was carried out using HP 3365 Series II Chemstation (version A.03.21; Hewlett-Packard, 1989-1992). Quantitation of the ethylformate was completed by plotting the peak area ratios of ethylformate to ethanol against the calibration concentrations using Chemistry Software for Windows software (ChemSW, Fairfield, CA 1993-1998). Linearity of the 2 calibration curves was established in a similar manner. Linear regression analysis of all standard curves produced $r^2 > 0.999$. Assay limit of quantification was 0.026 mmol/L, and intraassay and interassay accuracy and precision had coefficient of variation values <10%. Quality control samples at low,

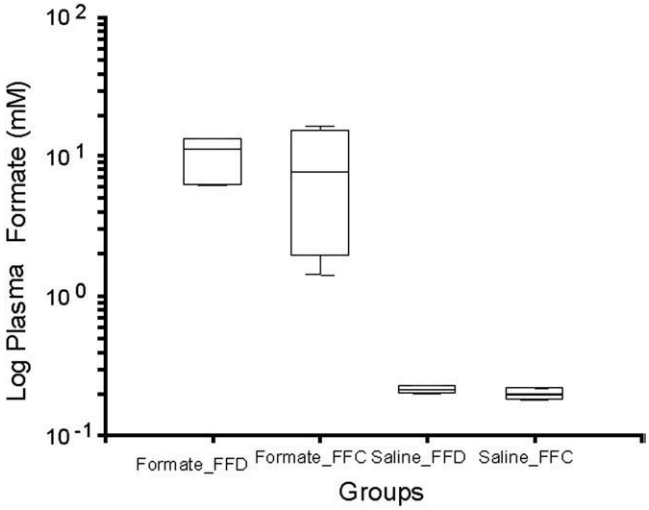

Fig. 1. Box plot of plasma formate levels (logarithmic scale) in FFD and FFC male pigs (n = 4, 31-38 kg) administered 351 mg/kg sodium formate (Formate_FFD and Formate_FFC) and normal saline (Saline_FFD and Saline_FFC). Animals were given a short intravenous infusion (10 minutes) via the left jugular vein.

medium, and high concentrations were analyzed in duplicate for each run as acceptance criteria for the analysis.

Mean ± SE plasma formate levels with time for the 2 groups were plotted using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA.). Group means were compared using a 2-sample equal-variance t test with $P < .05$. This was done after confirmation of equal variance in the data among the groups by modified Levene equal-variance test ($P = .068$). Pharmacokinetic modeling of the data was accomplished using a noncompartmental analysis with WinNonlin version 5.1 (Pharsight, Mountain View, CA.).

3. Results

To reach folate deficiency status, the designated group (FFD) of pigs was fed a folate-deficient diet for 6 weeks. Folate deficiency was successfully induced in this group at the end of 6 weeks as confirmed by plasma folate levels (26 ± 5 nmol/L, mean ± SE), which were at nadir (<30 nmol/L) and statistically different from the folate control diet–fed pigs (100 ± 25 nmol/L, $P = .007$). To determine whether folate deficiency altered formate pharmacokinetics, pigs were administered formate or saline via a short intravenous infusion. At the end of the infusion, plasma formate levels rose from a baseline value of 0.16 ± 0.01 mmol/L (mean ± SE) in folate deficiency (FFD) and 0.12 ± 0.05 mmol/L in folate control (FFC) to a maximum concentration (concentration at end of infusion) of 9.63 ± 0.57 and 10.04 ± 0.71 mmol/L, respectively. Formate levels in saline-administered animals did not rise significantly (Fig. 1). The FFD pigs accumulated more formate than the FCC pigs, as indicated by the ~2.5-fold higher area under the curve (AUC) values of FFD pigs relative to the FFC pigs (Fig. 2 and Table 2). The plot of formate concentration as a function of time indicated first-order elimination kinetics for both folate-replete and folate-deficient groups (Fig. 2). The slope of the log-linear terminal phase was much shallower and statistically different ($P = .005$) in folate-deficient pigs (FFD) (Fig. 2). The half-life ($t_{1/2}$) of formate in FFD pigs was approximately 2.5-fold higher relative to that in FFC pigs. Systemic clearance of formate was about 44% lower in FFD than FFC pigs, but volume of distribution under steady state (V_{SS}) remained approximately 18 L in both groups.

4. Discussion and conclusion

In the present study, formate levels increased ~70-fold after formate administration relative to the saline controls (Fig. 2). The amount of formate administered (237 mg formate per kilogram) and the resultant formate levels have not been associated with overt metabolic crisis [14,19,23-25], although measures to assess academia such as blood pH and plasma bicarbonate were not taken in these animals. No physical distress was observed in our animals during the experiment; that is, signs of acute intoxication such as mild central nervous system depression, tremors, ataxia, and recumbency were not observed. This is consistent with Dorman et al [23], Makar et al [14], and Tephly et al [19], who reported only minimum metabolic acidosis or bicarbonate depletion after administered doses of 425 or 500 mg/kg sodium formate (338 mg/kg formate).

Folate deficiency resulted in a significant decrease (55%) in the systemic clearance of formate but had no affect on the volume of distribution (~18 L, 0.53L/kg, $P = .377$). Hence, the significantly lower elimination rate constant (2.5-fold) and longer half-life (2.5-fold) in folate-deficient pigs were due to changes in systemic clearance alone. This is consistent with folate's critical role in formate metabolism. Consequently, folate-deficiency state results in higher

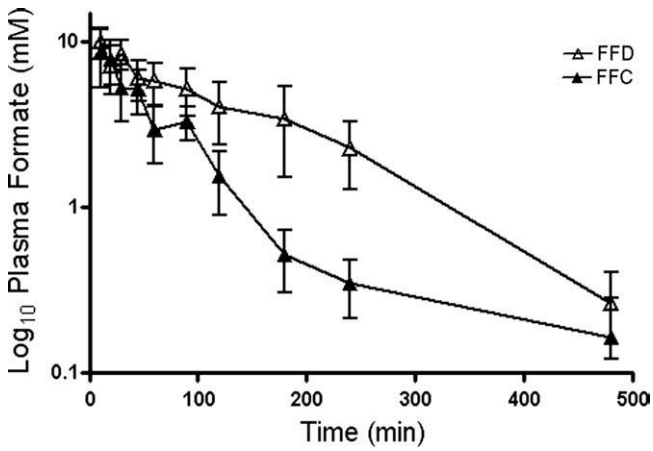

Fig. 2. Semilogarithmic plot of plasma formate (mean ± SE) concentration (in millimoles per liter) vs time plot of FFD and FFC male pigs (n = 4, 31-38 kg). Animals were given a short intravenous infusion (10 minutes) via the left jugular vein with 351 mg/kg sodium formate (237 mg/kg formate).

Table 2
Pharmacokinetic parameter estimates of formate in FFD and FFC male pigs

Parameter	Units	FFD ± SE	CV (%)	FFC ± SE	CV (%)	*P* value
Cend-inf	mmol/L	9.63 ± 0.57	5.87	10.04 ± 0.71	7.04	.6680
Cend-inf	mg/L	443 ± 26	5.88	462 ± 32	7.04	.6610
AUC	g min/L	72.37 ± 8.29	11.40	30.08 ± 2.58	8.58	.0028
K	min	0.0061 ± 0.0009	14.90	0.0154 ± 0.0020	12.88	.0054
$t_{1/2}$	min	113 ± 17	14.90	45 ± 6	12.85	.0093
MRT	min	163 ± 24	14.90	65 ± 8	12.80	.0082
Cl_S	L/min	0.12 ± 0.01	11.50	0.27 ± 0.02	8.59	.0005
V_{SS}	L	18.84 ± 1.05	5.89	17.21 ± 1.35	7.04	.3770

Pigs (31-38 kg) were administered 351 mg/kg sodium formate (237 mg/kg formate, n = 4 per group) via intravenous infusion (10 minutes) into the left jugular vein. Blood samples were collected over an 8-hour period. Values with the exception of coefficient of variation (percentage) represent mean ± SE. CV indicates coefficient of variation; AUC, area under the curve; K, elimination constant; $t_{1/2}$, elimination half-life; Cend-inf, concentration at end of infusion; Cl_S, systemic clearance; MRT, mean residence time; V_{SS}, volume of distribution under steady state.

formate exposure as observed by the 2.5-fold higher AUC and mean residence time (MRT) values in FFD pigs relative to FFC pigs (72.37 ± 8.29 vs 30.08 ± 2.58 g min/L and 163 ± 24 vs 65 ± 8 minutes, respectively). Mean residence time is the average total time molecules of a given dose spend in the body. Thus, MRT is another measure of the rate of elimination of a xenobiotic.

Increased formate exposure can lead to metabolic acidosis, free radical damage, and neurotoxicity [8,25-29]. Formaldehyde, a highly reactive intermediary metabolite of methanol and precursor of formic acid, can combine with a variety of cellular constituents forming relatively stable adducts [8]. For example, formaldehyde combines with glutathione to form *S*-formylglutathione. This may significantly alter glutathione levels, leading to reduced antioxidant capacity. The formic acid molecule dissociates completely at physiological pH into its conjugate base, formate, and a hydrogen ion. Formate distributes freely into total body water and is found in blood and cerebrospinal fluid. The increased amounts of hydrogen ions formed as a result of the dissociation eventually overwhelm the normal acid-base balance of the body, resulting in acidosis. Formate is also neurotoxic, leading to edema and atrophy of the optic nerve (resulting in permanent vision loss). Damage to the putamen of the basal ganglia of the brain (the area that controls gross intentional motor activities that are normally performed unconsciously) is often seen in autopsies of victims of methanol poisoning. These adverse effects are reportedly associated with prolonged blood formate elevation (>7 to 10 mmol/L) of greater than 24 hours. Although endogenous levels of formate are low, ranging form 0.12 to 0.28 mmol/L [30], it is conceivable to obtain much higher levels because of exposure from various sources (as a result of extensive use of formate in industry) in folate-deficiency state. Other high-folate–demand conditions such as pregnancy, disease, malnutrition, and convalescence [13] might also aggravate folate deficiency because of high folate demand and utilization.

Our observations of formate kinetics as demonstrated by $t_{1/2}$ in normal pigs seem to support that of other workers (Table 3) with the exception of Vss. The Vss obtained of approximately 18 L (0.53 L/kg) is much lower than that observed by others. For example, Hanzlik et al [32] found the Vss of formate in human female subjects given an oral dose of 3900 mg calcium formate to be 2.36 L/kg. By extrapolating the kinetic plots (lnC vs t) of Clay et al [33] to time 0 and dividing by the dose, Hanzlik and colleagues calculated the dose-independent Vss of formate as 2.25 L/kg. They also obtained a Vss of 2.58 L/kg by similar extrapolation of the kinetic plot of Eells et al [34]. This difference between our value and that of others is probably due to the fact that Hanzlik et al [32] and Eells et al [34] obtained Vss based on oral and intraperitoneal administration of formate. Thus, such an estimate of Vss is confounded by formate bioavailability (ie, the estimate is actually Vd/F).

Table 3
Formate elimination half-life in various species

Animal	Minipig [a]	Outbred swine [b]	Micropig [b]	Human [c, d]	Rat [e]	Monkey [f]	Human [g]
Formate $t_{1/2}$ (min)	50-112	87 ± 18	74.1 ± 6	69-120	12-23	31-51	205±90

Values in columns with footnotes *a*, *d*, *e*, *f*, and *g* represent ranges, whereas values in columns with footnote *b* represent mean ± SE.

[a] Data from Dorman et al [23].
[b] Data from Makar et al [14].
[c] Data from McMartin et al [29].
[d] Data from Hanzlik et al [32].
[e] Data from Clay et al [33].
[f] Data from McMartin [28].
[g] Data from Kerns et al [31].

Furthermore, the lack of the formate bioavailability in their work and in the literature leaves much speculation on how our value for Vss compares with others. Another possible reason for this difference may be due to our short-term infusion (~10 minutes). As a result, there may be certain error associated with a Vss derived in such a manner. The intravenous infusion over such a time (~10 minutes) was necessary to address concerns of formate toxicity that might arise in the folate-deficiency animals (351 mg/kg sodium formate administered in a 109- to 133-mL solution to each animal depending on the weight) as well as to comply with safe infusion guidelines for such animals [22].

We have demonstrated that folate deficiency significantly reduces systemic formate clearance, resulting in increased risk of formate toxicity in folate-deficient pigs. The experimental model described in this study (of folate-deficient pig) can serve as the basis of further research on modulation of effects of formate toxicity in folate deficiency in humans. The use of swine as an experimental model for formate toxicity in folate deficiency offers huge cost savings compared with the use of primates as has been done in most of the formate/methanol toxicity studies. Although strain as well as individual differences might exist in hepatic tetrahydrofolate levels in pigs (Table 1), they are significantly lower than those found in monkeys. Consequently, formate elimination is slower (Table 3). Our findings of the suitability of swine as a model for formate toxicity is consistent with those of Makar et al [14]. Dorman et al [23], however, found that swine was not a suitable model. This inconsistency can be explained by the fact that the animals used by Dorman et al were far younger than ours (8.5-13 kg compared with 31-38 kg). Their control animals also had much higher hepatic tetrahydrofolate levels than those of other workers such as Makar et al [14]. Thus, a true "folate-deficiency" state might not have been attained before methanol was administered. Further research is needed to elucidate the effects (subclinical and clinical) of formate during folate deficiency in tissues and biochemical parameters, and how this relates to disease manifestations.

Acknowledgment

The authors appreciate and recognize the contribution of Ms Rachel Kuehn toward the care and feeding of the research animals. The support of Natural Sciences and Engineering Research Council of Canada (JDH and GAZ) is gratefully acknowledged.

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