Study of pharmacokinetic interaction of ascorbic acid and phenytoin in rats

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ABSTRACT

Background and purpose of the study: There is great interest for researchers and therapists to study the interactions of phenytoin with other drugs or foods because of its enzyme inducing effects, saturable biodisposition and specific physicochemical properties. There are reports indicating that ascorbic acid (ASC) affects interacting properties of phenytoin. By considering pharmacokinetic aspects, the present study was carried out to evaluate the effect of ASC on single and multiple dose kinetics of phenytoin in rats.

Methods: Male Wistar rats weighting 200-225 g were randomly divided into 9 groups of 6. In groups 1 to 3, phenytoin was administered orally (p.o.) (30 mg/kg) for a week and saline (5 ml/kg) and ASC (200, 500 mg/kg) were given p.o. one hour before each phenytoin treatment respectively. In groups 4 and 5, animals were treated with saline and ASC (500 mg/kg) for a week and one hour before single dose of phenytoin (30 mg/kg). In groups 6 and 7, single dose of phenytoin was administered p.o. (60 mg/kg) whereas normal saline and ASC (500mg/kg) were administered concurrently intraperitoneally (i.p.). In groups 8 and 9, single dose of phenytoin (60 mg/kg) was administered i.p., whereas other treatments were similar to groups 6 and 7. Blood samples were taken at 0, 1, 2, 3, 4, 6, 8 and 12 hours after phenytoin administration and analyzed by HPLC method.

Results: It was found that $AUC_{0-\infty}$, AUC_{0-t}, C_{max}, T_{max}, and $T_{1/2}$ didn't change significantly in the test groups compared to the respected controls. T_{max} and $T_{1/2}$ were only parameters showed a significant increase in groups 3 and 5 compared to control groups. Acidic change in gut lumen and/or renal tubules may explain these interactions.

Conclusion: Results of this study indicate that ASC has no significant interaction with phenytoin bioavailability.

Keywords: Phenytoin, Ascorbic Acid (Vitamin C), Pharmacokinetic, Interaction, Rats

INTRODUCTION

Phenytoin, the commonly prescribed antiepileptic drug has a narrow therapeutic index and a change in bioavailability with other drugs or supplements administered concurrently may lead to toxic effects or therapeutic failure (1). There is great interest for researchers and therapists to study the interactions of phenytoin with other drugs and foods because of its enzyme inducing effects and special physicochemical and solubility properties (2, 3). Phenytoin has also a complex kinetics and saturable biodisposition (4) and as a result it is necessary that clinicians be aware of the possible increase of phenytoin toxicity or therapeutic failure during its coadministeration with ascorbic acid (vitamin C, ASC).

ASC is an essential vitamin, which is commonly used in clinic as antioxidant supplement and can alter the pH of sites for absorption and/or elimination of drugs (5-6). There are also some reports indicating that ASC can affect pharmacological effects of phenytoin and it has been found that ASC can augment resistant effects of phenytoin on urethane-induced loss of righting reflex in rats (7). Another study suggesting that this effect could be apparent on both latency to and/or duration of loss of righting reflex in rats when ASC was chronically given in combination with phenytoin (8). To our knowledge the potential of kinetic interaction of phenytoin with ASC has not been explored. The present study was designed to evaluate the effect of ASC on single and multiple dose pharmacokinetics of phenytoin in rats.

MATERIALS AND METHODS

Drugs
Phenytoin sodium (Amin Corp., Isfahan, Iran), and ASC (Merck, Darmstadt, Germany) were dissolved in normal saline before administration

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to the animals. Carbamazepine powder (Sobhan Corp., Tehran, Iran) was dissolved in methanol and used as internal standard for HPLC analysis. HPLC grade methanol and analytical grade sodium hydrogen mono-phosphate and sodium hydrogen diphosphate were obtained from Merck (Darmstadt, Germany). Double distilled deionized water (Amin Corp., Isfahan, Iran) was used for all preparations.

Animals
Male Wistar rats (local breed) weighting 200-225 g at the beginning of the experiment were used. The animals were kept in a temperature/ humidity- controlled environment and subjected to natural daily dark-light cycle with free access to a commercial rodent chow and tap water.

Grouping
The experimental rats were randomly divided into 9 groups of six animals
In groups 1 to 3, phenytoin was administered orally (p.o.) (30 mg/kg) for a week and saline (5 ml/kg) and ASC (200, 500 mg/kg) were given p.o. one hour before each phenytoin treatment respectively. In groups 4 and 5, animals were treated with saline and ASC (500 mg/kg) for a week and one hour before administration of a single dose of phenytoin (30 mg/kg) at the day of 8. In groups 6 and 7, single dose of phenytoin was administered p.o. (60 mg/kg) whereas normal saline and ASC (500 mg/kg) were administered concurrently intraperitoneally (i.p.) (5 ml/kg). In groups 8 and 9, single dose of phenytoin (60 mg/kg) was administered i.p., and animals were subjected to other treatments similar to groups 6 and 7. Blood samples (0.5 ml) were taken at 0, 1, 2, 3, 4, 6, 8, and 12 hours after phenytoin treatment by using microhematocrit capillaries and from retro-orbital plexus under light ether anesthesia and were collected in haparinized tubes.
The plasma were separated and stored at −20 ºC until assessed for phenytoin.

Apparatus
The HPLC system for the assay of phenytoin was consisted of three pumps (Shimadzu LC-6A, Japan), UV-VIS detector (Shimadzu SPD-6AV, Japan), Oven (Shimadzu CTO-6A, Japan), analytical column (ODS RP, 150 × 4.6 mm, 5 μm, Waters, USA), System controller (Shimadzu SCL-6A, Japan) and an integrator (Shimadzu CR-6A Chromatopac, Japan). The mobile phase was water- methanol (50:50, v/v) which adjusted to pH = 4.9 by addition of phosphate buffer (M=

0.052, pH= 4.9) and filtered through a 0.45 μm Millipore filter tissue. The flow rate was 1 ml/min. Detection was performed at a wavelength of 258 nm under a constant temperature (37 ± 1 ºC)

HPLC analysis
Phenytoin concentrations in plasma samples were estimated by a modified technique (9, 10), which was standardized in our laboratory. The plasma was mixed with 25 μl of internal standard (Carbamazepine, 100 μg/ml) and 0.2 ml of 1N HCl, and the mixture was then extracted with 3.5 ml of diethyl ether. After vortex mixing for 3 min, the mixture was centrifuged to dryness below 40 ºC and the residue was then dissolved in 0.1 ml of methanol. A 20 μl aliquot was injected onto the HPLC column for analysis.

Pharmacokinetic parameters
Peak plasma phenytoin concentration (C_{max}) and time to reach peak concentration (T_{max}) for single and multiple dose studies were calculated from actual plasma data of each rat. The area under the time plasma concentration (AUC_{0-12}) of phenytoin was calculated by trapezoidal rule, the $AUC_{12-\infty}$ was calculated by dividing the last plasma concentration of the drug (C_{12}) by elimination rate constant (K_{el}) of phenytoin which was calculated by least square regression analysis method. Finally elimination half life ($T_{1/2}$) was calculated by using the formula $T_{1/2}= 0.693/K_{el}$ (9, 11).

Statistical Analysis
One-way ANOVA test was utilized to find level of significance of difference between pharmacokinetic data of phenytoin in multiple dose study. For single dose treatments where two separate groups were studied, independent Student's *t*-test was used. $P<0.05$ was taken to be statistically significance.

RESULTS

Validitation of the method
Under the HPLC system described above, the retention times of phenytoin and carbamazepine as internal standard, were 5.9 ± 0.2 and 8.1 ± 0.2 min, respectively. The detection limit of the assay was approximately 0.25 μg/ml and the recovery rate of phenytoin in plasma averaged over 80 %. The calibration curve for phenytoin was linear (r^2 = 0.996) over the concentration range of 0.5-20 μg/ml. With the least- square method, a regression equation of y= 0.116 x + 0.087 was obtained. The intra-assay and inter-assay coefficient of variation were 4.1 % and 5.9 % respectively

Effect of concurrent oral doses of ASC (200 and 500 mg/kg) on multiple dose (30 mg/kg) kinetics of phenytoin

Figures 1 and 2 depict plasma phenytoin concentrations (mean ± SD) at different time intervals of six rats. It is evident that phenytoin could be detected in 12 hours samples of all animals after single dose administration.

Pharmacokinetic parameters are summarized in table 1. No significant difference (p>0.05) was found between parameters of multiple dose of phenytoin given concurrently with two different doses of ASC (200 and 500 mg/kg) and normal saline (group 1). T_{max} and $T_{1/2}$ were two main parameters of phenytoin increased significantly (p<0.05) after concurrent use of ASC in higher dose (500 mg/kg) compared to groups 1 and 2. After treatment with 200 mg/kg of ASC, C_{max} and AUC_{0-12} decreased by 23.1 % and 3.3 % but $AUC_{0-\infty}$, T_{max} and $T_{1/2}$ increased by 0.4 %, 5.3 % and 37.8 % respectively. After treatment with higher dose of ASC (500 mg/kg), AUC_{0-12}, $AUC_{0-\infty}$, and C_{max} decreased by 24.4 %, 18.3 %, and 29.4% respectively in comparison with control group (table 1, group 1, figure 1).

Effect of multiple oral doses of ASC (500 mg/kg) as pretreatment on single oral dose (30 mg/kg) kinetics of phenytoin

After pretreatment with normal saline (5 ml/kg) or high oral dose of ASC for a week, T_{max} (p<0.05) and $T_{1/2}$ (p<0.01) were the only two parameters that showed significant increase compared with respected control group (group 4). C_{max}, AUC_{0-12} and $AUC_{0-\infty}$ of phenytoin decreased by 24.6%, 6.5% and 2.3% respectively compared with control group (table1, group 4, figure 2).

Effect of concurrent intraperitoneal dose of ASC (500 mg/kg) on single oral dose (60 mg/kg) kinetics of phenytoin

By concurrent treatment of rats with normal saline (5ml/kg) or high dose of ASC (500 mg/kg) intraperitoneally, C_{max}, AUC_{0-12} and $AUC_{0-\infty}$ of phenytoin increased by 6.3 %, 20.4 %, and 24.9 % and T_{max} and $T_{1/2}$ decreased by 9.9 % and 21.3 % respectively. However, the differences were not significant (p>0.05) for all parameters in comparison with respected control group (table1, group 6, figure 3).

Effect of concurrent intraperitoneal dose of ASC (500 mg/kg) on single intraperitoneal dose (60 mg/kg) kinetics of phenytoin

In this phase of study, C_{max}, AUC_{0-12} and $AUC_{0-\infty}$ of phenytoin increased by 10.2 %, 14.3 %, and 15.2 % and T_{max} and $T_{1/2}$ decreased by 5.1 % and 18.7 % respectively. However when the higher dose of ASC was given concurrently, the differences were not significant (p>0.05) for all parameters in comparison with respected control group (table1, group 8, figure 3).

DISCUSSION

Phenytoin, a widely used anticonvulsant, interacts with many drugs and foods that for some of them the interaction may be clinically significant (5, 12). Recognition of the drugs and nutritional supplements that interact with phenytoin may help to increase the therapeutic efficacy and/or to reduce the toxicity of this drug (13, 14).

ASC is used commonly in clinic as supplement and can alter the pH of sites for absorption and/or elimination of drugs (5, 14-15). There are also reports suggesting that ASC can affect antiseizure effects of phenytoin in healthy rats and rabbits (7-8) so it is logical to expect that it may have the same effects in human. In the present work, the influence of ASC on the pharmacokinetics of phenytoin in rats was studied in order to identify the interactions between ASC and phenytoin and its possible mechanisms.

Results of this study demonstrate that multiple dose kinetics of phenytoin is not affected significantly by concurrent administration of two increasing doses of ascorbic acid. $T_{1/2}$ and T_{max} were only two parameters that increased significantly following the use of high dose of ascorbic acid (500 mg/kg). In single dose study, when pretreatment with ascorbic acid was continued for a week, similar changes in $T_{1/2}$ and T_{max} were also observed. Phenytoin is eliminated from the body by hepatic metabolism principally through process of hydroxylation (4, 9) so functial status of hepatic microsomal cytochrome P450 isoenzyme(s) is crucial. The increase in elimination half life ($T_{1/2}$) may indicate that decrease in metabolism or renal clearance of phenytoin when it is co-administered with single or multiple high dose of ascorbic acid. This is unlikely that ASC causes a reduction in metabolic oxidation because this vitamin acts as a reducing agent and may replenishes the cofactors required for oxidative pathways (15). This is also in contrary with the results obtained with pyridoxine (vitamin B6) (16) and folic acid (17) given in large doses with phenytoin in patients with epileptic disorders produced a reduction in serum phenytoin concentrations and rebound of seizure attacks which might be due to increase in phenytoin metabolism. Another site of interaction might be renal excretion. About 5 % of phenytoin is eliminated through the kidney as unchanged drug (4, 14). In high doses this amount tends to be increased because phenytoin inhibits its own metabolism to some extent (18-19). ASC in
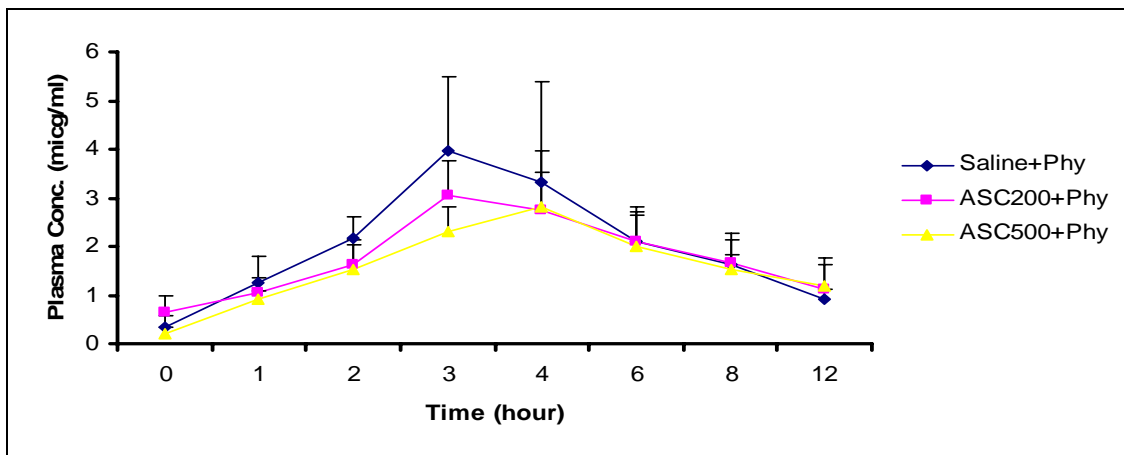
Figure 1. Plasma concentrations of phenytoin (mean ± SD) at different time intervals in rats (n=6). Normal saline (5ml/kg) or ascorbic acid (ASC, 200 and 500 mg/kg) were administered orally (p.o.) for a week with multiple doses of phenytoin (Phy, 30 mg/kg). Blood sampling was carried out at the day of 8 after an overnight fasting.
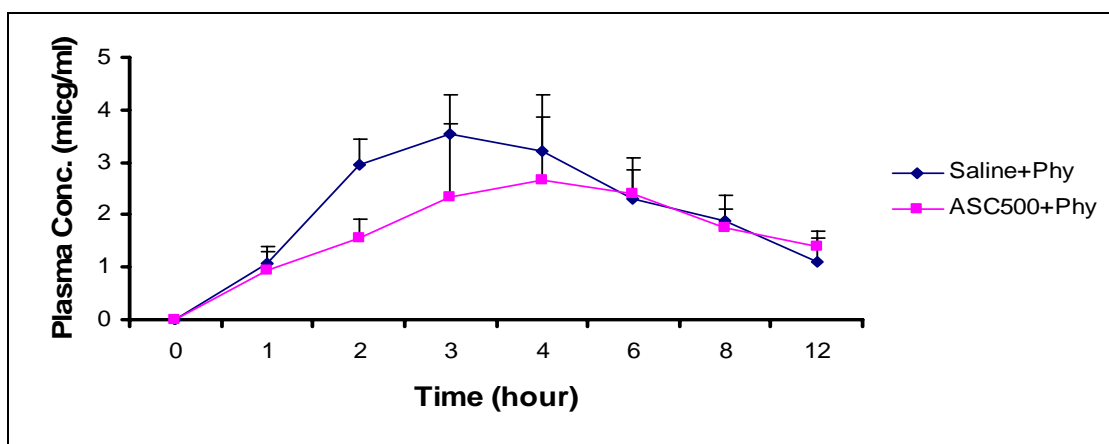
Figure 2. Plasma concentrations of phenytoin (mean ± SD) at different time intervals in rats (n=6). Normal saline (5ml/kg) or ascorbic acid (ASC, 500 mg/kg) were administered orally (p.o.) for a week with a single dose of phenytoin (Phy, 30 mg/kg). Blood sampling was carried out at the day of 8 after an overnight fasting.
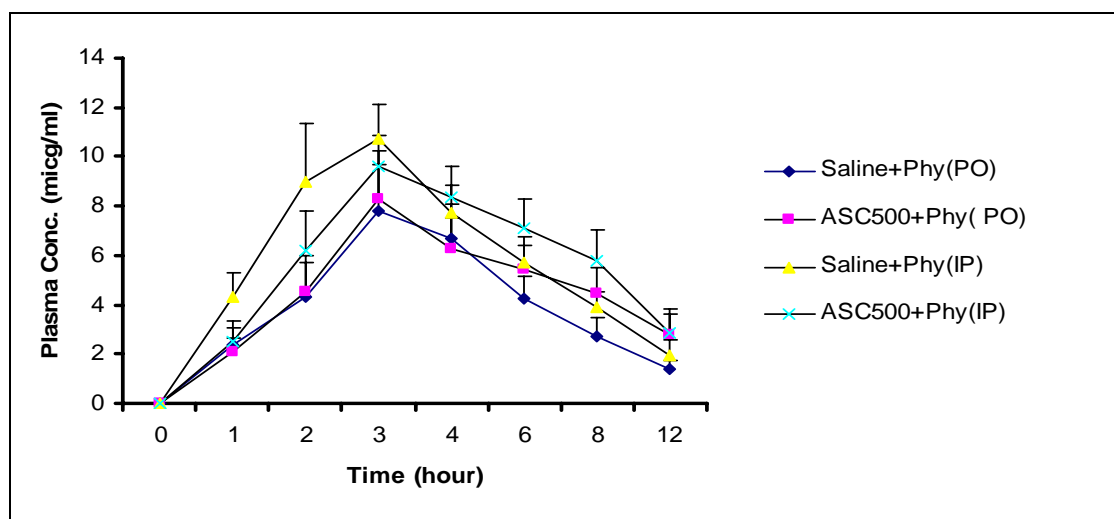
Figure 3. Plasma concentrations of phenytoin (mean ± SD) at different time intervals in rats (n=6). Normal saline (5ml/kg) or ascorbic acid (ASC, 200 and 500 mg/kg) were co-administered intraperitoneally (i.p.) with a single dose of phenytoin (Phy, 60 mg/kg) administered orally (p.o.) or intraperitoneally. Blood sampling was carried out after an overnight fasting.

Table1. Parameters of single dose (SD, 30 and 60 mg/kg) and multiple dose (MD, 30 mg/kg) kinetics of phenytoin (Phy) co-administered with saline (5 ml/kg, MD or SD as control) or ascorbic acid (ASC, 200, 500 mg/kg MD or SD) by different routes of administration. Data are Mean ± SD.

Groups of Treatment	AUC 0 – 12 (μg/ml) hr^{-1}	AUC 0 - ∞ (μg /ml) hr^{-1}	C max (μg /ml)	T max (hour)	T ½ (hour)
1- Saline (MD, PO)+Phy30 (MD, PO)	23.46 ± 6.09	25.88 ± 6.49	3.98 ± 1.51	3.16 ± 0.40	4.12 ± 1.10
2- ASC200 (MD, PO)+Phy30 (MD, PO)	22.68 ± 6.42	26.00 ± 7.35	3.06 ± 0.71	3.33 ± 0.51	5.68 ± 2.03
3- ASC500 (MD, PO)+Phy30 (MD, PO)	17.73 ± 6.28	21.13 ± 8.62	2.81 ± 0.72	4.33 ± 0.51*	6.92 ± 1.39*
4- Saline (MD, PO)+Phy30 (SD, PO)	22.36 ± 4.20	24.59 ± 5.72	3.53 ± 1.39	3.16 ± 0.40	4.04 ± 1.18
5- ASC500 (MD, PO)+Phy30 (SD, PO)	20.90 ± 6.25	24.03 ± 8.09	2.66 ± 0.67	4.33 ± 1.03*	7.48 ± 2.2**
6- Saline (SD, IP)+Phy60 (SD, PO)	41.00 ± 9.75	41.98 ± 10.57	7.82 ± 1.84	3.33 ± 0.51	2.25 ± 0.75
7- ASC500 (SD, IP)+Phy60 (SD, PO)	49.38 ± 10.16	52.45 ± 10.69	8.31± 1.92	3.00 ± 0.00	2.73 ± 0.35
8- Saline (SD, IP)+Phy60 (SD, IP)	58.25 ± 8.17	59.01 ± 8.27	10.7 ± 2.35	3.33 ± 0.51	1.66 ± 0.81
9- ASC500 (SD, IP)+Phy60 (SD, IP)	66.56 ± 13.52	68.01 ± 14.37	9.61 ± 1.27	3.16 ± 0.40	1.97 ± 0.18

* $p < 0.05$, ** $p<0.01$ represents significant difference in comparison with respected control group. PO: Per Oral. IP: Intraperitoneal

excess of body's needs is also rapidly eliminated unchanged in the urine and can acidify the urine (14). This tends to increase the lipid solubility of phenytoin and to enhance the tubular reabsorption which eventually results in delayed renal excretion. Another possible mechanism might be an increase in volume of distribution (V_d) for phenytoin, which may results in longer elimination half-life (10). Phenytoin is chemically a weak acid (pKa = 8.3) and is highly bound to plasma albumin (>90 %) (19) however; ASC has a low protein binding and it is unlikely that plasma protein binding plays an important role in our findings.

The increase in time to reach peak plasma concentration of phenytoin (T_{max}) represents a delay in onset and/or the rate of absorption in gut lumen. The delay in onset of absorption (damping phenomenon) is not acceptable because; no lag time could be found in plasma phenytoin profiles. In addition, ASC is readily absorbed from the gut lumen and there is no evidence for alteration in gastric emptying (14). On the other hand, phenytoin is predominantly absorbed from the small intestine and its absorption depends on its pKa and lipid solubility, the pH of the medium in which phenytoin is dissolved, its solubility in the medium and its concentration (10, 18). ASC, in high concentrations is strong enough (pKa = 4.1) to acidify the pH of gastrointestinal lumen and alter the solubility and eventually the lipid solubility of phenytoin. A 5 % solution of ASC in water has a pH of 2.1 to 2.6 (14). Phenytoin sodium remains in solution when the pH is considerable alkaline (about 10-12) (20), so it

seems likely that slowing phenytoin absorption would be partly due to alteration in pH of gut lumen. There have been reports of loss of clarity and precipitation when solutions of phenytoin sodium have been mixed with a variety of drugs or foods (20). It has been shown that phenytoin absorption (C_{max}) and bioavailability ($AUC_{0-\infty}$) increases in the presence of oral acidic beverage, Coca Cola, in healthy rabbits (21). Differences in study design especially on the time of ASC administration, acidic strength (Coca Cola versus ASC), constituents of Coca Cola as a beverage (*e.g.* xanthins) and animal species difference, might explain the discrepancies were between results of this work and the reported studies.

Single dose kinetics of phenytoin didn't significantly change by concurrent use of high dose of ascorbic acid injected intraperitoneally. In this phase of study, the dose of phenytoin was doubled to examine the possible role of saturated metabolism or biodisposition on the mechanisms that might be involved in pharmacokinetic interactions. However, our findings indicated no significant interaction in these groups compared with respected controls.

Taken together we conclude that ascorbic acid, after multiple or single administration at usual and high doses through different routes of administration had no significant interaction with phenytoin bioavailability.

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