Effect of Folic Acid on the Pharmacokinetics of Acutely Administered Phenytoin in Pregnant and Nonpregnant Rats

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Abstract □ The concentrations of both total and free phenytoin in the plasma of epileptic women tend to decrease during pregnancy, suggestive of a pregnancy-associated increase in the metabolic clearance of the drug. On the other hand, the metabolic clearance of free (unbound) phenytoin decreases during pregnancy in rats. One possible reason for this species difference is the routine dietary supplementation of folic acid in human pregnancy and the apparent ability of folic acid to lower phenytoin plasma concentrations even in nonpregnant humans. The purpose of this investigation was to determine the effect of treatment with folic acid on the pharmacokinetics of phenytoin in pregnant and female nonpregnant rats. In one experiment, the treated animals received folic acid in the drinking water, ~100–150 μg/kg/d, for 19 d. There was no apparent difference between the treated and untreated rats in the pharmacokinetics of a 10-mg/kg iv dose of phenytoin (which was administered to the pregnant rats on the 20th day of gestation), regardless of pregnancy status. In another experiment, pregnant and female nonpregnant rats received either folic acid, 400 μg/kg/d, or an equal volume of the solvent only, by gastric intubation for 19 d. The next day (which was the 20th day of gestation for the pregnant rats), the animals received an intravenous injection of phenytoin, 30 mg/kg. Again, pretreatment with folic acid had no apparent effect on the pharmacokinetics of phenytoin in both pregnant and nonpregnant rats. However, the results of this investigation confirm previous observations of dose-dependent phenytoin pharmacokinetics in rats and of decreased clearance of free phenytoin in late pregnancy. It is possible that the qualitative difference between humans and rats with respect to the effect of pregnancy on the pharmacokinetics of phenytoin may be due, at least in part, to a species difference in response to folic acid.

Keyphrases □ Pharmacokinetics—acutely administered phenytoin, folic acid, pregnant and nonpregnant rats □ Phenytoin—acutely administered, effect of folic acid on, pregnant and nonpregnant rats □ Folic acid—effect on acutely administered phenytoin, pregnant and nonpregnant rats

Long term use of phenytoin by epileptic patients is associated with decreased concentrations of folic acid or folate activity in serum, erythrocytes, and cerebrospinal fluid (1–3). Decreased folate activity has also been observed during pregnancy in nonepileptic women who are not taking phenytoin or other drugs (4, 5). Pregnant women taking phenytoin are, therefore, considered to be at particular risk of folate deficiency, and it is customary to give them supplementary folic acid.

Plasma concentrations of total and free phenytoin tend to decrease in women during pregnancy (6). The occurrence of decreased concentrations of free phenytoin suggests that the intrinsic metabolic clearance of the drug is increased in human pregnancy. The opposite is found in rats, *i.e.*, the metabolic clearance of free phenytoin is decreased during pregnancy (7). The reasons for this unusual, qualitative, apparent species difference are unclear (7), particularly since both humans and rats eliminate phenytoin primarily by the same metabolic pathway, *i.e.*, *p*-hydroxylation. One possibility is that it may be related to the dietary supplementation of folate in pregnant women. A number of studies on nonpregnant human subjects have shown that administration of folic acid can cause phenytoin plasma concentrations to decrease (8–11). The question arises, therefore, whether folate administration to rats will alter the pharmacokinetics of phenytoin in these animals, particularly during pregnancy.

EXPERIMENTAL SECTION

Female inbred Lewis rats were used in this investigation. Some of the animals were mated while others were designated as nonpregnant controls. The rats were then housed individually in suspended wire cages to prevent or minimize coprophagy. In one experiment, some of the pregnant and nonpregnant animals had folic acid and sodium bicarbonate, 1 μg/mL each, added to the drinking water while the others received drinking water containing only sodium bicarbonate. After 10 d, the folic acid concentration in the drinking water of pregnant animals was increased to 1.2 μg/mL in case these rats did not increase their water intake in proportion to their increased body weight. The total period of folic acid administration was 19 d; on the 20th day (day 20 of gestation of the pregnant rats) the rats received an intravenous injection of phenytoin, 10 mg/kg. Venous blood samples were collected periodically for determination of plasma phenytoin concentrations by HPLC. A large volume of blood was obtained from the abdominal aorta at the end of the experiment for determination of the plasma free fraction of phenytoin by immediate ultrafiltration at 37°C. The anticoagulant was heparin for the serial blood samples intended for phenytoin assay and EDTA for the large sample intended for plasma ultrafiltration.

In another experiment, some of the pregnant and nonpregnant rats received folic acid, 400 μg/kg as a 200 μg/mL freshly prepared aqueous solution containing 200 μg/mL sodium bicarbonate, daily for 19 d by gastric intuba-

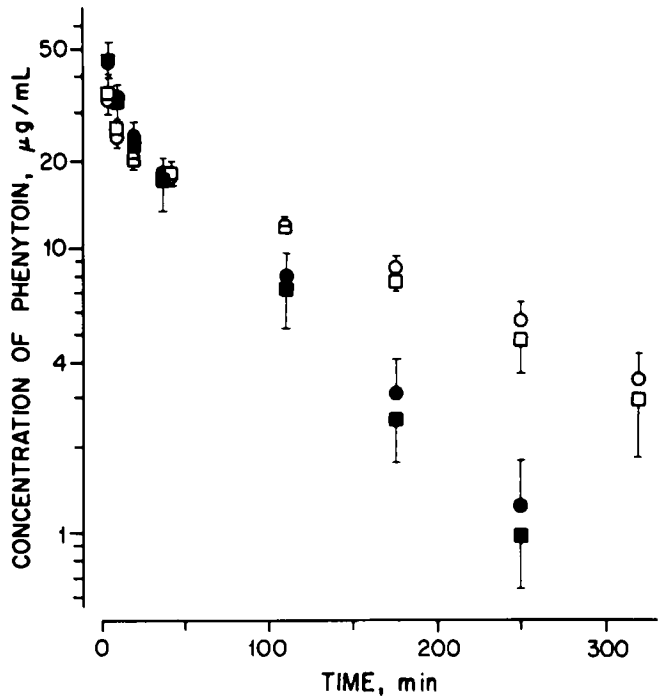

Figure 1—*Effect of pretreatment with folic acid (400 μg/kg/d orally for 19 d) on the pharmacokinetics of a 30-mg/kg iv dose of phenytoin in pregnant (day 20 of gestation) (open symbols) and nonpregnant (solid symbols) rats. Key: (□, ■) folic acid pretreated rats; (○, ●) rats that did not receive supplementary folic acid. Mean of 5 animals per group; vertical bars indicate + or − SD.*

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Table I—Effect of Treatment with Folic Acid on the Pharmacokinetics of a 10 mg/kg Dose of Phenytoin in Pregnant (Day 20 of Gestation) and Female Nonpregnant Inbred Lewis Rats [a]

Variable	Pregnant		Nonpregnant	
	Folic Acid Treated	Untreated	Folic Acid Treated	Untreated
Number of animals	4	3	7	6
Body weight, g	301 ± 31	303 ± 23	246 ± 17	229 ± 24
Hematocrit, %	37.1 ± 2.5	36.5 ± 1.5	44.4 ± 2.0	45.0 ± 2.1
Total plasma clearance, mL/min	4.90 ± 0.48	4.98 ± 0.35	3.85 ± 1.02	4.24 ± 0.63
mL/min/kg	16.3 ± 1.0	16.5 ± 0.9	15.7 ± 4.3	18.5 ± 1.9
Apparent volume of distribution, mL	297 ± 66	309 ± 100	180 ± 25	169 ± 22
mL/kg	979 ± 119	1009 ± 245	733 ± 94	745 ± 116
Plasma free fraction, %	21.7 ± 4.2	20.2 ± 4.5	13.5 ± 1.1	15.3 ± 2.5
Apparent volume of distribution of free drug, mL/kg	4594 ± 711	4987 ± 186	5419 ± 394	4949 ± 1008
Half-life, min	41.9 ± 6.6	42.7 ± 11.6	33.8 ± 6.9	28.1 ± 4.9
Free plasma clearance, mL/min	22.9 ± 2.4	25.3 ± 4.0	28.4 ± 7.0	27.9 ± 4.0
mL/min/kg	77.2 ± 14.9	84.4 ± 18.9	116 ± 29	123 ± 21

[a] Results are reported as mean ±*SD*. The treated animals received approximately 100–150 μg/kg/d of folic acid in drinking water for 19 d before the experiment. There were no statistically significant differences between treated and untreated pregnant or nonpregnant animals.

Table II—Effect of Treatment with Folic Acid on the Pharmacokinetics of a 30 mg/kg Dose of Phenytoin in Pregnant (Day 20 of Gestation) and Female Nonpregnant Inbred Lewis Rats [a]

Variable	Pregnant		Nonpregnant	
	Folic Acid Treated	Untreated	Folic Acid Treated	Untreated
Number of animals	5	5	5	5
Body weight, g	278 ± 11	281 ± 6	226 ± 5	222 ± 10
Hematocrit, %	36.5 ± 1.7	35.6 ± 1.8	44.9 ± 1.8	45.4 ± 1.6
Total plasma clearance, mL/min	2.13 ± 0.20	2.02 ± 0.13	2.79 ± 0.69	2.47 ± 0.37
mL/min/kg	7.64 ± 0.64	7.18 ± 0.54	12.3 ± 2.8	11.1 ± 1.7
Apparent volume of distribution, mL	339 ± 81	356 ± 41	199 ± 41	195 ± 23
mL/kg	1210 ± 257	1268 ± 147	878 ± 163	874 ± 91
Plasma free fraction, %	24.3 ± 3.7	23.9 ± 3.1	15.3 ± 1.8	14.8 ± 1.4
Apparent volume of distribution of free drug, mL/kg	4958 ± 478	5368 ± 898	5769 ± 1126	5898 ± 400
Half-life, min	111 ± 31	123 ± 21	49.7 ± 2.8	55.5 ± 9.5
Free plasma clearance, mL/min	8.96 ± 1.79	8.57 ± 1.43	18.4 ± 4.8	16.7 ± 2.7
mL/min/kg	32.3 ± 6.9	30.5 ± 5.3	81.0 ± 19.3	75.2 ± 11.6

[a] Results are reported as mean ±*SD*. The treated animals received 400 μg/kg/d of folic acid by gavage for 19 d before the experiment. There were no statistically significant differences between treated and untreated pregnant or nonpregnant animals.

tion. The other animals received only the solvent. On the 20th day (day 20 of gestation of the pregnant rats), a single intravenous injection of phenytoin, 30 mg/kg, was administered, and blood samples were collected periodically for assay and a large terminal sample was collected for ultrafiltration. All technical details of the pharmacokinetic study were identical to those already described (7).

RESULTS

The nonpregnant rats consumed 26.8 ± 2.8 (mean ± *SD*) mL/d of folic acid solution (equivalent to 109 ± 15 μg/kg/d of folic acid), 28.0 ± 2.4 mL/d of sodium bicarbonate solution, or 28.3 ± 3.3 mL/d of pure water (the latter determined in rats not used in the pharmacokinetic study). The pregnant rats consumed increasingly larger volumes of water with increasing gestation time: 16.3 ± 3.3, 23.5 ± 3.0, and 36.8 ± 6.3 mL/d on the 4th, 10th, and 15th day of gestation, respectively. These are combined figures for folic acid and sodium bicarbonate solutions since there were no apparent differences between the two. The average dose of folic acid ingested with the drinking water was 151 ± 21 μg/kg/d at the end of gestation.

The results of the pharmacokinetic study with the 10 mg/kg dose of phenytoin are summarized in Table I. There was no statistically significant difference between the folic acid treated and untreated rats in any of the parameters that describe the pharmacokinetics of phenytoin, including the plasma protein binding. This applies to pregnant and to nonpregnant rats, considered separately. A comparison of phenytoin pharmacokinetics in untreated pregnant and nonpregnant rats, with a larger number of animals per group, has been reported (7).

The results of the pharmacokinetic study with a 30 mg/kg dose of phenytoin are summarized in Table II, and the average plasma concentration *versus* time profiles are presented in Fig. 1. As already reported (7), the plasma clearance of the 30 mg/kg dose is considerably lower than that of the 10 mg/kg dose and is significantly decreased in pregnancy; but again, supplementation with folic acid had *no* apparent effect on the pharmacokinetics of phenytoin in either the pregnant or the nonpregnant animals. Nor did folic acid treatment affect body weight, hematocrit, or plasma protein binding of phenytoin.

One out of 10 folic acid treated and 4 out of 12 sodium bicarbonate treated impregnated (spermatozoa-positive vaginal smear) rats were not pregnant on the 20th day after mating, most likely due to resorption of fetuses. The difference between the two groups is not statistically significant by comparison of two success probabilities (12). Only the rats that were actually pregnant were used in the pharmacokinetic studies.

DISCUSSION

Female Sprague–Dawley rats fed a diet containing 1.6-mg folic acid per kg of feed[1] had 52% lower serum folate concentrations on the 21st day of pregnancy than did nonpregnant controls (13). The type of cages used to house these animals was not specified. The rats in the present investigation were fed a diet[2] containing 0.89-mg folic acid per kg of feed[1], and they were housed in suspended wire-mesh cages to minimize coprophagy, by which the animals could obtain folate synthesized by intestinal bacteria (14). It is not practical to put pregnant rats on a folate-deficient diet since this results in a high incidence of fetal resorption (15).

Blake *et al.* (16) have determined the effect of folic acid supplementation (100 μg/kg daily by gavage, from the 7th to the 20th day of gestation) on the *in vitro* activity of hepatic microsomal phenytoin hydroxylase in pregnant Sprague–Dawley rats on a diet containing 1.7 mg folic acid per kg of feed[1]. Enzyme activity per gram of liver increased fourfold relative to that of rats that did not receive folic acid supplementation. The authors concluded that this may explain the decreased plasma phenytoin concentrations observed when patients receive extra folic acid. Contrary to the *in vitro* findings by Blake *et al.* (16), the results of the present *in vivo* investigation indicate that

[1] Information obtained from the manufacturer's product literature.
[2] RMA 1000; Charles River.

folic acid supplementation has no apparent effect on the pharmacokinetics of phenytoin in pregnant as well as in nonpregnant rats. Significantly, neither of these two studies involved prior or chronic administration of phenytoin.

The published clinical data concerning decreased phenytoin plasma concentrations during pregnancy were obtained, necessarily, from patients on a chronic oral regimen of the drug. While it would seem feasible to administer a single intravenous tracer dose of stable isotope labeled phenytoin to these individuals before, during, and after pregnancy, this has apparently not been done. In an acute (single dose) clinical study on nonpregnant individuals, pretreatment with 15- or 30-mg folic acid orally everyday for several weeks had no effect on the biological half-life of phenytoin (17). Plasma concentrations of phenytoin in male Wistar rats 1.5 and 5 h after a single intraperitoneal dose of phenytoin, were similar in animals given a single concomitant oral dose of folic acid, 5 mg/kg, and in controls (18).

One possibility not explored in the present investigation is that folic acid supplementation affects the *in vivo* clearance of phenytoin only in rats that have been treated chronically with phenytoin. If that possibility can be excluded, then the qualitative species difference between humans and rats, with respect to the effect of pregnancy on the pharmacokinetics of phenytoin, may be due, at least in part, to the difference in response to folate. If, however, increased dietary folic acid causes an increase in the metabolic clearance of phenytoin in both humans and rats on chronic phenytoin therapy, then the apparent species difference in the effect of pregnancy on phenytoin pharmacokinetics may be related to the common practice of supplementing the diet of pregnant women, but not of pregnant rats, with folate.

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NOTE ADDED IN PROOF

Subsequent to the submission of this manuscript, Carl and Smith (19) reported that oral folate supplementation (20 mg/kg/d for 6 d, then every 12 h for 4 d) had no apparent effect on phenytoin concentrations in the liver, brain, and plasma of male Sprague-Dawley rats who received oral phenytoin (100 mg/kg/d) for 6 d, then every 12 h for 4 d) concomitantly. In a prospective study of epileptic women during pregnancy, Hiilesmaa *et al.* (20) found a negative correlation between folate and phenytoin concentrations in serum ($r = -0.56$, $p = 0.002$, $n = 39$) but no association between serum folate concentrations and the number of seizures during pregnancy.

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Synthesis of Methyl 2,3-*Bis*(hydroxymethyl)-5-phenyl-7-oxabicyclo[2.2.1]hepta-2,5-diene-6-carboxylate *Bis*(*N*-methylcarbamate) Derivatives as Potential Antitumor Agents

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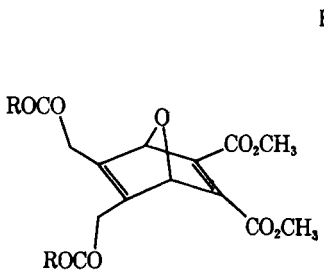
Abstract □ Methyl 2,3-*bis*(hydroxymethyl)-5-phenyl-7-oxabicyclo[2.2.1]-hepta-2,5-diene-6-carboxylate *bis*(*N*-methylcarbamate) along with the *p*-chlorophenyl and *p*-nitrophenyl analogues were synthesized using a Diels Alder reaction. The title compound and the *p*-chlorophenyl analogue were inactive against murine P388 lymphocytic leukemia.

Keyphrases □ *Bis*-carbamates—synthesis, antitumor activity □ 7-Oxabicyclo[2.2.1]hepta-2,5-diene derivatives—synthesis, antitumor activity

The discovery that certain derivatives of 7-oxabicyclo[2.2.1]heptane (1) showed cytotoxic activity in the 9KB tissue culture assay led to the synthesis and evaluation of related compounds. Dimethyl 2,3-*bis*(acetoxymethyl)-7-oxa-



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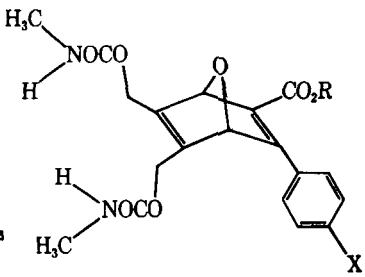

IIA: X=H; R=C_2H_5,
IIB: X=Cl; R=CH_3,
IIC: X=NO_2; R=CH_3,

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