PHARMACOKINETIC INVESTIGATION OF THE INTERACTION OF AZAPROPAZONE WITH PHENYTOIN

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- 1 We have investigated the interaction of azapropazone with phenytoin in five healthy volunteers.
- 2 From steady-state plasma phenytoin concentrations of about 17 $\mu\text{mol/l}$ there was at least a two-fold increase following the introduction of azapropazone.
- 3 The main mechanism of the interaction was a decrease in phenytoin clearance, attributable to competitive inhibition by azapropazone of phenytoin *p*-hydroxylation.
- 4 Protein-binding of phenytoin in the plasma (as assessed by salivary phenytoin concentrations) was significantly reduced from 92 to 90% by azapropazone and similar changes occurred in *in vitro* studies of [^3H]-phenytoin protein binding.

Introduction

We have previously shown that steady-state plasma phenytoin concentrations, measured by radioimmunoassay, increase during concomitant treatment with azapropazone (Geaney *et al.*, 1982). This interaction may result in phenytoin intoxication (Roberts *et al.*, 1981; Geaney *et al.*, 1982).

We now report the results of our further pharmacokinetic investigations of this interaction. We have extended our previous observations in the same subjects (Geaney *et al.*, 1982) by measuring plasma, urinary and salivary concentrations of phenytoin and its metabolites, this time using high performance liquid chromatography. We have also studied the effect of azapropazone on *in vitro* phenytoin protein binding.

Methods

Five healthy male volunteers, aged 30-37 years, took part in the study. Each took phenytoin (Epanutin, Parke-Davis) in a dose of 100 mg every evening for 2 weeks, and plasma phenytoin concentrations were then measured. The daily dose of phenytoin was then increased to either 200 mg or 300 mg, depending on the plasma phenytoin concentration found during treatment with 100 mg/day. Plasma phenytoin concentrations were measured again after 2 weeks. The dosage of phenytoin was then adjusted on the basis of these steady-state plasma phenytoin concentration measurements to achieve a steady-state plasma phenytoin concentration of around 20 $\mu\text{mol/l}$. We checked that this concentration had been achieved on

three occasions after a further 2 weeks of treatment. Azapropazone (Rheumox, A.H. Robins) was then introduced in the conventional therapeutic dose of 600 mg twice daily orally. Thereafter blood for plasma phenytoin and azapropazone concentration measurements was taken before most morning doses of azapropazone.

Following the introduction of azapropazone plasma phenytoin concentrations rose, but we were unable to allow a new steady state to be reached because of symptoms of toxicity in two subjects. We therefore reduced the dosages of phenytoin and subsequently measured steady-state phenytoin concentrations during treatment with two lower dosages of phenytoin. The details are given in the results section below.

In addition to blood samples, mixed, unstimulated saliva samples were taken at the same time for measurement of salivary phenytoin concentrations. In three subjects, 24 h urine collections were made during treatment with phenytoin, once before and once during azapropazone treatment, for measurement of urinary concentrations of phenytoin and its main metabolite, *p*-hydroxyphenylphenylhydantoin (*p*-HPPH). We also measured total *p*-HPPH in the plasma samples.

Analytical techniques

Phenytoin and *p*-HPPH were extracted from plasma, saliva, and urine using a method based on that described by Sawchuk & Cartier (1980). After extraction, analysis was by high performance liquid

chromatography using an Altex model 110 pump (Altex Scientific, USA), a Rheodyne model 7125 injection valve fitted with a 20 μ l sample loop (Rheodyne, USA), and a 15 cm $\times$ 4.6 mm (internal diameter) stainless steel analytical column with 5 μ m diameter Ultrasphere ODS particles (Altex Scientific). The analytical column was fitted with a 5 cm $\times$ 4.6 mm (internal diameter) pre-column packed with 30–38 μ m diameter Co-Pell ODS (Whatman, UK). The detection system was a model LC-UV variable wavelength ultra-violet detector (Pye Unicam, UK).

The mobile phase consisted of 0.15 mmol/l H_3PO_4 : methanol (50:50, vol./vol.), final pH = 3.5, filtered through a 0.5 μ m Fluropore membrane filter (Millipore, UK) before use. The mobile phase flow rate was 1 ml/min. The detector wavelength was set at 240 nm.

The internal standard used was sodium phenobarbitone. The k' values for the individual solutes were: *p*-HPPH, 1.22; phenobarbitone, 1.65; phenytoin, 4.16.

Plasma azapropazone concentrations were measured using the spectrophotometric assay of Leach (1976) after first precipitating plasma proteins with perchloric acid.

In vitro protein-binding studies

We measured the binding of [^3H]-phenytoin (N.E.N.) to plasma proteins *in vitro*, in the presence and absence of azapropazone, using the method of Monks *et al.* (1978). The concentrations of phenytoin were varied between 40 and 400 $\mu\text{mol/l}$ and those of azapropazone between 167 and 500 $\mu\text{mol/l}$ (50 and 150 $\mu\text{g/ml}$).

Mathematical methods

The apparent K_m and $V_{\max}$ for phenytoin metabolism were calculated from steady-state concentration measurements using the method of the direct linear plot (Eisenthal & Cornish-Bowden, 1974). Total body clearance of phenytoin was then calculated from the relationship:

$$\text{CL} = \frac{V_{\max}}{K_m + C_{ss}}$$

on the assumption that phenytoin absorption was unaffected during the interaction (see **Discussion**).

Renal clearance (CL_R) of phenytoin was calculated from the relationship:

$$\text{CL}_R = (\text{Amount excreted in urine in one dosage interval at steady state})/(\text{plasma concentration at steady state}).$$

The apparent K_D and $B_{\max}$ for the binding of

phenytoin to plasma proteins in the presence and absence of azapropazone were calculated using the method of Scatchard (1949).

Values of K_i for both the *in vitro* protein binding displacement interaction and the inhibition of metabolism *in vivo* were calculated from the relationship:

$$K_{\text{obs}} = K \left[1 + \frac{[I]}{K_i} \right]$$

where K_{obs} is the apparent K_D (or K_m) in the presence of concentration $[I]$ of the inhibitor, azapropazone, and K is the apparent K_D (or K_m) in the absence of the inhibitor.

Statistical comparisons were made using the two-tailed Mann-Whitney rank sum test, except where otherwise stated.

Results

Plasma phenytoin concentrations

In Figure 1 (a) is shown the change in plasma phenytoin concentrations following introduction of azapropazone, and during subsequent changes in phenytoin dosage at a constant dose of azapropazone.

Steady-state plasma phenytoin concentrations on daily doses of phenytoin of 125–250 mg (median 200 mg), immediately before the introduction of azapropazone, were 11–20 $\mu\text{mol/l}$ (median 17 $\mu\text{mol/l}$). Within one day after the introduction of azapropazone plasma phenytoin concentrations fell to 9–19 $\mu\text{mol/l}$ (median 13 $\mu\text{mol/l}$), but this difference was not significant. Thereafter there was a steady rise in plasma phenytoin concentrations to 20–43 $\mu\text{mol/l}$ (median 39 $\mu\text{mol/l}$). These values were significantly higher than the pre-azapropazone values ($P = 0.02$). At that point two of the volunteers complained of severe drowsiness, and although plasma phenytoin concentrations were clearly not yet at steady state, we decided to withhold phenytoin for a day and restart at a lower dose. At a new daily dose range of 75–175 mg (median 150 mg) plasma phenytoin concentrations reached a plateau of 13–34 $\mu\text{mol/l}$ (median 31 $\mu\text{mol/l}$). Phenytoin was again withheld for a day and the subsequent daily dose of phenytoin further reduced to 50–125 mg (median 100 mg). Plasma phenytoin concentrations then reached a plateau of 8–21 $\mu\text{mol/l}$ (median 19 $\mu\text{mol/l}$). Thus plasma phenytoin concentrations on a median daily dose of 100 mg during azapropazone therapy were similar to those on a median daily dose of 200 mg of phenytoin taken alone.

Phenytoin K_m , $V_{\max}$, and clearances (total and renal)

The K_m and $V_{\max}$ of phenytoin metabolism were calculated from the steady-state plasma concentrations

Figure 1 Plasma phenytoin concentrations (a), plasma total *p*-HPPH concentrations (b), and the ratio of *p*-HPPH/phenytoin (c) before and during treatment with azapropazone. Results and dosages of phenytoin are shown as median and complete range. The plasma phenytoin concentrations shown in the uppermost panel, measured by h.p.l.c., may be compared with the analysis by radioimmunoassay previously reported (Geaney *et al.*, 1982).

measured at three different doses before azapropazone treatment and at two different doses during azapropazone treatment. The values in each individual are given in Table 1 along with the corresponding values of total body and renal phenytoin clearances. Two examples of the original data are shown in direct linear plot in Figure 2. During azapropazone treatment the median K_m of phenytoin metabolism trebled ($P = 0.05$) but there was no significant change in the V_{max} .

In Figure 3 are shown the total phenytoin clearances at each dose, calculated for each individual, using the steady-state values of plasma phenytoin

concentration actually measured. As expected there was a linear decrease in total clearance with increasing daily dosage before the introduction of azapropazone (open symbols). After the introduction of azapropazone (closed symbols) total phenytoin clearances were lower and fell less with increasing daily dosage. The values of total phenytoin clearance, calculated from the measured K_m and V_{max} , and assuming a steady-state plasma phenytoin concentration of $20 \mu\text{mol/l}$ are given in Table 1. Total phenytoin clearance fell by 35–59% (median 48%, $P = 0.01$) after the introduction of azapropazone.

The K_i for inhibition of phenytoin metabolism by

Table 1 Phenytoin K_m , V_{max} , and total body and renal clearance before and during azapropazone treatment, and K_i of azapropazone

Subject	K_m ($\mu\text{mol/l}$)		K_i ($\mu\text{mol/l}$)	V_{max} (mg/day)		CL^* (ml/min)		CL_R (ml/min)	
	Before	During		Before	During	Before	During	Before	During
1	29.8	64.5	200	570	523	32	17	0.60	0.54
2	19.5	72.6	172	485	469	34	14	0.12	0.26
3	20.4	52.8	104	306	280	21	11	0.30	0.26
4	35.2	93.5	161	650	656	33	16		
5	12.5	22.0	417	239	193	20	13		
Median	20.4	64.5	172	485	469	32	14		
	$P = 0.05$			NS		$P = 0.01$		NS	

* Calculated for a steady-state phenytoin concentration of 20 $\mu\text{mol/l}$.

Figure 2 Direct linear plots of steady-state plasma phenytoin concentrations at different daily doses of phenytoin in subjects 1 and 2. — before azapropazone; ---- during azapropazone.

Figure 3 Relationship between total phenytoin clearance and daily dose of phenytoin at steady-state in all five subjects. Open symbols, before azapropazone; closed symbols, during azapropazone.

azapropazone was calculated using as inhibitor concentration the mean plasma azapropazone concentration at the times of phenytoin steady-state (Table 1). The values of K_i were 104–417 $\mu\text{mol/l}$ (median 172 $\mu\text{mol/l}$, 57 $\mu\text{g/ml}$). The K_i was generally lower than the plasma azapropazone concentrations found during treatment (see below).

Renal phenytoin clearance, in the three subjects in whom it was measured (Table 1), was low and did not change during azapropazone treatment ($P > 0.2$, t -test based on range).

Plasma *p*-HPPH concentrations

The changes in plasma total (i.e. conjugated and unconjugated) *p*-HPPH following the introduction of azapropazone are shown in Figure 1 (b). Immediately after the introduction of azapropazone there was a significant fall in plasma total *p*-HPPH concentrations ($P = 0.02$) but thereafter changes in plasma total *p*-HPPH concentrations mirrored the changes in plasma phenytoin concentrations. At their peak during the interaction, however, plasma total *p*-HPPH concentrations were still lower than pre-azapropazone *p*-HPPH concentrations, despite a doubling of plasma phenytoin concentrations. When azapropazone was withdrawn, plasma total *p*-HPPH concentrations rose significantly ($P = 0.02$). The relationship of these changes in plasma total *p*-HPPH concentrations to the changes in plasma phenytoin concentrations are better seen in the graph in Figure 1 (c), in which are plotted the changes in the ratio of total *p*-HPPH to phenytoin concentrations during the period of study. Following the introduction of azapropazone this ratio fell significantly ($P = 0.02$) but

then remained steady until azapropazone was withdrawn, when it rose significantly ($P = 0.02$).

Urinary phenytoin and *p*-HPPH recoveries

The total urinary recoveries of phenytoin and *p*-HPPH during a 24 h period at steady state, before and during azapropazone treatment in the three subjects studied are given in Table 2, both as absolute amounts recovered and as percentage recoveries related to the daily dose at steady state. These measurements were made at times when median plasma phenytoin concentrations were similar (17 $\mu\text{mol/l}$ before azapropazone, and 19 $\mu\text{mol/l}$ during azapropazone on half the steady-state phenytoin dose). Phenytoin urinary excretion as a percentage of total daily dose increased in all three cases ($P = 0.055$, t -test based on range, after arcsin transformation). There was a reduction in the urinary excretion of *p*-HPPH, but the recovery of *p*-HPPH as a percent of the administered dose was unchanged. This was because these measurements were made at times of equivalent plasma phenytoin concentrations, the dose of phenytoin being about half as high during the interaction as before the interaction.

Salivary phenytoin concentrations

Phenytoin concentrations in saliva before the introduction of azapropazone were 0.5–1.6 $\mu\text{mol/l}$ (median 1.3 $\mu\text{mol/l}$) and rose during azapropazone therapy to a maximum of 1.9–4.0 $\mu\text{mol/l}$ (median 3.8 $\mu\text{mol/l}$). The saliva:plasma ratio before azapropazone was 5–9% (median 8%) and this ratio was consistently raised during steady-state azapropazone treatment. For example, when salivary and plasma phenytoin concentrations were maximal, the saliva:plasma ratio was 8–11% (median 10%) ($P = 0.05$ compared with pre-azapropazone values) and similar values were found when both salivary and serum concentrations were lower.

Plasma azapropazone concentrations

Throughout the period of treatment with azapropazone plasma azapropazone concentrations were very variable, with median values varying between 250 $\mu\text{mol/l}$ (75 $\mu\text{g/ml}$) and 367 $\mu\text{mol/l}$ (110 $\mu\text{g/ml}$). However, there was no consistent pattern to the variation throughout the treatment period.

In vitro phenytoin protein-binding

In Figure 4 are shown the Scatchard plots for phenytoin plasma protein-binding *in vitro*, in the presence and absence of azapropazone. The apparent K_D of binding of phenytoin in the absence of azapropazone was 47.1 $\mu\text{mol/l}$ with a B_{max} of 1.25 mol phenytoin/

Table 2 Urinary recovery of phenytoin and *p*-HPPH during two dosage intervals at steady state before and during azapropazone treatment

Subject	Phenytoin		Total <i>p</i> -HPPH (free plus conjugated)	
	Before	During	Before	During
1	3.65 (1.83%)	2.89 (2.89%)	110.0 (55.0%)	37.0 (37.0%)
2	2.16 (1.44%)	2.02 (2.69%)	71.2 (47.5%)	36.4 (48.5%)
3	0.60 (0.30%)	1.78 (1.78%)	88.7 (44.4%)	41.1 (41.1%)
	P = 0.055		NS	

Data are given as mg/day (% of daily dose).

Measurements before and during azapropazone were made at times of equivalent plasma phenytoin concentrations, not equivalent phenytoin dosage.

mol of albumin, at an albumin concentration of 35 g/l. Azapropazone in concentrations of 167–500 $\mu\text{mol/l}$ (50–150 $\mu\text{g/ml}$) increased the apparent K_D but did not alter the B_{max} , suggesting competitive inhibition. The K_i of inhibition, calculated from the values of apparent K_D at the concentrations of azapropazone which we found during azapropazone therapy (see **Methods** above), was 964 $\mu\text{mol/l}$ (289 $\mu\text{g/ml}$). The *in vitro* free fraction of phenytoin at a phenytoin concentration of 40 $\mu\text{mol/l}$ in the absence of azapropazone was 7.3% and this increased to 10.0% in the presence of 250 $\mu\text{mol/l}$ (100 $\mu\text{g/ml}$) azapropazone.

Discussion

The addition of azapropazone during phenytoin administration increased steady-state plasma phenytoin concentrations at least two-fold at daily phenytoin doses of 125–250 mg. The possible mechanisms of this pharmacokinetic interaction include:

(1) *Increased absorption of phenytoin.* Although we have not studied this aspect, it is unlikely that it is a relevant mechanism, since the absorption of phenytoin (Epanutin, Parke-Davis) is virtually complete (Jusko *et al.*, 1976).

(2) *Decreased hepatic clearance of phenytoin.* We think that this is the most important feature of the interaction. The total body clearance of phenytoin (phenylphenylhydantoin), most of which is accounted for by *p*-hydroxylation in the liver to *p*-hydroxyphenylphenylhydantoin (*p*-HPPH) (see Richens, 1979), was halved during the interaction (Table 1). In contrast renal phenytoin clearance, which accounted for only about 1% of the total clearance, was unchanged. The changes in plasma total *p*-HPPH concentrations during the interaction suggest that the change in non-renal phenytoin clearance is due, at least in part, to inhibition of phenytoin *p*-hydroxylation, since the ratio of *p*-HPPH to phenytoin in the plasma decreased significantly during the interaction and stayed constant thereafter, rising again following

Figure 4 The effect of azapropazone on *in vitro* binding of [^3H]-phenytoin to human plasma proteins. Azapropazone concentrations ($\mu\text{mol/l}$): $\blacktriangle$ 0, $\blacksquare$ 167, $\bullet$ 250, $\square$ 333, $\circ$ 500.

the withdrawal of azapropazone (Figure 1). Since there was no change in the rate of excretion of *p*-HPPH relative to the steady-state phenytoin dosage during the interaction (Table 2), the fall in plasma *p*-HPPH concentrations cannot have been due to an increase in the rate of renal *p*-HPPH clearance, supporting the suggestion that it is due to decreased production of *p*-HPPH from phenytoin.

The increase in phenytoin K_m without change in V_{max} during the interaction (Table 1) also supports the suggestion that azapropazone inhibits phenytoin metabolism and furthermore suggests that that inhibition is *competitive*. In passing, one may note that azapropazone has been demonstrated by others to decrease the clearance of tolbutamide, probably by inhibition of its metabolism (Andreasen *et al.*, 1981).

The K_i for inhibition of phenytoin metabolism by azapropazone was 172 $\mu\text{mol/l}$ (Table 1), a value considerably lower than the azapropazone concentrations we found during the interaction. Thus, at the usual recommended doses of azapropazone, phenytoin *p*-hydroxylation is likely to be inhibited by at least 50%.

(3) *Protein-binding displacement.* The results of our *in vitro* and *in vivo* studies suggest that azapropazone causes a small degree of displacement of phenytoin from plasma proteins. In the *in vitro* studies of [^3H]-phenytoin protein binding, azapropazone reduced phenytoin binding from about 93% to about 90% at a phenytoin concentration of 40 $\mu\text{mol/l}$. The ratio of salivary phenytoin concentrations to plasma phenytoin concentrations showed a similar change during the interaction, and since salivary phenytoin concentrations are thought to be representative of unbound phenytoin concentrations in plasma (Knott *et al.*, 1982), this suggests that there is *in vivo* protein-binding displacement of phenytoin during the interaction.

This change in phenytoin plasma protein binding may have contributed to a small decrease in plasma phenytoin concentrations which we observed immediately after the introduction of azapropazone, since the rate of clearance of phenytoin, a drug with a low hepatic extraction ratio, is proportional to the unbound fraction in the plasma. This phenomenon is comparable with that observed by Neuvonen *et al.*

(1979) when they described the interaction of phenytoin with phenylbutazone and ascribed the changes in plasma phenytoin concentrations (similar to those we since observed here) to protein-binding displacement and inhibition of metabolism of phenytoin by phenylbutazone, which is related in structure to azapropazone.

Although any effect of azapropazone on total (bound and unbound) plasma phenytoin concentrations due to its effect in protein-binding displacement is outweighed by its effects on phenytoin clearance, it should be noted that interpretation of plasma phenytoin concentrations during the interaction must take into account the alteration in unbound fraction since the unbound concentration will be underestimated by at least 25% during the interaction.

(4) *Alteration in the apparent volume of distribution of phenytoin.* Displacement of phenytoin from its protein-binding sites will result in an increase in its apparent volume of distribution. However, we have not directly measured the apparent volume of distribution of phenytoin and are unable to say whether there is a net change during the interaction. Any change in the apparent volume of distribution of phenytoin which might have occurred would not, however, have affected steady-state plasma concentrations, which at any given dose depend only on total body clearance and systemic availability.

Conclusions

We believe that azapropazone inhibits the *p*-hydroxylation and therefore the metabolic clearance of phenytoin by competitive inhibition, and that that is the major mechanism for the interaction. We also believe that azapropazone displaces phenytoin from its sites of binding to plasma proteins and that that effect might alter one's interpretation of the steady-state plasma total phenytoin concentration.

In view of these effects we believe that azapropazone is better avoided in patients taking phenytoin.

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