Atovaquone has no effect on the pharmacokinetics of phenytoin in healthy male volunteers

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The potential pharmacokinetic interaction between atovaquone and phenytoin was investigated in 12 healthy male volunteers. Each volunteer received a single 600 mg oral dose of phenytoin in the two treatment periods. On one occasion phenytoin was taken alone and on the other after pre-treatment with 2000 mg atovaquone taken as two doses of 1000 mg as a microfluidized suspension. The mean ($\pm$ s.d.) peak plasma concentrations ($C_{\max}$), apparent total clearance (CL/F) and terminal half-life ($t_{1/2}$) for phenytoin when administered alone were 10.6 (1.8) mg l⁻¹, 24.3 (7.7) ml min⁻¹ and 25 (8) h, respectively. When administered together with atovaquone, phenytoin $C_{\max}$, CL/F and $t_{1/2}$ were 10.9 (2.0) mg l⁻¹, 23.8 ml min⁻¹ and 24 (6) h, respectively. There were no statistically significant differences in any of these plasma pharmacokinetic parameters. There were also no statistically significant differences in the fraction of circulating drug not bound to plasma protein or urinary excretion of 5-hydroxyphenyl-phenyl-hydantoin. In conclusion, there was no effect of atovaquone on the pharmacokinetics of phenytoin or its major metabolite after a single dose.

Keywords atovaquone phenytoin pharmacokinetics drug interactions

Introduction

The hydroxynaphthoquinone, atovaquone (2-*trans*-[4-(4'-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone) is a potent broad spectrum antiprotozoal compound with activity against *Plasmodium* species, *Pneumocystis carinii* and *Toxoplasma gondii* [1, 2].

Clinical trials in patients with mild or moderate *P. carinii* pneumonia (PCP) have suggested that atovaquone is nearly as effective as co-trimoxazole but has fewer haematological and other adverse effects [3]. The bioavailability of the tablet is limited due to low solubility and is dependent on concomitant dietary fat intake [4]. Thus a suspension with a very small particle size and superior bioavailability has been developed [5]. Atovaquone is >99.6% bound to plasma proteins.

Atovaquone has activity *in vitro* and *in vivo* against the tachyzoites and tissue cysts of *Toxoplasma gondii* in mice [6] and has shown promising efficacy in the treatment of cerebral toxoplasmosis in AIDS patients intolerant of standard therapies [7]. Such patients are likely to be receiving concomitant treatment with an

anticonvulsant, commonly phenytoin. In addition, a study in HIV infected patients has indicated that atovaquone may reduce the clearance of the anti-retroviral drug, zidovudine, through inhibition of glucuronidation [10].

As atovaquone and phenytoin are likely to be given together in the management of cerebral toxoplasmosis and both drugs are highly bound to plasma proteins, the possible effects of atovaquone on phenytoin pharmacokinetics were examined.

Methods

Study population

Twelve healthy male volunteers aged 20–30 years (median 24 years), height 167–194 cm (median 183 cm) and weight 56–92 kg (median 70 kg) were recruited. The protocol was approved by Medeval's independent Ethics Committee.

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Study design

This was an open, randomised, balanced two period cross-over design in which 12 volunteers received a single 600 mg oral dose of phenytoin (Epanutin® 100 mg capsules, Parke-Davis) on two occasions. On one occasion phenytoin was taken alone, whilst on the other it was taken after pre-treatment with atovaquone as a microfluidised suspension. In the latter case, each volunteer took an oral dose of 1000 mg atovaquone with food (a standard meal containing 23 g fat) on the evening prior to the study day and a further 1000 mg dose was taken with food at the same time as phenytoin. There were at least 3 weeks between study occasions, to allow the complete elimination of atovaquone.

Analytical methods

Phenytoin was analysed in plasma by h.p.l.c. with u.v. (210 nm) absorbance detection. Plasma (250 µl) was mixed with 250 µl perchloric acid (1.6 M), to precipitate plasma proteins. After centrifugation, 20 µl of supernatant was injected onto a Spherisorb S3 ODS2 column. The mobile phase was 980 ml 0.1 M phosphate buffer, 550 ml methanol and 240 ml acetonitrile, flowing at 0.75 ml min⁻¹. The limit of quantification was 1.23 mg l⁻¹.

Phenytoin and total HPPH (i.e. the sum of both unconjugated and conjugated HPPH) were analysed in urine simultaneously under similar h.p.l.c. conditions. Urine was first hydrolysed, then extracted with ethyl acetate: dichloromethane, (1:2). Unconjugated HPPH was extracted as above, omitting the hydrolysis step. The limit of quantification was 11.1 mg l⁻¹ for total HPPH and 0.37 mg l⁻¹ for unconjugated HPPH in urine. The extent of plasma protein binding of phenytoin was assessed by ultrafiltration.

Atovaquone was assayed in plasma using a validated h.p.l.c. assay, with a limit of detection of 0.25 mg l⁻¹ [11].

Data analysis

Phenytoin $C_{\max}$ (peak plasma concentration), $t_{\max}$ (time to peak plasma concentration), $AUC_{0-\infty}$ (total area under the plasma concentration-time curve), $t_{\frac{1}{2},z}$ (terminal half-life) CL/F (apparent total plasma clearance) and V/F (apparent volume of distribution, estimated from the terminal phase) were estimated using standard methods [12].

The cumulative amount of metabolite HPPH excreted in the urine over all the collection intervals was calculated, both for total HPPH ($A_{e(tot)}$) and unconjugated HPPH ($A_{e(u)}$). The total amount of conjugated HPPH ($A_{e(c)}$) was then calculated as the difference between $A_{e(tot)}$ and $A_{e(u)}$.

The fraction unbound in plasma was calculated for phenytoin at $t = 0, 2, 10, 24$ and 72 h using the ratio of unbound to total phenytoin concentration for each plasma sample. Trapezoidal area under the five unbound

plasma concentration-time points (AUC_{unb}) was calculated for each subject, for comparative purposes.

Phenytoin pharmacokinetic parameters were compared by analysis of variance on the log transformed data (GLM procedure of SAS for Windows, version 6.10, SAS Institute, Cary, NC 27513, USA). Urinary $A_{e(c)}$ and $A_{e(u)}$ were compared without log transformation. Total plasma phenytoin $t_{\max}$ values were compared using Wilcoxon's test.

Results

Mean total plasma phenytoin concentration-time data are plotted in Figure 1. The mean ($\pm$ s.d.) pharmacokinetic parameters for phenytoin (Table 1) were very similar in the presence and absence of atovaquone. The mean percentage of phenytoin in plasma not bound to proteins was 6.7% in the absence and 7.0% in the presence of atovaquone. Atovaquone total plasma concentrations ranged between 1.5 and 25.9 mg l⁻¹, well within PCP therapeutic levels. No phenytoin was detected in urine. There were no statistically significant differences (significance level $P = 0.05$) in the parameters $C_{\max}$, $t_{\max}$, $t_{\frac{1}{2},z}$, AUC , CL/F or V/F for total plasma phenytoin, AUC_{unb} for unbound plasma phenytoin, or urinary A_e for either conjugated or unconjugated HPPH.

Discussion

All 12 subjects successfully completed both periods of the study. Both treatments were well tolerated and there were no clinically important adverse experiences. Phenytoin pharmacokinetics agreed well with previous reports [7]. As the mean peak concentration of both drugs were within their respective therapeutic ranges, a clinically important pharmacokinetic interaction between atovaquone and phenytoin in clinical use is remote. Furthermore, atovaquone does not appear to

Figure 1 Mean ($\pm$ s.d.) plasma phenytoin concentration-time data. ■, phenytoin alone. ▲, phenytoin with atovaquone.

Table 1 Mean ($\pm$ s.d.) plasma pharmacokinetic parameters of phenytoin and urinary excretion of its major metabolite, HPPH.

	Phenytoin alone	Phenytoin with atovaquone	95% confidence interval
Phenytoin			
$C_{\max}$ (mg l ⁻¹)	10.57 (1.84)	10.93 (1.97)	0.99–1.08 ^b
$t_{\max}$ (h)	6.0 (3–10) ^a	4.0 (3–10) ^a	–5–1 ^c
AUC _{unb} (mg l ⁻¹ h)	21.7 (11.0)	22.4 (12.1)	0.88–1.22 ^b
AUC (mg l ⁻¹ h)	456 (163)	464 (152)	0.95–1.10 ^b
$t_{1/2}$ (h)	25 (8)	24 (6)	0.91–1.09 ^b
CL/F (ml min ⁻¹)	24.3 (7.7)	23.8 (8.2)	0.91–1.06 ^b
V_z/F (l)	48 (9)	46 (9)	0.94–1.00 ^b
HPPH			
$A_{e(\text{tot})}$ (mg)	269 (86)	277 (94)	–44.4–61.7 ^d
$A_{e(u)}$ (mg)	4.0 (1.8)	4.3 (1.5)	1.10–1.57 ^d
$A_{e(c)}$ (mg)	265 (85)	273 (93)	–43.4–60.2 ^d

^a Median (range).^b Ratio of parameter in the presence of atovaquone against the parameter in the absence of atovaquone.^c Interval for median difference between treatments.^d Interval for difference between treatments.

inhibit the glucuronidation of HPPH. The explanation may be that, due to the high degree of plasma protein binding (99.6%), the free concentration of atovaquone was not high enough to lead to inhibition of the enzyme. Unbound atovaquone was not quantifiable, as concentrations achieved were too low. In conclusion, there was no effect of atovaquone on the pharmacokinetics of phenytoin or its major metabolite after a single dose. Hence a clinically important pharmacokinetic interaction is unlikely.

References

- Hudson AT, Randell AW, Fry M, *et al.* Novel antimalarial naphthoquinones with potent broad spectrum antiprotozoal activity. *Parasitol* 1985; **90**: 45–55.
- Fry M, Pudney M. Site of action of the antimalarial hydroxy naphthoquinone, 2-trans[4-(4'-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone (566C80). *Biochem Pharmacol* 1992; **43**: 1545–1553.
- Hughes W, Leoung G, Kramer F, *et al.* Comparison of atovaquone (566C80) with trimethoprim-sulfamethoxazole to treat *Pneumocystis carinii* pneumonia in patients with AIDS. *N Engl J Med* 1993; **328**: 1521–1527.
- Spencer CM, Goa KL. Atovaquone. A review of its pharmacological properties and therapeutic efficacy in opportunistic infections. *Drugs* 1995; **50**: 176–196.
- Pozniak A, Yoganathan K, McNicol S, *et al.* Improved bioavailability of atovaquone from a new suspension formulation in healthy volunteers and HIV infected patients [abstract]. *9th International Conference on AIDS*, Berlin, June 1992.
- Araujo FA, Huskinson J, Remington JS. Remarkable *in vitro* and *in vivo* activities of the hydroxynaphthoquinone 566C80 against tachyzoites and tissue cysts of *Toxoplasma gondii*. *Antimicrob Agents Chemother* 1991; **35**: 293–299.
- Kovacs JA. Efficacy of atovaquone in the treatment of toxoplasmosis in patients with AIDS. *Lancet* 1992; **340**: 637–638.
- Perucca E, Richens A. Drug interactions with phenytoin. *Drugs* 1981; **21**: 120–137.
- Richens A. Clinical pharmacokinetics of phenytoin. *Clin Pharmacokin* 1979; **4**: 153–169.
- Lee BL, Taüber MG, Sadler B, *et al.* Atovaquone inhibits the glucuronidation and increases the serum concentrations of zidovudine [abstract]. In *34th Interscience Conference on Antimicrobial Agents and Chemotherapy* (1994): 81.
- DeAngelis DV, Long JD, Kanics LL, Woolley JL. High performance liquid chromatographic assay for the measurement of atovaquone in plasma. *J Chromatogr* 1994; **652**: 211–219.
- Rowland M, Tozer TN. *Clinical pharmacokinetics: Concepts and applications*. 2nd edition, 1989; Lea & Febiger, Philadelphia and London.

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