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Diltiazem and verapamil elevate plasma phenytoin concentrations in the rat

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Abstract

The effects of the calcium channel blockers, diltiazem and verapamil on the pharmacokinetics of phenytoin were investigated in rats. Animals were given phenytoin alone or phenytoin together with either diltiazem or verapamil and the plasma samples were collected at different time intervals. The concentration of phenytoin was measured using a high performance liquid chromatographic method (HPLC). Diltiazem and verapamil significantly ($p < 0.05$) increased the area under the curve (AUC), the maximum plasma concentration (C_{max}) and elimination half-life ($t_{1/2}$) of phenytoin. These results suggest that a potentially harmful drug-drug interaction may occur if phenytoin is administered concurrently with either diltiazem or verapamil.

Keywords: Calcium channel blocker; Phenytoin; Pharmacokinetic interactions; Rat

1. Introduction

Phenytoin is a potent anticonvulsant drug which has successfully been used in the management of generalized tonic-clonic seizures and partial seizures (Elson and Penry, 1981). It is also useful in the treatment of trigeminal neuralgia (Zakrzewska and Patsalos, 1992). In addition, the drug has been shown to be effective in correcting several experimental and clinical tachycardias (Dreifus and Watanabe, 1970).

Calcium channel blockers have been shown to exhibit anticonvulsant activity in various experimental models of epilepsy, such as amygdala-kindled seizures in rats (Ashton and Wauquier, 1979a) and dogs (Wauquier et al., 1979), allyl glycine-induced seizures in rats (Ashton and Wauquier, 1979b), cefazolin-induced seizures in rats (De Sarro et al., 1986), audiogenic seizures in mice (De Sarro et al., 1988), and pentylenetetrazole-induced seizures in rats (Walden and Speckmann, 1988). In addition, these drugs were reported to enhance the protective effects of antiepileptics against electrically induced convulsions in mice (Czuczwar et al., 1990a), and pentylenetetrazol-induced seizures in mice (Czuczwar et al., 1990b).

Phenytoin has the ability to induce hepatic microsomal enzymes and to increase the metabolism of other drugs (Roger et al., 1992). Hence, the clearance of other drugs which are metabolised by the mixed function oxidase system

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in the liver is enhanced when these drugs are administered together with phenytoin. It has been shown that treatment with phenytoin increases the metabolic clearance and/or reduces the steady-state serum concentrations of antibiotics (Penttila et al., 1974), carbamazepine (Dam et al., 1975), dexamethasone (Boylan et al., 1976), diazepam (Dhillon and Richens, 1981), digoxin (Rameis, 1985), oral contraceptives (Back and Orme, 1990), and azoles (Tucker et al., 1992). On the other hand, a variety of drugs have been reported to inhibit phenytoin metabolism leading to a rise in its serum concentration and increase in the risk of its toxicity. For example, it has been reported that valproic acid, izoniazid and non-steroidal anti-inflammatory drugs inhibit the metabolism of phenytoin and increase its plasma concentration (Miller et al., 1979; Neuvonen et al., 1979; Bruni et al., 1980).

Phenytoin is extensively bound to plasma proteins (Hooper et al., 1974) and a number of drugs have been reported to displace it from these binding sites. For instance, tolbutamide, aspirin and diazoxide have been shown to displace phenytoin from its plasma protein binding sites leading to elevation of its plasma levels (Roe et al., 1975; Wesseling and Mols-Thurkow, 1975; Paxton, 1980).

Since some calcium channel blockers have been shown to be effective as adjunctive therapy with certain antiepileptics (Overweg et al., 1984; Macphee et al., 1986; Larkin et al., 1988) the present work was undertaken to investigate the possible pharmacokinetic interaction following the concurrent administration of phenytoin and the calcium channel blockers, diltiazem or verapamil to rats.

2. Materials and methods

2.1. Materials

Phenytoin (5,5-diphenylhydantoin), diltiazem hydrochloride and verapamil hydrochloride, were purchased from Sigma Chemical Co., St. Louis, MO. Phenytoin was suspended in Tween 80, whereas diltiazem hydrochloride and verapamil hydrochloride were dissolved in normal saline. Acetonitrile (HPLC grade) and phenacetin were purchased from Merck, Darmstadt, Germany.

2.2. Methods

2.2.1. Pharmacokinetic study

Male Wistar rats weighing 300–350 g were obtained from the Animal House, College of Medicine, King Saud University. The animals were housed under standard laboratory conditions with free access to food and water ad libitum.

The rats were anaesthetised with ether and the right femoral artery was surgically exposed and was cannulated using a fine flexible polyethylene tube. The other end of the tube was then drawn under the skin to an incision in the back region and was connected to a syringe containing heparinised saline. Rats were given 1000 IU kg^{-1} heparin through the cannula prior to drug treatment.

After surgery, each animal was housed individually in specially made wooden cages (22 × 10 × 7 cm) with a metal grid top and kept in a temperature-controlled room (21 ± 1°C). As soon as the animals had recovered from the anaesthesia, they were divided into three different groups, each group comprising five rats. Groups 1 and 2 were injected intraperitoneally (i.p.) with 5 mg/kg diltiazem and 5 mg/kg verapamil, respectively. The third group of rats served as control and was injected intraperitoneally with saline only. Thirty minutes later, phenytoin (20 mg/kg) was administered i.p. to both the drug- and the saline-treated groups. Blood samples (0.3 ml) were withdrawn via the cannula and collected into heparinised Eppendorff tubes before treatment with the drugs and thereafter at 0.083, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 h. The last measurable plasma phenytoin concentration following the administration of phenytoin alone was at 6 h post-dose. Hence, plasma samples were collected for up to 6 h in the group which had received phenytoin alone. The cannula was flushed with an equal volume of heparinised saline after each sample withdrawal.

2.2.2. Determination of plasma phenytoin concentration

The plasma concentrations of phenytoin were determined by a modification of the method described by Kabra et al. (1977). Briefly, blood samples from rats which had received phenytoin alone or a combination of phenytoin together with either diltiazem or verapamil were centrifuged at 2100 × *g* for 10 min on a Gallenhamp Angle Head Centrifuge. An aliquot (200 μl) of the plasma was added to 200 μl of chromatography grade acetonitrile solution containing phenacetin (20 μg ml^{-1}) as an internal standard. The suspension was vortexed and then centrifuged at 5400 × *g* for 5 min on a Select-a-fuge 24 Biodynamics centrifuge. Twenty μl of the acetonitrile supernatant was injected into an HPLC system consisting of a Waters (Milford, MA) M-510 pump and Waters reverse phase (NOVA-PAK C-18, 3.9 mm × 1.5 cm) column. The column was eluted at the rate of 1.5 ml min^{-1} with a prefiltered and degassed mobile phase consisting of 45% methanol and 0.01 M potassium dihydrogen phosphate (KH_2PO_4).

Phenytoin was detected by a Waters M-441 variable wavelength UV absorption detector at 214 nm wavelength with a sensitivity of 0.01 absorbance units of full scale (AUFS) and a chart speed of 2 mm min^{-1}. To determine the plasma concentrations of phenytoin, several standard solutions of phenytoin were freshly prepared in concentrations of 50–1000 μg^{-1} to which was added 100 μl of a solution of 20 μg ml^{-1} phenacetin. Twenty μl of these standard phenytoin concentrations were injected into the column using a 50-μl Hamilton syringe. A linear relationship was obtained when the various phenytoin concentrations were plotted against their corresponding peak heights. The concentration of phenytoin in plasma was calculated from a previously constructed standard curve. This procedure provided a low detection limit of 0.1 μg ml^{-1}.

When the procedure described above was used for the detection of phenytoin and phenacetin in plasma samples, it resulted in peaks which corresponded to the retention times of the standard solutions of phenytoin and phenacetin. To eliminate the possibility of interference from either diltiazem or verapamil in the assay of phenytoin, a sample of blank rat's plasma was spiked with these drugs and treated as described above. Injection of phenytoin into the column under the same experimental conditions as that used for the assay showed no peaks for diltiazem or verapamil at the wavelength used and within the retention time observed for phenytoin.

2.2.3. Analysis of data

The area under the plasma concentration-time curve to the last sampling time (AUC) was estimated by the linear trapezoidal method. The elimination rate constant was determined by linear regression analysis. Terminal half-lives were calculated from the log-linear part of the slope. The significant difference between respective treatment groups was analyzed using the unpaired Student's *t*-test; *p* values less than 0.05 were considered significant.

3. Results

Fig. 1 shows the mean plasma concentration versus time profiles of phenytoin administered alone or in combination with either diltiazem or verapamil. The absorption process was completed with a mean t_{max} of 1.0 ± 0.0 and 2.0 ± 0.0 units for the phenytoin alone and drug combination groups, respectively. Diltiazem and verapamil induced an upward shift in the time-concentration curve of phenytoin. A concentration decay being evident within 2 and 4 h following drug administration for phenytoin alone and drug combination groups, respectively. Phenytoin was not detectable after 6 h in the phenytoin-treated groups; whereas it was detectable up to 12 h in the groups which had received either diltiazem or verapamil together with phenytoin.

Table 1 summarises the various pharmacokinetic parameters. Treatment of rats with diltiazem and verapamil significantly increased the mean area under the curve (AUC) for phenytoin from 4.90 ± 0.40 to 20.20 ± 1.11 (diltiazem) and 25.20 ± 1.50 μg ml^{-1} h^{-1} (verapamil) ($p < 0.05$). Maximum plasma concentration (C_{max}) and the elimination half-life of phenytoin were also

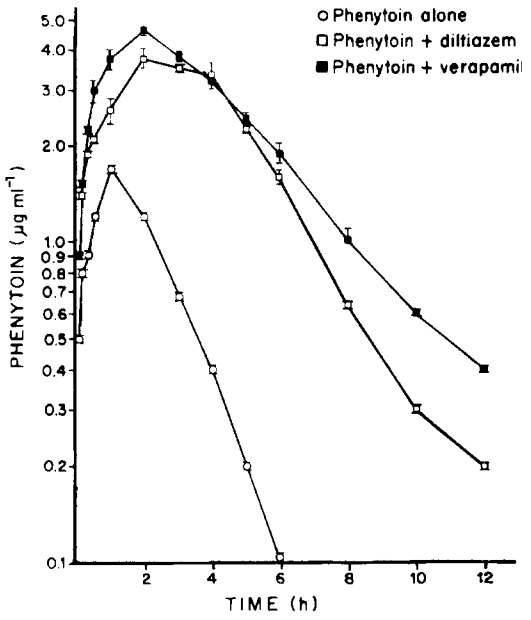

Fig. 1. Fig. 1. Plasma phenytoin concentration-time profiles for phenytoin alone (○), phenytoin together with diltiazem (□) or phenytoin together with verapamil (■). Each point represents the mean ± S.E.M. of five observations.

significantly higher than those obtained when phenytoin was administered alone ($p < 0.05$). The time to reach C_{max} (t_{max}) was also significantly prolonged in all treated groups ($p < 0.05$).

4. Discussion

The present study shows that when diltiazem or verapamil are administered concurrently with phenytoin they significantly increase the area under the curve (AUC), maximum plasma concentration (C_{max}) and elimination half-life ($t_{1/2}$) of the latter drug in rats.

Phenytoin is widely used for the treatment of convulsive disorders (Eadie, 1984). Some calcium channel blockers were reported to be effective as adjunctive therapy with the available antiepileptic drugs in patients with partial complex seizures (Overweg et al., 1984). Calcium channel blockers are also widely used in the management of cardiovascular disorders (Stone et al., 1980). Some patients with epilepsy may have a cardiovascular disease for which a calcium channel blocker may be prescribed.

It has been shown that the hepatic mixed function oxidase system is responsible for the metabolism of phenytoin (Roger et al., 1992) as well as the calcium channel blockers (Triggle, 1981). Several drugs have been reported to inhibit phenytoin metabolism and precipitate intoxication in patients who had been previously well stabilized on phenytoin (Richens, 1977). Recent studies have demonstrated that the calcium channel blockers depress the activity of cytochrome P450-dependent mixed function oxidase system and consequently inhibit the metabolism of other drugs (Renton, 1985; Currum et al., 1986). It is thus possible that the increased levels of phenytoin observed in this study following the administration of either diltiazem or verapamil may result, at least in part, from inhibition of liver mixed-function oxidase enzymes by the latter drugs.

Table 1
Serum pharmacokinetic parameters of phenytoin (20 mg/kg, i.p.) administered alone or together with diltiazem (5 mg/kg, i.p.), or verapamil (5 mg/kg,i.p.) in rats

Parameter	Phenytoin	Phenytoin + diltiazem	Phenytoin + verapamil
C_{max} (μg ml^{-1})	1.70 ± 0.10	3.80 ± 0.23[a]	4.64 ± 0.20[a]
t_{max} (h)	1.00 ± 0.0	2.00 ± 0.0[a]	2.00 ± 0.0 [a]
AUC (μg ml^{-1} h)	4.90 ± 0.40[b]	20.20 ± 1.11[a]	25.20 ± 1.50[a]
$t_{1/2}$ (h)	1.11 ± 0.20	2.0 ± 0.10[a]	2.60 ± 0.10[a]

Each value is the mean ± S.E.M. of five observations.
[a]Statistically significant from the values obtained for phenytoin alone ($p < 0.05$; Unpaired t-test).
[b]The last measurable plasma phenytoin concentration following the administration of phenytoin alone was at 6 h post-dose.

Phenytoin and calcium channel blockers have been reported to bind to plasma proteins (Hooper et al., 1974; Perruca, 1980; Godfraind, 1982). It is, therefore, possible that these drugs when given concurrently may displace each other from these plasma protein binding sites. It is tempting to speculate that the calcium channel blockers may have displaced phenytoin from these binding sites. This together with their ability to inhibit drug metabolism in the liver may be the reason for the elevation of phenytoin concentrations observed in this study.

The gastrointestinal tract as a possible site for interaction between the calcium channel blockers and phenytoin is unlikely because all drugs used in this study were given by the i.p. route. However, it would be of further interest to determine the effect of chronically administered oral calcium channel blockers on the blood levels of phenytoin when it is given intraperitoneally.

In conclusion, the concurrent administration of calcium channel blockers, diltiazem and verapamil, together with phenytoin to rats produced a significant increase in the serum concentrations of the latter. Such increased levels of phenytoin may predispose to the toxicity of the drug. Although our results may not be extrapolated to humans, caution should be exercised when diltiazem or verapamil are administered together with phenytoin.

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