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Examination of some factors responsible for a food-induced increase in absorption of atovaquone

P. E. ROLAN, A. J. MERCER, B. C. WEATHERLEY, T. HOLDICH, H. MEIRE[1], R. W. PECK, G. RIDOUT & J. POSNER
Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS and [1]Department of Radiology, Kings College Hospital, Denmark Hill SE5 9RS

1 Atovaquone is a potent antiprotozoal slowly and irregularly absorbed after administration as tablets to fasting volunteers. A series of studies was performed to investigate the effects of food, bile and formulation on atovaquone absorption.

2 In 18 healthy male volunteers, a high-fat breakfast administered 45 min before 500 mg atovaquone as tablets increased AUC by 3.3-fold (95% CI 2.8–4.0) and C_{max} 5.3-fold (4.3–6.6) compared with fasting.

3 The absorption of atovaquone from tablets was examined in 12 healthy male volunteers after an overnight fast, following toast alone, toast with 28 g butter (LOFAT), or toast with 56 g butter (HIFAT). Compared with absorption when fasted, toast had no significant effect but LOFAT increased AUC 3.0-fold (2.1–4.2) and C_{max} 3.9-fold (2.6–5.8). HIFAT increased AUC 3.9-fold (2.7–5.5) and C_{max} 5.6-fold (3.8–8.4).

4 The absorption of atovaquone was examined in nine healthy fasting male volunteers from tablets, an aqueous suspension, and an oily solution/suspension in miglyol (fractionated coconut oil). Compared with tablets, AUC following the aqueous suspension was increased 1.7-fold (1.0–2.7) and C_{max} 2.4-fold (1.7–3.5). Following miglyol, AUC was increased to the same extent but C_{max} was only increased 1.8-fold (1.2–2.6).

5 Atovaquone absorption was examined in eight healthy fasting male volunteers following an i.v. infusion of cholecystokinin octapeptide (CCK-OP) which decreased gallbladder volume by 82% (73%–90%) on occasion 1 or saline on occasion 2. AUC(0,12) was increased following CCK-OP by 1.6-fold (1.1–2.4) and C_{max} by 1.5-fold (0.98–2.4). However, total AUC was not significantly increased (mean ratio 1.2, 95% CI 0.85–1.8).

6 In four healthy male volunteers who received 750 mg atovaquone containing 100 μCi [^{14}C]-atovaquone as a suspension following a high-fat meal, no drug was detected in chylomicrons separated from plasma.

7 The substantial increase in absorption of atovaquone from tablets after a fatty meal can be accounted for quantitatively by the fat content of the meal. Absorption from suspensions in water and miglyol was superior to that from tablets but both were inferior to the tablets following a high fat meal. Bile release may make some contribution to the food effect. Food probably increases atovaquone absorption by increasing its solubility in the gut lumen.

Keywords atovaquone 566C80 absorption miglyol cholecystokinin food

Correspondence: Dr P. E. Rolan, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

Introduction

Atovaquone (566C80; 2-hydroxy-3-*trans*-4′(4-chlorophenyl)cyclohexyl-1,4-naphthoquinone; Figure 1) is a potent antiprotozoal compund which inhibits mitochondrial pyrimidine biosynthesis [1]. *In vitro* it has activity against *Plasmodia*, *Pneumocystis* and *Toxoplasma* species [1, 2, 3], and efficacy has been confirmed in *P. carinii* pneumonia in AIDS patients, with a low incidence of adverse reactions [4, 5].

An initial pharmacokinetic study in fasting healthy volunteers with a tablet formulation of atovaquone showed that absorption was slow and irregular and that AUC increased less than proportionally with doses above 450 mg (unpublished data). In asymptomatic HIV-infected patients taking atovaquone with food there was considerable variation both within and between subjects in plasma drug concentrations [6]. In AIDS patients being treated for *P. carinii* pneumonia, dosing regimens of 750 mg two, three or four times daily with food produced similar steady-state plasma drug concentrations [4]. These data suggest that the absorption of atovaquone is variable and limited, which may be due to its extremely low aqueous solubility. In 133 AIDS patients treated for *P. carinii* pneumonia with a fixed dose of 750 mg atovaquone three times a day with food there was an approximately 10-fold variation in steady-state plasma drug concentration. A close relationship was demonstrated between steady-state plasma drug concentration and the probability of therapeutic response. The overall efficacy rate was 62% and the mean concentration was 13.9 μg ml^{-1}: it was estimated that the response rate could be increased to 95% if a steady-state drug concentration of > 15 μg ml^{-1} could be achieved. Hence a moderate improvement in absorption could have significant clinical benefit. We have now examined the effect of some modifications of the gastrointestinal environment on the absorption of atovaquone.

Methods

Protocols

All studies were performed in healthy male volunteers aged 19–48 years following satisfactory screening within 2 weeks of the start of the study, consisting of history, medical examination, ECG, haematology, plasma biochemistry, urinalysis and a negative urinary screen for drugs of abuse. Studies 1, 2, 4 and 5 were approved by the Wellcome Protocol Review Committee and the Ethics Committee of Camberwell Health Authority; study 3 was approved by the BIOS Ethics Committee. Volunteers gave written consent following a full written and oral explanation of the study.

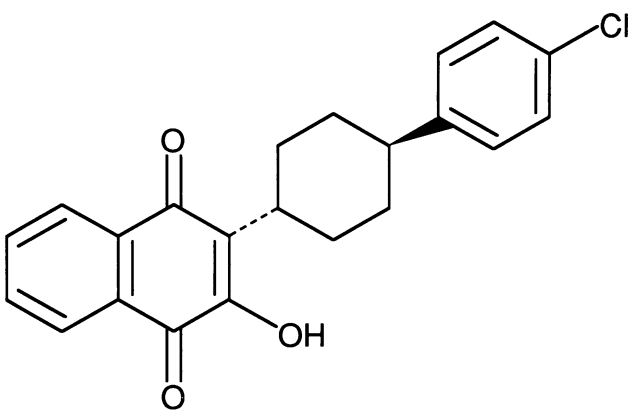

Figure 1 Structure of atovaquone.

The dose of atovaquone was 500 mg per occasion in studies 1–4 and 750 mg in study 5. All atovaquone formulations were prepared by the Pharmaceutical Development Laboratories, Wellcome Foundation Ltd, Dartford, Kent. All tablets used contained 250 mg atovaquone.

Study 1

The objective of this study was to examine the effect of food on atovaquone absorption. It was of randomised, balanced, two-limb, crossover design in 18 healthy volunteers. Atovaquone was administered as two 250 mg tablets, either following an overnight fast or 45 min after eating a standard high fat breakfast. The meal consisted of 100 ml orange juice, 100 ml homogenised milk, 25 g cereal (cornflakes), 1 boiled egg, 2 slices of white toast, 15 g butter and 30 g marmalade. The total fat content was 23 g, carbohydrate content 97 g and protein content 18 g. Blood samples were taken before drug dosage and at 1, 2, 3, 4, 6, 8, 10, 12, 24, 32, 48, 56, 72, 96, 192, 264, 360, 432 and 528 h after dosage.

Study 2

The objective of this study was to examine whether the effect of food observed in study 1 could be accounted for by the fat content of the meal. It was of randomised, balanced, incomplete block, crossover design. Twelve healthy volunteers who had fasted overnight took part on three occasions and received two 250 mg tablets of atovaquone under three of the following four dietary conditions: fasted, following 2 slices of toast, following 2 slices of toast with 28 g butter (LOFAT) or 2 slices of toast with 56 g butter (HIFAT). The amount of butter used in the LOFAT limb contained 23 g fat, equal to that in the standard breakfast. Meals were given at 45 min before drug ingestion (as in study 1). Blood samples were taken before drug dosage and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 24, 32, 48, 72, 96, 120, 168, 264 and 336 h thereafter.

Study 3

The objective of this study was to examine the absorption of atovaquone from an oily vehicle in which the drug was partly in solution. A suspension of 500 mg atovaquone in 30 ml miglyol was used. Miglyol (fractionated coconut oil) was selected as it had the highest solubility of atovaquone (4 mg ml^{-1}) of the edible oils examined and is an acceptable pharmaceutical excipient. The volume of 30 ml was very similar to the amount of butter used in study 2, and it was calculated

that approximately one quarter of the atovaquone would be in solution, the remainder being in suspension. An aqueous suspension of the same dose of atovaquone and volume using 0.25% w/v methylcellulose as a suspending agent was used as another suspension control in addition to two 250 mg tablets. Nine healthy volunteers took part, and received each formulation after an overnight fast, according to a randomised balanced three-way crossover design. Blood samples were taken at the same times as in study 2.

Study 4

The objective of this study was to assess the contribution of native bile to the increase in absorption of atovaquone observed with food and fat. The study was of single-blind, two limb, crossover design in eight volunteers who had fasted overnight. On the first occasion bile release was stimulated by the synthetic cholecystokinin octapeptide (CCK-OP; 'Kinevac', E. R. Squibb, Montreal, Canada). An i.v. infusion of CCK-OP at the rate of 75 pmol kg^{-1} h^{-1} was delivered for 30 min by a syringe pump using a glass syringe commencing 45 min prior to drug ingestion. This rate was selected as it had previously been demonstrated to produce a rate of gallbladder emptying comparable with that after a high fat test meal [7]. Gallbladder volume was assessed by abdominal ultrasound [8] to ensure that adequate contraction (> 55% decrease in volume) had been attained before atovaquone administration. A saline infusion of similar volume was given on the second occasion and gallbladder volume was also measured. On each occasion volunteers received two 250 mg tablets of atovaquone. Blood samples were taken at the same times as on study 2 with the addition of samples at 0.5, 1.5, 2.5 h and omission of samples at 32 h.

Study 5

As part of a metabolism study the extent of uptake of atovaquone into chylomicrons was examined. Four healthy male volunteers received a 25 ml suspension of 750 mg atovaquone containing 100 μCi [^{14}C]-atovaquone, 45 min after a high fat meal similar to that in study 1. Blood samples for chylomicron preparation were taken at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after drug dosage.

Sample handling and assay

Following blood sampling, plasma was separated rapidly and frozen at −20° C until analysis. Atovaquone in plasma was measured using a GC assay with electron-capture detection in study 1 and an h.p.l.c. assay for studies 2–4. To separate chylomicrons in study 5, 4 ml plasma were mixed with 2 ml 0.9% w/v NaCl solution (d = 1.006 g ml^{-1}) in a 10 ml clear ultracentrifuge tube. The mixture was overlayered with a further 4 ml NaCl solution and centrifuged at 22 000 *g* for 30 min in a Beckman L855 centrifuge with a 50TI rotor at a temperature of 16° C. The top 4 ml containing the chylomicrons were then aspirated and frozen before assay [9].

Assay 1: GC-ECD

Five μl of a 20 μg ml^{-1} solution of internal standard (*trans*-2-hydroxy-3-(4-phenylcyclohexyl)-1,4-naphthoquinone) in 1% v/v dimethylformamide in methanol, plasma (0.1 to 0.5 ml) and 0.5 ml 0.5 M acetic acid were briefly vortex mixed and extracted twice with 5 ml 2% v/v isoamyl alcohol in *n*-hexane. The extracts were dried under nitrogen at 60° C and methylated at room temperature for 5 min with 0.9 ml of 0.3 M diazomethane in diethyl ether using 100 μl of methanol as a reaction aid. The dried derivatives were resuspended in 100 μl of 1% v/v dimethylformamide in toluene. One μl was injected onto a 25 m × 0.25 mm i.d. methylsilicone capillary column and the analytes were detected by a ^{63}Ni-electron capture detector. The concentrations of the test samples were interpolated from a plot of peak height analyte:internal standard ratio against atovaquone concentration constructed from a set of calibrating standards covering the expected concentration range. The method was linear over the range 0.01 to 2.0 μg ml^{-1} of atovaquone and precision was < 15% at all concentrations in this range. Samples above this range were diluted into range. The bias was < 5% for quality control samples (n = 43) at 0.34 μg ml^{-1}.

Assay 2: H.p.l.c.-u.v.

Twenty μl of a 100 μg ml^{-1} solution of internal standard (*trans*-2-hydroxy-3-(4-phenylcyclohexyl)-1,4-naphthoquinone) in 1% v/v dimethylformamide in methanol, plasma (0.25 to 0.5 ml) and 0.5 ml 0.5 M acetic acid were briefly vortex mixed and extracted with 5 ml 2% v/v isoamyl alcohol in *n*-hexane. The extracts were dried under nitrogen at 60° C and resuspended in 200 μl methanol. Ten μl were injected on to a 20 cm × 3 mm i.d. column packed with 5 μm Chrompack Chromspher C-8 reverse phase material (London, UK). The mobile phase was 70:30 acetonitrile:0.4% TFA (aq) v/v pumped at 0.6 ml min^{-1}. Atovaquone concentrations were calculated as in Assay 1. The method was linear over the range 0.05 to 40 μg ml^{-1} and precision was < 15% at all concentrations in this range. The bias was < 3% for quality control samples (n = 129) at 5 μg ml^{-1}.

Assay of radiolabelled atovaquone in plasma and chylomicrons

Radioactivity in plasma and separated chylomicrons was measured by liquid scintillation counting (Beckman LS5000CE and LS5081).

Data analysis

Pharmacokinetic analysis was by standard non-compartmental methods. Total AUC was calculated as $AUC(0,t) + C(t)/\lambda_z$, where $AUC(0,t)$ was calculated by the linear trapezoidal method, $C(t)$ was the concentration at the last time point t and λ_z was calculated by linear regression of the terminal part of the log drug concentration-time curve (Siphar, Simed, Creteil, France). For statistical analysis of AUC and C_{max} data, individual results were log transformed and a full

analysis of variance taking into account variation due to subjects, periods and treatment was performed. Point estimates and 95% confidence intervals were calculated for the difference between a formulation/dietary condition, compared with the control limb of tablets/fasted. These point estimates and confidence intervals were then back transformed to give ratios of AUC and C_{max} between treatments. Median values of t_{max} were calculated, and a 95% non-parametric confidence interval for the difference in median t_{max} between treatments was calculated.

Results

Geometric mean and standard deviation AUC, C_{max} and median and range t_{max} data, with confidence intervals, are presented in Table 1 and mean concentration-time curves for the four studies are shown in Figures 2–5. In study 1, there was an increase of 3.3-fold in the extent of absorption of atovaquone following the fatty breakfast. In study 2, toast had little effect but LOFAT produced a significant 3-fold increase in absorption, and HIFAT produced a further improvement to 3.9-

Table 1 Pharmacokinetic summary of studies 1 to 4

Study	*Formulation/Diet*	*Mean (s.d.) AUC ($\mu g\ ml^{-1}\ h$)*	*Mean (95% CI) for AUC ratio compared with tablets fasted*	*Mean (s.d.) C_{max} ($\mu g\ ml^{-1}$)*	*Mean (95% CI) for C_{max} ratio compared with tablets fasted*	*Median (range) t_{max} (h)*	*95% CI for t_{max} difference compared with tablets fasted*
1	Tablets/Fasted	94 (46)		0.82 (0.34)	–	28 (2–56)	–
1	Tablets/Fed	288 (77)	3.3 (2.8–4.0)	4.4 (2.5)	5.3 (4.3–6.6)	7 (3–56)	
2	Tablets/Fasted	121 (74)	–	1.5 (1.3)	–	5 (3–120)	–
2	Tablets/Toast	147 (144)	1.2 (0.86–1.7)	1.7 (0.6)	1.2 (0.8–1.8)	5 (2–48)	
2	Tablets/LOFAT	356 (165)	3.0 (2.1–4.2)	5.7 (1.3)	3.9 (2.6–5.8)	4 (2–5)	
2	Tablets/HIFAT	469 (152)	3.9 (2.7–5.5)	8.3 (2.0)	5.6 (3.8–8.4)	4 (3–6)	
3	Tablets/Fasted	86 (43)	–	1.1 (0.6)	–	5 (2–48)	–
3	Aqueous/Fasted	144 (48)	1.7 (1.0–2.7)	2.7 (1.1)	2.4 (1.7–3.5)	2 (1–4)	−15 to −6
3	Miglyol/Fasted	144 (124)	1.7 (1.0–2.7)	2.0 (1.8)	1.8 (1.2–2.6)	10 (7–32)	−2 to +7
4	Tablets/Fasted	148 (59)	–	1.1 (0.37)	–	20	–
	Saline	7.4 (6.3); AUC(0,12)				(4–72)	
4	Tablets/Fasted	180 (47)	1.2 (0.85–1.8)	1.7 (0.90)	1.5 (0.98–2.4)	2.5	−42 to +19
	CCK-OP	11.8 (14); AUC(0,12)	1.6 (1.1–2.4)			(1.5–96)	

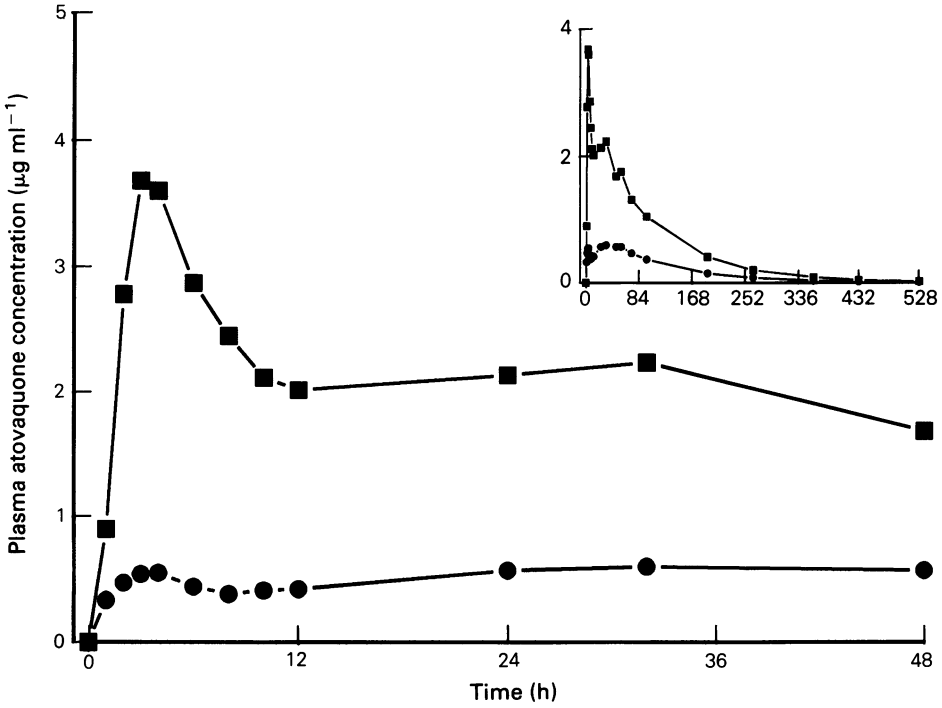

Figure 2 Mean plasma concentrations of atovaquone over 48 h (main figure) and 528 h (inset) in study 1. Atovaquone (500 mg) was administered fasted (•) or 45 min after a high-fat meal (■).

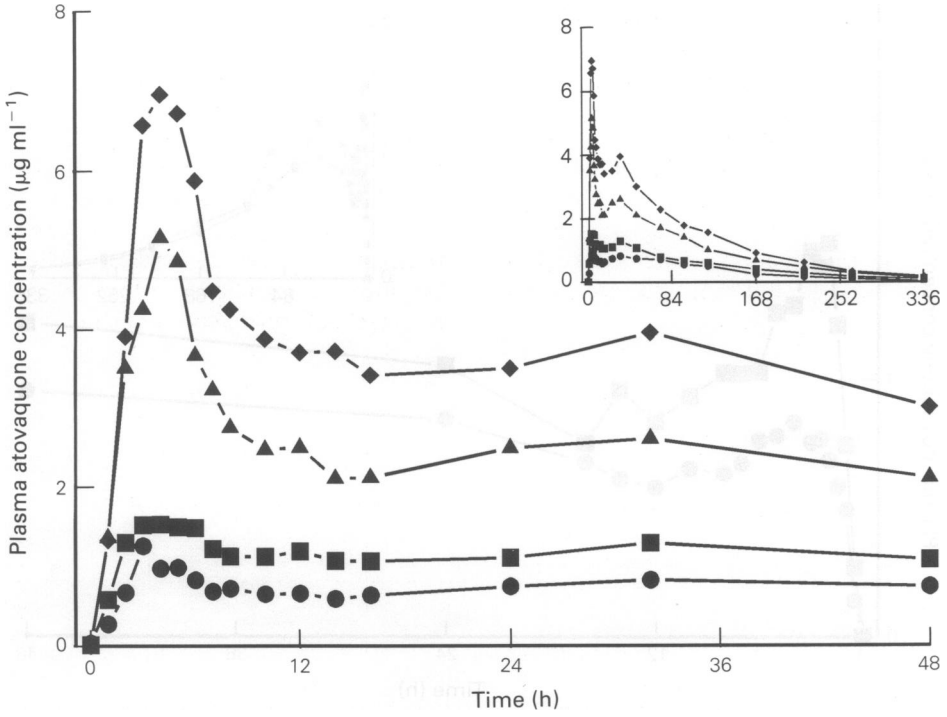

Figure 3 Mean plasma concentrations of atovaquone over 48 h (main figure) and 336 h (inset) in study 2. Atovaquone (500 mg) was administered fasted (●) or 45 min after toast alone (■), toast with 28 g butter (LOFAT) (▲) or toast with 56 g butter (HIFAT) (◆).

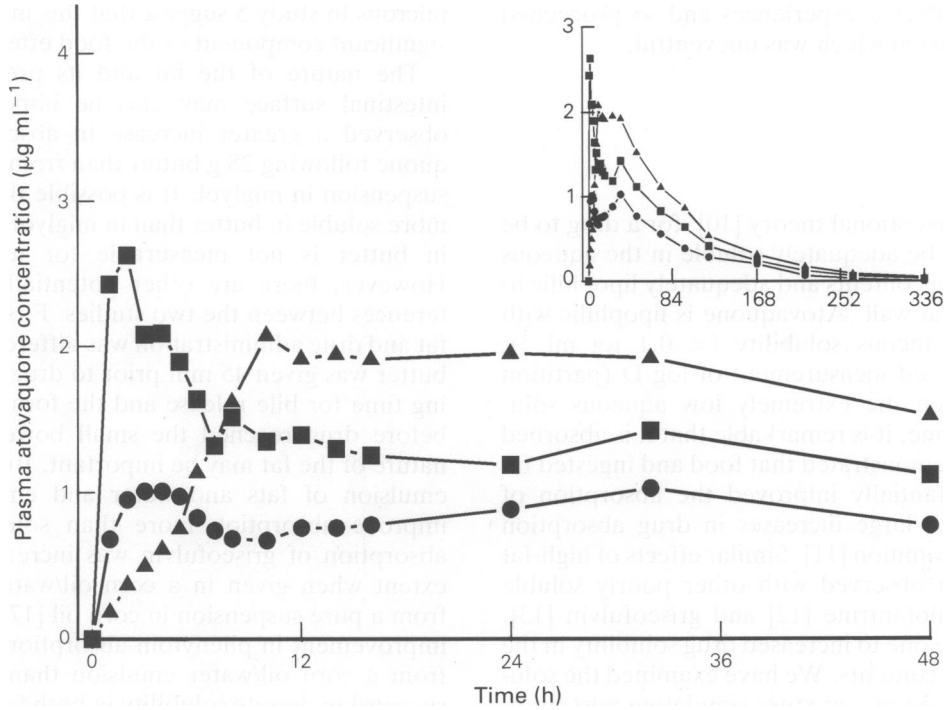

Figure 4 Mean plasma concentrations of atovaquone over 48 h (main figure) and 336 h (inset) in study 3. Atovaquone (500 mg) was administered fasted as tablets (●), an aqueous suspension (■) or an oily suspension (▲).

fold. In study 3, similar increases in absorption (1.7-fold) occurred following both suspensions, smaller than that following food. In study 4, gallbladder volumes decreased by 82% (95% CI: 73–90%) after CCK-OP but increased non-significantly after saline. Absorption over the first 12 h following the CCK-OP infusion was increased 1.6-fold (1.1–2.4) compared with saline. In study 5, plasma concentrations of total radioactivity were attributable to atovaquone alone but no radioactivity was detected in chylomicrons.

There were no adverse experiences attributable to atovaquone. The fatty meals and formulations in studies 2 and 3 were also well tolerated. CCK-OP was well tolerated during the infusion but one volunteer reported faintness, headache and nausea between 5 and 8 h from dosing. He had previously received atova-

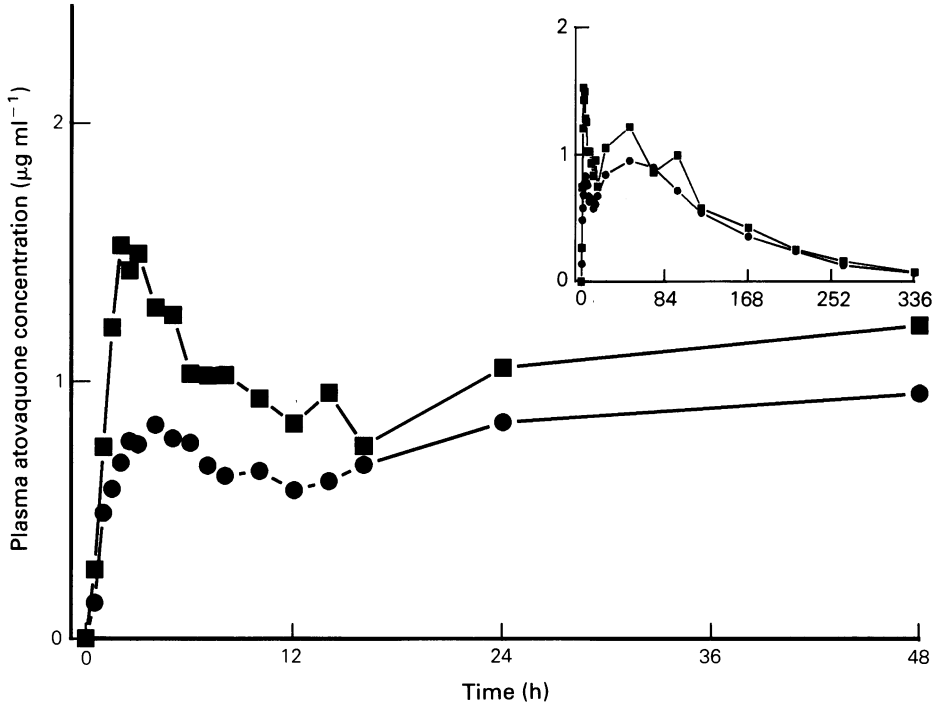

Figure 5 Mean plasma concentrations of atovaquone over 48 h (main figure) and 336 h (inset) in study 4. Atovaquone (500 mg) was administered fasted as tablets following an infusion of CCK-OP (■) or saline (●).

quone without adverse experiences and so proceeded to the saline occasion which was uneventful.

Discussion

According to conventional theory [10], for a drug to be absorbed it must be adequately soluble in the aqueous medium of the gut contents and adequately lipophilic to cross the intestinal wall. Atovaquone is lipophilic with extremely low aqueous solubility (< 0.1 μg ml^{-1}), which has precluded measurement of log D (partition coefficient). Given the extremely low aqueous solubility of atovaquone, it is remarkable that it is absorbed at all. We have demonstrated that food and ingested fat (as butter) substantially improved the absorption of atovaquone. Such large increases in drug absorption with food are uncommon [11]. Similar effects of high-fat meals have been observed with other poorly soluble drugs such as halofantrine [12] and griseofulvin [13]. This is likely to be due to increased drug solubility in the post-prandial gut contents. We have examined the solubility of atovaquone in a mixture simulating post-prandial gut contents [14, 15]. The solubility of atovaquone in the micellar phase was as high as 0.1 mg ml^{-1}, provided that drug was added after the formation of micelles, confirming significantly increased solubility (unpublished observations). Digestible lipid can also increase the absorption of lipophilic substances by causing the formation of chylomicrons in the intestinal cell. The chylomicrons containing dissolved drug are then transported into the intestinal lymphatics and then to the systemic circulation. This has been demonstrated to be an important route of absorption for alpha-tocopherol [16]. The absence of atovaquone in chylomicrons in study 5 suggest that this mechanism is not a significant component of the food effect.

The nature of the fat and its presentation to the intestinal surface may also be important. We have observed a greater increase in absorption of atovaquone following 28 g butter than from a 30 ml solution/suspension in miglyol. It is possible that atovaquone is more soluble in butter than in miglyol but the solubility in butter is not measurable for technical reasons. However, there are other potentially significant differences between the two studies. Firstly, the timing of fat and drug administration was different. In study 2 the butter was given 45 min prior to drug ingestion, allowing time for bile release and the formation of micelles before drug reached the small bowel. Secondly, the nature of the fat may be important. Butter is a complex emulsion of fats and water and emulsions seem to improve absorption more than solutions. Thus the absorption of griseofulvin was increased to a greater extent when given in a corn oil/water emulsion than from a pure suspension in corn oil [17]. Also, a greater improvement in phenytoin absorption in rats was seen from a corn oil/water emulsion than from a corn oil suspension despite solubility in both formulations being the same [18]. Miglyol also differs from butter in that it is a medium chain triglyceride which produces a lower stimulus for bile release than the longer chain fats in butter. A study in rats on the absorption of the lipophilic pesticide DDT [19] has also shown that miglyol improved absorption less than a longer chain oil (arachis oil), although both improved absorption compared with an aqueous suspension. Thus lipids of different chain lengths improve the absorption of poorly aqueous soluble lipophilic compounds to different extents.

The improvement in absorption from the aqueous suspension in study 3 compared with tablets was not

expected as *in vitro* tests had shown good disintegration of the tablets. The superior absorption from the aqueous suspension is probably due to its finer dispersion allowing faster dissolution of the drug. Preliminary results from a pharmacokinetic study in HIV infected patients at risk for *P. carinii* pneumonia using an atovaquone suspension of reduced particle size confirm improved absorption [20]. Mean (range) steady-state plasma drug concentrations in six patients receiving 1000 mg twice daily were 23.1 (13.3–33.1) $\mu g\ ml^{-1}$ when drug administration was separated by at least 2 h from food. This compares favourably with the mean concentration of 13.9 $\mu g\ ml^{-1}$ using 2250 mg daily of the tablets with food [5].

Bile has been demonstrated to be an important determinant of absorption of some other poorly soluble lipophilic substances such as cyclosporin A [21], tetracycline [22] and vitamins A, D, E, and K [23]. However, the effect of bile in study 4 was modest, perhaps partly because of poor mixing of the bile with the tablet granules as suggested above but also probably because no dietary fat was present so that there would be little micelle formation. It is possible that a greater effect of the CCK-OP infusion would have been seen in study 4 if the drug had been given as a suspension, to maximise the likelihood of bile mixing well with the drug.

In conclusion, the absorption of atovaquone can be improved substantially by modifying the gastrointestinal environment. Improved solubility with dietary fat, particularly in an emulsion, results in the greatest increase. Not all oils and fats increase absorption equally. Bile release stimulated by food may make a contribution to the food effect. An aqueous suspension is better absorbed than tablets. The potential effect of food on drug absorption should be considered early in the development of poorly soluble drugs as the effect can be clinically significant.

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References

1 Hammond DJ, Burchell JR, Pudney M. Inhibition of pyrimidine biosynthesis *de novo* in *Plasmodium falciparum* by 2-(4-*t*-butylcyclohexyl)-3-hydroxy-1,4-naphthoquinone *in vitro*. *Mol Biochem Parasitol* 1985; **14**: 97–109.

2 Hughes WT, Gray VL, Gutteridge WE, Latter VS, Pudney M. Efficacy of a hydroxynaphthoquinone 566C80 in experimental *Pneumocystis carinii* pneumonitis. *Antimicrob Agents Chemother* 1990; **34**: 225–228.

3 Huskinson-Mark J, Araujo FG, Remington JS. Evaluation of the effect of drugs on the cyst of *Toxoplasma gondii*. *J infect Dis* 1991; **164**: 101–171.

4 Falloon J, Kovacs J, Hughes W, *et al.* A preliminary evaluation of 566C80 for the treatment of Pneumocystis pneumonia in patients with the acquired immunodeficiency syndrome. *New Engl J Med* 1991; **325**: 1534–1538.

5 Hughes W, Leoung, Kramer F, *et al.* Comparison of atovaquone (566C80) with trimethoprim-sulphamethaxazole to treat *Pneumocytsis carinii* pneumonia in patients with AIDS. *New Engl J Med* 1993; **328**: 1521–1527.

6 Hughes WT, Kennedy W, Shenep JL, *et al.* Safety and pharmacokinetics of 566C80, a hydroxynaphthoquinone with anti-*Pneumocystis carinii* activity: A Phase I study in human immunodeficiency virus (HIV)-infected men. *J infect Dis* 1991; **163**: 843–848.

7 Forgacs IC, Maisey MN, Murphy GM, Dowling RH. Influence of gallstones and ursodeoxycholic acid therapy on gallbladder emptying. *Gastroenterology* 1984; **87**: 299–307.

8 Palframan A, Meire HB. Real-time ultrasound. A new method for studying gall-bladder kinetics. *Br J Radiol* 1979; **52**; 801–803.

9 Mills GL, Lane PA, Weech PK. *A guide to lipoprotein technique*. New York: Elsevier, 1984.

10 Rowland M, Tozer TN. *Clinical pharmacokinetics*, 2nd edition. Philadelphia: Lea and Feabiger, 1989.

11 Welling PG. Effects of food on drug absorption. *Pharmac Ther* 1989; **43**: 425–441.

12 Milton KA, Edwards G, Ward SA, Orme ML, Breckenridge AM. Pharmacokinetics of halofantrine in man: effects of food and dose size. *Br J clin Pharmac* 1989; **28**: 71–77.

13 Kabasakalian P, Katz P, Rosencrantz B, Townley E. Paramaters affecting absorption of griseofulvin in a human subject using urinary extraction data. *J pharm Sci* 1970; **59**: 595–600.

14 Badley BW, Murphy GM, Bouchier IAD. Intraluminal bile-salt deficiency in the pathogenesis of steatorrhoea. *Lancet* 1969; **ii**: 400–402.

15 Hofmann AF. The function of bile salts in fat absorption. The solvent properties of dilute micellar solutions of conjugated bile salts. *Biochem J* 1963; **89**: 57–68.

16 Traber MG, Ingold KU, Burton GW, Kayden HJ. Absorption and transport of deuterium-sustained 2*R*,4′R,8′*R*-α-tocopherol in human lipoproteins. *Lipids* 1988; **23**: 791–797.

17 Bates TR, Sequeira JA. Bioavailability of micronized griseofulvin from corn oil-in-water emulsion, aqueous suspension, and commercial tablet dosage forms in humans. *J pharm Sci* 1975; **64**: 793–797.

18 Chakrabarti S, Belpaire FM. Bioavailability of phenytoin in lipid containing dosage forms in rats. *J Pharm Pharmac* 1978; **30**: 330–331.

19 Palin KJ, Wilson CG, Phillips AJ. The effects of oils on the lymphatic absorption of DDT. *J Pharm Pharmac* 1982; **34**: 707–710.

20 Pozniak A, Yoganathan K, McNicol S, Dixon R, Posner J, Rolan P. Improved bioavailability of atovaquone from a suspension formulation in HIV infected patients. *Abstract for the IXth International Conference on AIDS*, Berlin, June 1993.

21 Mehta MU, Venkataramanan R, Burckart GJ, *et al.* Effects of bile on cyclosporin absorption in liver transplant patients. *Br J clin Pharmac* 1988; **25**: 579–584.

22 Aukee S, Venho VM, Jussila J, Karjalainen P. Drug absorption in patients with T-tube after cholecystectomy. *Ann clin Res* 1975; **7**: 42–46.

23 Forsgren L. Studies on the intestinal absorption of labelled fat-soluble vitamins (A, D, E and K) via the thoracic-duct lymph in the absence of bile in man. *Acta Chirug Scand* Supplement 1969; **399**: 1–29.

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