ABSORPTION AND ELIMINATION OF FORMATE FOLLOWING ORAL ADMINISTRATION OF CALCIUM FORMATE IN FEMALE HUMAN SUBJECTS

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ABSTRACT:

Calcium formate is a water-soluble salt of an essential mineral nutrient with potential for use as a dietary calcium supplement. Formate ion is a product of endogenous and xenobiotic metabolism, but sustained high plasma formate concentrations (such as occur in cases of methanol poisoning) are toxic to the retina and optic nerve. Humans and primates have reduced capacity for formate oxidation compared with rodents and dogs and are thus more sensitive to methanol (and formate) intoxication. To assess the potential for accumulation of formate ion upon repeated administration of calcium formate as a potential dietary calcium supplement, we measured plasma concentrations of formate in 14 adult human subjects before and after oral administration of a single large dose of calcium formate (3900 mg; ca. 3–6 times the anticipated dose for calcium supplementation). Plasma formate concentrations increased briskly from 0.024 ± 0.008 mM (endogenous formate) to reach C_{\text{max}} (0.50 ± 0.04 mM) at 60 min postdose and then declined with a half-life of 59 ± 7 min. By 225 min postdose, plasma formate concentration had returned to baseline. With such a short half-life, repeated use of calcium formate as a dietary supplement, even three times daily, should not lead to progressive accumulation of formate. These findings are discussed in light of the production of formate by endogenous and xenobiotic metabolism and the kinetics of formate during methanol poisoning.

Calcium formate is a soluble salt of an essential mineral nutrient that is deficient in many modern diets (Levenson and Vockman, 1994; Porter, 2003). Formic acid and its conjugate base formate are essential endogenous one-carbon metabolites in most living organisms. The fact that calcium formate contains 30.8% calcium by weight, whereas the remainder is an endogenous substance, has made it potentially attractive for use as a dietary calcium supplement (DeLuca, 2003). Formic acid and the formate ion participate in the one-carbon pool of intermediary metabolism (Cook et al., 2001; Fu et al., 2001). For this reason, their metabolism and disposition has been extensively studied in animals, but more recently they have also received considerable attention because of their role in the human toxicity of methyl alcohol and especially its effects on the visual system (Johlin et al., 1987; Makar et al., 1990; Liesivouri and Savolainen, 1991; Tephly, 1991; Cook et al., 2001).

As discussed below, rodents and dogs metabolize formate much more efficiently than primates or humans and are thus not good models for studying formate kinetics in humans. In view of the fact that some dietary supplements are taken several times per day on a chronic basis, and the notorious connection of sustained high plasma formate concentrations to the toxic effects of methanol poisoning (discussed further below), it becomes important to examine the absorption and elimination of formate from high-load oral calcium formate in human subjects to assess the potential for accumulation of formate upon repeated dosing. In this article, we present the results of such a study, as well as an analysis of previous literature on the disposition of formate in animals and humans. The purpose of the latter is to provide perspective on important distinctions between the role of formate in endogenous metabolism and nutrition versus its disposition and toxicological actions when present in great excess for sustained periods, such as occurs in cases of methanol poisoning.

Materials and Methods

Subjects. This study of formate absorption and elimination was conducted as part of a larger study (manuscript in preparation) of the absorption of calcium from calcium formate compared with calcium citrate and calcium carbonate. This overall study was patterned after an earlier study of sodium formate in human subjects (Malorny, 1969) and on a preliminary study (unpublished results, Nephro-Tech 1, LLC) of oral calcium formate (4550 mg) in six healthy adult male volunteers. In the present study, the subjects were 14 normal, healthy adult females between 19 and 33 years of age. Their body mass was between 51 and 93 kg (64.5 ± 11.2, mean ± S.D., n = 14), and they were free of diabetes, hyperparathyroidism, thyroid excess or other endocrine disorder, bone disease, kidney stones, nephrolithiasis, renal disease, peptic or duodenal ulcer, bowel disease, intestinal resection or malabsorption, regional enteritis, chronic diarrheal conditions, and liver disease. In addition, none of the subjects were pregnant or breastfeeding. At no time during the study or the 7 days preceding it did any of the subjects take vitamin or mineral supplements, anticonvulsants, diuretics, steroids (other than oral contraceptives), bisphosphonates [e.g., alendronate sodium (Fosamax)], or other medications...
that could affect calcium or vitamin D metabolism. The study protocol was approved by the Institutional Review Board of the Heart of America Research Institute (Kansas City, Kansas), and signed written informed consent was obtained from each subject before the study began.

Study Design. On different days separated by at least 48 h, each subject ingested either a placebo (methyl cellulose tablets) or a single oral dose of calcium formate administered as six capsules of 650 mg of calcium formate (3900 mg total; 30 mmol of calcium, 60 mmol of formate). The placebo and calcium formate tablets were custom formulated by Opti-Med Inc. (Seymour, IN). On each study day, subjects arrived at the clinic prior to 8:00 AM after having fasted for ca. 10 h overnight. Between 8:00 and 9:00 AM, each subject ingested either the placebo or the calcium formate tablets along with a minimum of 240 ml (8 ounces) of water. During the 4.5 h subsequent to tablet ingestion, subjects were allowed water ad libitum, but no other beverage or food was allowed until after the last blood sample was collected. Venous blood samples for determination of plasma formate were taken immediately prior to ingestion of placebo or calcium compound (time 0) and at 30, 60, 90, 135, 180, 225, and 270 min postdose. Blood samples were collected in evacuated tubes, allowed to clot, and centrifuged at 2000 rpm for 5 min. The supernatant was drawn off carefully and deproteinized by the addition of 30 ml of 7.5% ZnSO4 per 100 ml of serum followed by brief centrifugation at 17,000 rpm. Formate concentrations were determined on this supernatant using the fluorometric assay of Makar and Tephly (1982).

Pharmacokinetic analyses were performed using Microsoft Excel X. Elimination rate constants (k1) were calculated as minus the slope of a plot of InC versus time and half-lives (t1/2) from the relationship t1/2 = ln2/k1. AUC values were computed by trapezoidal integration. Oral clearances and apparent volumes of distribution were calculated as (CL/F) = dose/ΔAUC and (Vd/F) = (CL/F)/k1, respectively, where ΔAUC refers specifically to the increment in formate AUC above the baseline area (Table 1, placebo). Results are reported as mean ± S.E. (n = 14).

Results and Discussion

The concentrations of formate in serum from venous blood samples drawn immediately prior to oral administration of a placebo or 3900 mg of calcium formate were 0.020 ± 0.0010 and 0.028 ± 0.0013 mM, respectively. After placebo, serum formate concentrations remained relatively constant to 270 min (Fig. 1A), whereas after calcium formate, they rose to an average Cmax of 0.50 ± 0.04 mM by around 60 min postdose (Fig. 1B and Table 1). After peaking at 60 min, serum formate concentrations declined monoexponentially with an average half-life of 59 ± 7 min and returned to baseline values by 225 min post. During the first 225 min after dosing, the AUC for total formate was 49.4 ± 6.2 mmol·min, but of this 8.7 ± 2.0 mmol·min is attributable to endogenous formate (i.e., placebo, Table 1). Thus, the net additional exposure (ΔAUC0–225) resulting from oral

![FIG. 1. Plots of plasma formate concentration versus time for 14 adult human female subjects following oral administration of (A) placebo (methyl cellulose) or (B) calcium formate (3900-mg total). Derived pharmacokinetic data are reported in Table 1.](image-url)
administration of 3900 mg of calcium formate was 41.2 ± 5.8 mM · min. From this and the dose of formate given (60 mmol/subject), CL/F is 1.95 ± 0.31 l/min, and from this and the apparent elimination rate constant, the Vₜ/F is 156 ± 27 liters or 2.36 ± 0.38 l/kg (Table 1).

There are few data in the literature to which the above values may be compared. In an earlier study of sodium formate administered orally to two subjects, Malorny (1969) reported more rapid formate absorption with peak plasma concentrations occurring less than 30 min postdose. It is likely that the more rapid absorption of sodium formate is related to its greater water solubility and greater degree of ionic dissociation compared with calcium formate. In the two individuals studied by Malorny, the half-lives of formate were 45 and 46 min, which is only slightly faster than we observed. After i.v. administration of sodium formate to monkeys, Clay et al. (1975) observed that the half-life of formate increased from 31 to 51 min with increases in dose size (0.73–6.91 mmol/kg). This may indicate that metabolic half-life of formate increased from 31 to 51 min, which is only slightly faster than we observed. After i.v. administration of calcium formate was 41.2 ± 5.8 mM · min. From this and the dose of formate given (60 mmol/subject), CL/F is 1.95 ± 0.31 l/min, and from this and the apparent elimination rate constant, the Vₜ/F is 156 ± 27 liters or 2.36 ± 0.38 l/kg (Table 1).

On the other hand, the exhalation of 14CO₂ accounts for up to 80% of administered doses of 14C-formate with most of this being recovered in the first few hours, even after oral administration (Sperling et al., 1953; Friedmann et al., 1954; Malorny, 1969) and is limited by a renal transport system that recovers formate and a proton in exchange for chloride and sodium (Karniski and Aromson, 1985). On the other hand, the exhalation of 14CO₂ accounts for up to 80% of administered doses of 14C-formate with most of this being recovered in the first few hours, even after oral administration (Sperling et al., 1953; Friedmann et al., 1954; Malorny, 1969) and is limited by a renal transport system that recovers formate and a proton in exchange for chloride and sodium (Karniski and Aromson, 1985). Extensive evidence indicates that excessive production of formic acid is produced by the catabolism of several amino acids including serine, glycine, histidine, and tryptophan and by the recycling of methylthioadenosine from the polyamine biosynthesis pathway (Cook et al., 2001; Fu et al., 2001). It is also produced by the oxidation of formaldehyde generated during cytochrome P450-catalyzed N- and O-demethylation reactions (Keef et al., 1987) and during the hydrolysis of certain produgs containing acyloxymethyl substituents as protecting groups, such as Fosphenytoin (Stella, 1996) and its analogs (Pozzo and Acquasaliente, 1992). Carbon atoms from formate or formaldehyde are interconvertible via oxidation or reduction, respectively, of the cofactors CH₃-H₄PteGlu₅ and HCO-H₄PteGlu₅, which donate single carbon atoms used in pyrimidine and purine biosynthesis (Fu et al., 2001). In addition to its utilization for biosynthesis, formate is also oxidized to CO₂ and water, primarily by the action of 10-formyltetrahydrofolate dehydrogenase on HCO-H₄PteGlu₅ but under some conditions by at least two other pathways including erythrocyte catalase (Cook et al., 2001). Oxidation to CO₂ is the primary means of eliminating formate that is not utilized biosynthetically.

Another formate precursor, methanol, is produced endogenously and is present in human blood at concentrations around 1 mg/liter (range 0.3–2.5 mg/liter) (Osterloh et al., 1996). Dietary sources of methanol include fruit juices (average 140 mg of methanol/liter; range 12–640 mg/liter) and fermented beverages which can contain up to 1.5 g of methanol/liter (Stegink et al., 1983; Kavat and Nauss, 1990). Consumption of large quantities of methanol by humans or nonhuman primates leads to toxic consequences including metabolic acidosis, visual disturbances progressing to permanent blindness, and death (Tong, 1982; Sejersted et al., 1983; Hantson and Mahieu, 2000; Timbrell, 2000; Fox and Boyes, 2001; Barceloux et al., 2002). The toxic dose of methanol is estimated to be between 0.3 and 1.0 g/kg. Extensive evidence indicates that excessive production of formic acid leads to metabolic acidosis and elevated blood formate ion concentrations which can reach 10 to 20 mM or more in severe human cases (Tong, 1982; Sejersted et al., 1983; Hantson and Mahieu, 2000; Timbrell, 2000; Fox and Boyes, 2001; Barceloux et al., 2002).

The conversion of formate to HCO-H₄PteGlu₅ consumes 1 mol of ATP per mol formate. As a moderate inhibitor of cytochrome c oxidase (Kᵣ = 6 mM) (Erecinska and Wilson, 1980), formate impairs tissue utilization of oxygen (Tong, 1982; Timbrell, 2000) resulting in
excess lactic acid production (which exacerbates the acidosis due to formic acid) and to depletion of ATP and ADP in retinal tissue and presumably other tissues as well (Seme et al., 2001). Persistence of these changes during the protracted metabolism of larger quantities of ingested methanol can lead to extensive damage to the pancreas and kidney as well as the retina and optic nerve. Neurons in the retina and optic nerve appear to be particularly sensitive to prolonged elevations in serum formate concentrations. However, clinical case reports and experimental investigations in nonhuman primates suggest that for irreversible damage to occur, formate concentrations in blood must exceed 7 mM for at least 24 h (Kavat and Nauss, 1990; Eells, 1992; Eells et al., 1996, 2000).

Nonprimates including mice, rats, rabbits, and dogs are resistant to methanol intoxication. In contrast, humans and nonhuman primates are uniquely sensitive to methanol poisoning. The primary reason for this appears to be the much greater ability of nonsusceptible species to eliminate the methanol metabolite formate. Thus, in humans and nonhuman primates, formate accumulates to much greater than endogenous concentrations, but it does not accumulate in resistant species (mice, rats, and rabbits). The species difference in formate accumulation is attributed to differences in hepatic tetrahydrofolate status. Hepatic tetrahydrofolate concentrations are significantly greater in rodents than in monkeys and humans (Eells et al., 1982; Black et al., 1985; Johlin et al., 1987). Furthermore, treatments which alter the levels of hepatic tetrahydrofolate modify formate metabolism and the toxic response to methanol. In rats, folate deficiency or selective reduction of tetrahydrofolate results in formic acidemia and metabolic acidosis following methanol administration, whereas no accumulation of formate or alteration in blood pH or bicarbonate is observed in control rats (Palese and Tophy, 1975; Eells et al., 1981, 1982; Makar and Tophy, 1982; Black et al., 1985; Johlin et al., 1987). Recent studies have also demonstrated ocular toxicity in folate-deficient monkeys (Lee et al., 1994) and tetrahydrofolate-deficient rats (Eells et al., 1996, 2000; Seme et al., 1999). In monkeys, folate deficiency or selective reduction of tetrahydrofolate increases formate accumulation and potentiates methanol toxicity, whereas treatment with folate derivatives decreases formate accumulation and can prevent or reverse the development of the methanol poisoning syndrome (Noker et al., 1980; Eells et al., 1983). In addition, administration of buffered sodium formate to monkeys, which like humans metabolize formate slowly, causes changes identical to those seen in methanol poisoning (optic disk edema; loss of pupillary response to light) but without the acidosis associated with rapid metabolism of methanol to formic acid (Martin-Amat et al., 1978).

In summary, the absorption of formate from oral calcium formate is rapid, but not as rapid as from sodium formate. After a large oral load of calcium formate, serum formate concentration peaks transiently around 60 min postdose and declines rapidly thereafter with a half-life of 59 min. Thus, the increments in serum formate concentration and formate AUC resulting from a large single oral dose of calcium formate were very much lower than those associated with methanol poisoning. Furthermore, the rate of elimination of serum formate in humans is sufficiently rapid to prevent its accumulation during chronic use of calcium formate as a dietary calcium supplement.

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