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Antipneumocystis Activity of 17C91, a Prodrug of Atovaquone

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The prophylactic efficacy of 17C91, a carbamate prodrug of atovaquone (ATQ), was investigated in a severe combined immunodeficient mouse model of *Pneumocystis carinii* pneumonia (PCP). At an oral dosage equivalent to 100 mg of ATQ per kg of body weight per day, 17C91 protected 9 of 10 mice from PCP and had a prophylactic efficacy comparable to that of co-trimoxazole (at 250 mg of sulfamethoxazole plus 50 mg of trimethoprim per kg per day orally). The intensity of *P. carinii* infection (infection score) of mice treated with 17C91 correlated with the concentration of ATQ in the plasma, with clearance of the infection associated with plasma ATQ levels of >35 μg/ml. 17C91 given orally provided enhanced levels of ATQ in the plasma compared with the conventional ATQ formulation. Additional studies reported in this paper demonstrate that the prophylactic activity of 17C91 against PCP in severe combined immunodeficient mice is comparable to that of a new oral microparticulate formulation of ATQ.

Atovaquone (ATQ) (566C80, Mepron, or Wellvone) has shown clinical activity in the treatment of malaria (12) and two of the most important microbial opportunistic infections in AIDS patients, *Pneumocystis carinii* pneumonia (PCP) and toxoplasmosis (4, 6, 7, 9). Advantages associated with ATQ are its excellent safety record in AIDS patients, its long half-life, its oral route of administration, and its novel mechanism of action. However, oral dosing has been complicated by variable plasma ATQ levels, which were an important determinant of therapeutic outcome (6). In clinical trials with a conventional tablet formulation, it was observed that a therapeutic response against PCP depended on steady-state levels in plasma; the same relationship may also exist for toxoplasmosis (13). In addition, maximal absorption occurs when ATQ is taken with a meal, preferably one with a high fat content (7, 9).

ATQ prodrugs have been investigated at The Wellcome Research Laboratories as one approach to improving oral bioavailability by raising aqueous solubility. These studies led to the synthesis of 17C91, a novel carbamate prodrug of ATQ (Fig. 1) (8, 15). We now report the prophylactic evaluation of 17C91 in an experimental severe combined immunodeficient (SCID) mouse model of PCP (1) and present data which show that when 17C91 is given orally it results in enhanced plasma ATQ levels, which were associated with improved efficacy relative to the administration of the conventional ATQ formulation.

MATERIALS AND METHODS

PCP mouse model. Full details of this model have recently been reported (1, 2). Briefly, the protocol involved starting *Pneumocystis*-free female SCID mice (weight, 20 to 25 g) on dexamethasone dissolved in the drinking water (at 2 mg/liter) 7 days prior to intratracheal inoculation with a single dose of cryopreserved mouse *P. carinii* (~20,000 cysts). Each drug or control group consisted of at least 10 mice. Drugs were evaluated for prophylaxis by administration once a day from day 1 postinfection until day 42 postinfection (i.e., 42 doses). Drugs were administered orally, by gavage, except for our positive control, Septrin, which was given in the drinking water. All drug trials were terminated on day 43 postinfection, 24 h after the last drug dose. Mice were anesthetized with halothane and killed by cervical dislocation, the lungs were removed en bloc, and impression smears were prepared. The presence of *P. carinii* in lung impression smears was rapidly and unambiguously identified by immunofluorescence. The intensity of the PCP was graded by scanning the impression smears and assigning, on a semilogarithmic basis, one of the following infection scores: 0, no infection evident; 1, very weak infection; 2, mild infection; 3, moderate infection; 4, heavy infection. Scores were an agreed-upon assessment, reached following two independent blind examinations. Results are presented as the calculated mean infection scores (±standard error of the mean) and the ratio of the number of mice infected with *P. carinii* to the total number of mice remaining in each group at the end of the experiment. Since the data did not follow a pattern of normal distribution, nonparametric statistics (Mann-Whitney U test) were used to compare the infection scores of selected individual groups in the same drug study.

Drugs. Micronized ATQ (batch ref., Q20M; Wellcome) was suspended in 0.25% (wt/vol) celacol (methylcellulose) in distilled water and ball milled for 48 h prior to dosing to ensure even suspension. ATQ oral microparticulate suspension (ATQ suspension) (batch ref., WPDL/92/0036/86) (median particle diameter, 1.4 μm, with 90% of the particles <2.3 μm) was diluted with an inert suspending vehicle and briefly mixed prior to use. Fresh drug suspensions were used each week and were kept refrigerated prior to use. 17C91 (batch WB ref., WDCP 91/18/52/1 and batch CH ref., ZTAA/89/63/33a; Wellcome) was dissolved in distilled water and was soluble at all concentrations used in this study (up to 30 mg/ml). Doses of 17C91 were adjusted to the equivalent dose of ATQ liberated in solution. A fresh drug solution of 17C91 was made daily and administered to mice within 1 h of preparation. All mice were dosed orally (by gavage) with 0.1 ml of drug suspension or solution per 10 g of body weight. Septrin (sugar-free pediatric suspension, Lot no. A6604A, containing 200 mg of sulfamethoxazole plus 40 mg of trimethoprim per 5 ml of flavored suspension; Wellcome) was diluted with 12.5 ml of suspension added per 250 ml of drinking water. Diluted Septrin solution in the water bottles was changed every Monday, Wednesday, and Friday. The amount of Septrin added, to give the required dosage of 250 mg of sulfamethoxazole plus 50 mg of trimethoprim per kg of body weight per day, was calculated with the assumption that each mouse drinks a minimum of 2.5 ml per day.

Plasma ATQ concentration. At the end of some studies (24 h after the last dose), mice anesthetized with halothane were exsanguinated by cardiac puncture, and plasma was collected. Plasma was stored frozen before quantitation of the ATQ levels. Following solvent extraction from the plasma, the ATQ concentration was determined by a reversed-phase high-pressure liquid chromatography method, involving UV detection (14).

RESULTS

17C91 at a dosage equivalent to 100 mg of ATQ per kg per day protected 9 of 10 mice from PCP, was well tolerated, showed significantly better oral activity compared with that of ATQ itself (the conventional micronized drug suspended in 0.25% celacol), and showed activity comparable to that of Septrin (250 mg of sulfamethoxazole plus 50 mg of trimethoprim per kg per day) (Table 1). The effect of 17C91 on the prophylaxis of mouse PCP was dose related insofar as

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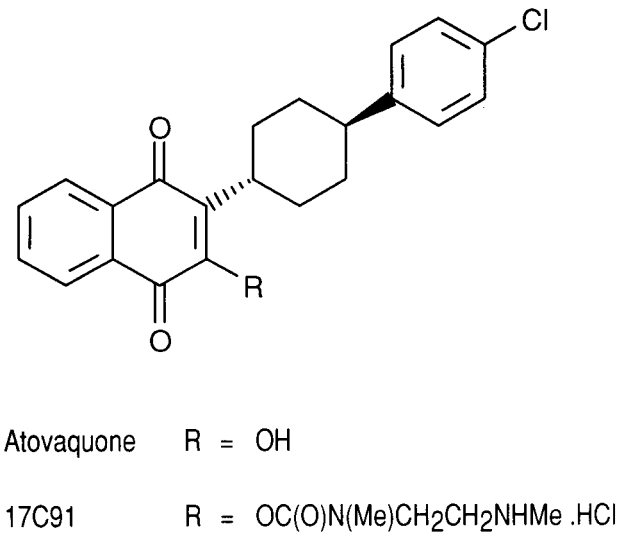

FIG. 1. Structure of ATQ and its carbamate prodrug, 17C91.

lower doses were less effective (50 mg/kg) or inactive (25 mg/kg). The prophylactic efficacy of 17C91 was proportional to the levels of ATQ in the plasma of *P. carinii*-infected SCID mice taken 24 h after the last dose of 17C91 (Fig. 2). At a dosage of 17C91 equivalent to 100 mg of ATQ per kg per day, the levels of ATQ in mouse plasma taken 24 h after the last dose (i.e., steady-state trough levels) were approximately threefold higher with 17C91 compared to that with ATQ (micronized drug). Plasma ATQ levels after administration of 17C91 at a dose equivalent to 50 mg of ATQ per kg were similar to those obtained after dosing with ATQ (conventional micronized drug) at 100 mg/kg. None of the intact 17C91 was observed in plasma at any dose level. The intensity of the PCP (infection score) in mice treated with 17C91 correlated with the concentration of ATQ in the plasma, and clearance of *P. carinii* (infection score, 0) was associated with levels in plasma of >35 μg/ml (mean level, ~56 μg/ml) (Fig. 3). In a separate study, the comparative efficacies of 17C91 and the ATQ suspension were evaluated against PCP prophylaxis in the SCID mouse

TABLE 1. Effect of 17C91[a] and conventional ATQ (micronized drug suspended in 0.25% celacol) on the prophylaxis of PCP in SCID mice

Group no.	Drug	Dosage (mg/kg/day p.o.)[b]	Infection score		No. of mice infected/total[d]	Statistical significance[e]
			Mean	SEM[c]		
1	None (control)		3.60	0.21	10/10	
2	17C91	100	0.10	0.09	1/10	B, C
3	17C91	50	1.90	0.30	9/10	
4	17C91	25	3.20	0.13	10/10	A
5	Micronized ATQ	100	2.44	0.17	9/9	
6	Septrin	250-50	0.10	0.09	1/10	

[a] 17C91 batch WB.
[b] Dosages of 17C91 are quoted with respect to ATQ content; dosage of Septrin is given as milligrams of sulfamethoxazole/milligrams of trimethoprim. p.o., orally.
[c] SEM, standard error of the mean.
[d] Total, total number of mice at the end of the experiment.
[e] A, not significantly different ($P < 0.05$) from results for the controls (group 1); B, significantly different ($P < 0.05$) from values obtained with the same dosage of micronized ATQ (group 5); C, not significantly different ($P > 0.05$) from values obtained with Septrin (group 6).

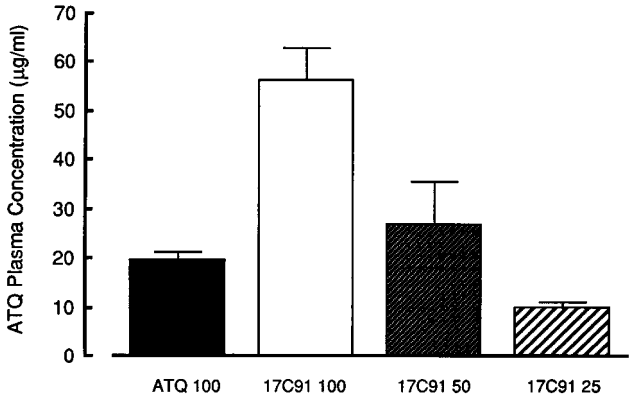

FIG. 2. Levels of ATQ in the plasma of *P. carinii*-infected SCID mice after receiving 17C91 or conventional ATQ (micronized drug suspended in 0.25% celacol) prophylaxis as detailed in Table 1. Mouse plasma samples were taken 24 h after the last dose of 17C91 or ATQ. Results are mean drug levels in plasma ± standard error of the mean. Dosages are in milligrams per kilogram per day orally, and for 17C91 they are quoted with respect to ATQ content.

(Table 2). Overall, 17C91 had activity comparable to that of the ATQ suspension, but the efficacy of 17C91 was marginally superior at the 100-mg/kg dose. The activity of 17C91 at doses delivering 200 and 100 mg of ATQ per kg was similar to that of Septrin (250 mg of sulfamethoxazole plus 50 mg of trimethoprim per kg), while the ATQ suspension at 100 mg/kg was less active than Septrin (Table 2). The small differences in the data for activity of 17C91 presented in Tables 1 and 2 are probably due to biological variability between studies and variation between the batches of 17C91 used.

DISCUSSION

ATQ has low oral bioavailability, which has been partly attributed to poor water solubility (0.05 μg/ml at pH 7.4). In contrast, 17C91, a carbamate prodrug of ATQ, has significantly improved water solubility (15). ATQ is released from 17C91 by a pH-dependent mechanism that does not rely on enzyme activity and occurs very rapidly at physiological pH ($t_{1/2}$, ~3 min at pH 7.4) (15). Animal studies (with rats and dogs) have confirmed that 17C91 provides enhanced levels of ATQ in plasma and improved bioavailability compared with ATQ itself

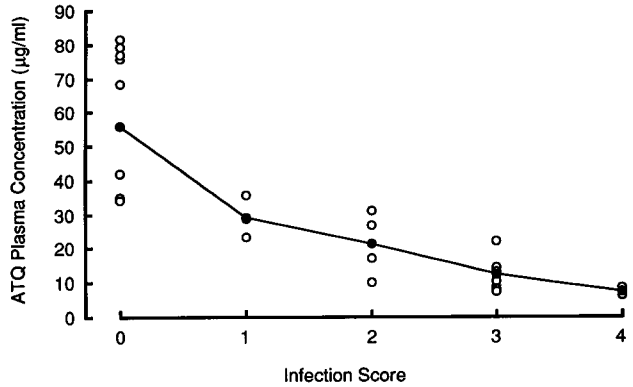

FIG. 3. Concentration of ATQ in the plasma of *P. carinii*-infected SCID mice taken 24 h after the last dose of 17C91 matched with the extent of PCP as assessed by the infection score (see Materials and Methods for details). Each datum point on the plot represents one 17C91-treated SCID mouse. The line is plotted through the mean plasma drug concentration at each infection score.

TABLE 2. Effect of 17C91[a] and ATQ suspension on the prophylaxis of PCP in SCID mice

Group no.	Drug	Dosage (mg/kg/day p.o.)[b]	Infection Score		No. of mice infected/total[d]	Statistical significance[e]
			Mean	SEM[c]		
1	None (control)		3.55	0.15	11/11	
2	17C91	200	0.10	0.09	1/10	B
3	17C91	100	0.60	0.25	4/10	A, B
4	17C91	50	2.70	0.20	10/10	
5	ATQ suspension	200	0.36	0.15	4/11	B
6	ATQ suspension	100	2.09	0.16	11/11	
7	ATQ suspension	50	2.90	0.17	10/10	
8	Septrin	250-50	0.11	0.10	1/9	

[a] 17C91 batch CH.
[b] Dosages of 17C91 are quoted with respect to ATQ content; dosage of Septrin is given as milligrams of sulfamethoxazole/milligrams of trimethoprim. p.o., orally.
[c] SEM, standard error of the mean.
[d] Total, total number of mice at the end of the experiment.
[e] A, significantly different ($P < 0.05$) from values obtained with the same dosage of ATQ suspension (group 6); B, not significantly different ($P > 0.05$) from values obtained with Septrin (group 8).

(15). In dogs, the bioavailability of ATQ after oral administration of 17C91 was enhanced, with peak levels in plasma two to three times higher than that seen after dosing with ATQ (15). Evaluation of 17C91 against PCP in the SCID mouse demonstrated that it was possible to improve upon the prophylactic activity of micronized ATQ itself and achieve an efficacy comparable to that of co-trimoxazole (Septrin at 250 mg of sulfamethoxazole plus 50 mg of trimethoprim per kg). At a dosage of 17C91 delivering the equivalent of 100 mg of ATQ per kg per day, the levels of ATQ in mouse plasma taken 24 h after the last dose (i.e., steady-state trough levels) were approximately threefold higher with 17C91 than with the conventional ATQ (micronized drug). Interestingly, the clearance of PCP in SCID mice was associated with a mean plasma ATQ level of ~56 μg/ml. This value is not too dissimilar from the result of previous studies of ATQ in the rat (10), where PCP was cleared when the ATQ levels were >60 μg/ml. Clearly, to achieve these plasma ATQ levels (after a dose of 100 mg of micronized ATQ per kg), the pharmacokinetic profile of ATQ in the rat must be markedly different from that in the SCID mouse.

In conclusion, our studies with 17C91 have confirmed the usefulness of the PCP SCID mouse model (1) and have demonstrated that by reformulating ATQ as a prodrug it is possible to improve upon the prophylactic efficacy of conventional ATQ (micronized drug), because of the enhanced bioavailability. 17C91 is currently in preclinical development, and one of the issues being addressed is the fate and potential for toxicity of metabolites arising from the carbamate moiety.

An alternative approach to improving the oral absorption of ATQ which has been investigated at Wellcome is the development of the oral microparticulate suspension. The latter is a homogeneous suspension of ATQ formulated in an inert suspending vehicle, with the majority of the drug particles at a defined particle size (~1.5 μm). Additional studies mentioned in this paper demonstrate that the prophylactic activity of 17C91 against PCP in SCID mice is comparable to that of the ATQ suspension. Clinical trials for prophylaxis of PCP with the ATQ suspension are now under way (3).

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