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Effect of Atovaquone and Atovaquone Drug Combinations on Prophylaxis of *Pneumocystis carinii* Pneumonia in SCID Mice

JOHN C. W. COMLEY* AND ALETHEA M. STERLING

Experimental Biology Group, Biology Division, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, United Kingdom

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The prophylactic efficacies of atovaquone (ATQ) alone and in combination with azithromycin, clarithromycin, rifabutin, proguanil, PS-15, trimethoprim, co-trimoxazole, or dapsone were investigated in a SCID mouse model of *Pneumocystis carinii* pneumonia (PCP). ATQ alone was shown to have a significant dose-related effect, and at 200 mg/kg of body weight per day administered orally, the efficacy of ATQ was comparable to that of Septrin (co-trimoxazole). Of the drugs investigated orally in combination with ATQ, only dapsone (25 mg/kg/day) and to a lesser extent PS-15 (5 mg/kg/day) had any noteworthy antipneumocystis activity (at the doses examined) when administered alone. ATQ drug combinations affected the prophylactic efficacy of a subcurative dosage of ATQ (50 mg/kg/day given orally) in the following ways: dapsone (25 mg/kg/day) or co-trimoxazole (25 mg of sulfamethoxazole plus 5 mg of trimethoprim per kg/day) had no significant effect on ATQ, azithromycin (200 mg/kg/day) or clarithromycin (200 mg/kg/day) had a slight additive effect with ATQ, trimethoprim (100 mg/kg/day) or PS-15 (5 mg/kg/day) had an additive effect with ATQ, and proguanil (25 mg/kg/day) or rifabutin (200 mg/kg/day) had a marked synergistic effect on ATQ. The last result was particularly noteworthy as neither proguanil nor rifabutin was effective against PCP when administered alone. None of the drugs examined antagonized the prophylactic activity of ATQ in experimental PCP in SCID mice. The results suggest that clinical trials of ATQ with synergistic drug combinations may now be justified, particularly if such drug combinations improve ATQ's efficacy and broaden its spectrum of activity.

The hydroxynaphthoquinone atovaquone (ATQ) (566C80, Mepron, or Wellvone) is now marketed in the United States, Canada, South Africa, and many European Union countries for the oral treatment of mild to moderate *Pneumocystis carinii* pneumonia (PCP) in AIDS patients, who do not tolerate co-trimoxazole (COTRIM). The original demonstration of efficacy of ATQ against PCP was established with an experimental rat model of *P. carinii* infection (11). This animal model of PCP involved a chemically immunosuppressed latent infection by *P. carinii* in rats that were not virus free or guaranteed to be free of specific pathogens and in the case of ATQ was shown to be predictive of antipneumocystis activity in humans (7, 9). Subsequently, work carried out at The Wellcome Research Laboratories, Beckenham, Kent, United Kingdom, using this rat model confirmed that in vivo, ATQ was the most active of a range of hydroxynaphthoquinone analogs (8). Investigations with ATQ and other novel drugs served to highlight the inadequacies of this rat model, namely, the marked variation between rats in infection, the susceptibility of immunosuppressed rats to secondary infections, the difficulty in the United Kingdom of obtaining rats harboring a latent *Pneumocystis* infection, the reduced susceptibility of alternative virus-free rats to *P. carinii* infection, and the large amount of drug required (100 g or more) for limited investigation. Combined, these difficulties severely hampered work on the experimental evaluation and optimization of ATQ and led to a decision at Beckenham to seek an alternative PCP model in mice. Uppermost in our thoughts was the desire to significantly reduce (by about 90%) the amount of compound required for the experimental evaluation of novel drugs.

We recently reported the development and optimization of a mouse model for PCP involving intratracheal infection of severely combined immunodeficient (SCID) mice with a cryopreserved inoculum of mouse *P. carinii* (6). This model was validated through the evaluation of COTRIM, and evidence suggesting limited synergy between sulfamethoxazole (SMX) and trimethoprim (TMP) (the components of COTRIM) was presented (6).

We now describe our experiences with this SCID mouse model of PCP in the prophylactic evaluation of ATQ alone and of ATQ administered in combination with a range of other antimicrobial agents. ATQ drug combinations have so far been extensively investigated in experimental toxoplasmosis (1, 2, 13) and against *Plasmodium falciparum* in vitro (3), but to date we are aware of no comparable investigations of ATQ against experimental PCP. The latter is of some importance in the chemotherapy of opportunistic infections, since increasingly AIDS patients are receiving a cocktail of drugs, and it is desirable to establish at the preclinical level whether any of these drugs may affect the antipneumocystis activity of ATQ. If drug combinations are shown to lack antagonism or be synergistic, significant reduction in their dosages might be possible, which may improve drug tolerance and patient compliance in severely immunocompromised patients. Increasingly, it is feared that it is only a matter of time before drug-resistant strains of *P. carinii* appear, and the use of appropriate synergistic drug combinations may reduce the onset of resistance to ATQ or may possibly improve ATQ's efficacy and broaden its spectrum of activity.

* Corresponding author.

TABLE 1. Effect of ATQ on prophylaxis of PCP in SCID mice

Group no.	Drug	Dose(s) (mg/kg/day p.o.)	Infection score		No. of mice infected/total[a]	Statistical significance[b]
			Mean	SEM		
1	None (control)		3.90	0.09	10/10	
2	ATQ	200	0.36	0.15	4/11	B
3	ATQ	100	2.09	0.16	11/11	
4	ATQ	50	2.90	0.17	10/10	
5	ATQ	25	3.45	0.20	11/11	A
6	Septrin	250/50[c]	0.11	0.10	1/9	

[a] Total number of mice remaining at the end of the experiment.
[b] A, not significantly different ($P > 0.05$) from results for the controls (group 1); B, not significantly different ($P > 0.05$) from values obtained with Septrin (group 6).
[c] 250 mg of SMX and 50 mg of TMP.

MATERIALS AND METHODS

PCP mouse model. Prophylactic antipneumocystis activity in SCID (C.B.-17Icr *cru-scid*) mice artificially infected with *P. carinii* was determined. Investigations outlining the development, optimization, and validation of this PCP mouse model have recently been fully reported elsewhere (see reference 6 for further details of the assay methodology). Briefly, the protocol involved starting *Pneumocystis*-free female SCID mice (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 treatment 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). All drugs were administered orally (p.o.), by gavage, except for our positive control Septrin, which was given in the drinking water. In combination studies, mice were always dosed with ATQ first, with the second drug administered immediately afterwards. 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, and the lungs were removed en bloc and impression smears were prepared. The presence of *P. carinii* in lung impression smears was rapidly and unambiguously determined 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 assessment reached following two independent blind examinations. Results are presented as the calculated mean infection scores (± standard errors of the means [SEM]) and the ratios 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) was used to compare the infection scores of selected individual groups in the same drug study.

Drugs. TMP (Sigma), COTRIM (SMX [Sigma] and TMP mixed at a fixed ratio of 5:1), dapsone (DAP) (Sigma), PS-15 [*N*-3-(2,4,5-trichlorophenoxypropyloxy)-*N'*-(1-methylethyl)imidocarbonimidic diamide hydrochloride] (12) (batch reference ZTAA/89/63/151a; Wellcome), clarithromycin (CLARI) (lot no. 37-024 VC; Abbott Laboratories), azithromycin (AZITH) (lot no. 17419-131-1F; Pfizer), and rifabutin (RIF) (batch 3027F155; Pharmacia Farmitalia Carlo Erba) were all suspended in 0.25% (wt/vol) celacol (methylcellulose) in distilled water and ball-milled for 48 h prior to dosing to ensure even suspension. Proguanil (PROG) (paluridine HCl; reference PD/RS 708/46; Zeneca) was all dissolved in distilled water. ATQ oral microparticulate suspension (batch reference WPDL/92/0036/86 [volume median diameter, 1.4 μm; 90% of particles <2.3 μm in diameter] and batch reference WPDL/93/0046/83 [volume median diameter, 1.4 μm; 90% of particles <2.9 μm in diameter]; Wellcome) was diluted with inert suspending vehicle. All mice were dosed p.o. with 0.1 ml of drug suspension or solution per 10 g of body weight. Fresh drug suspensions or solutions were prepared weekly and were kept refrigerated prior to use. Septrin (Wellcome; sugar-free pediatric suspension, lot no. A6604A, containing 200 mg of SMX plus 40 mg of TMP per 5 ml of flavored suspension) 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 dose of 250 mg of SMX and 50 mg of TMP per kg of body weight per day, was calculated on the assumption that each mouse drinks a minimum of 2.5 ml per day.

RESULTS

Effect of ATQ alone on the prophylaxis of mouse PCP. Prophylactic studies confirmed that ATQ had potent antipneumocystis activity at 200 mg/kg/day administered p.o. by gavage; the mean infection score was 0.36 versus 3.90 for the untreated control, with the infection cleared in 7 of 11 mice (Table 1). A dose-related effect was observed: lower doses of ATQ were less effective (100 mg/kg, mean infection score of 2.09; 50 mg/kg, mean infection score of 2.90) or ineffective (25 mg/kg, mean infection score of 3.45). In the same study, our positive control, Septrin (250 mg of SMX plus 50 mg of TMP per kg per day), was almost completely effective in the prophylaxis of mouse PCP (mean infection score, 0.11), with the infection cleared in eight of nine mice. In other studies, Septrin was slightly less effective (see Tables 2 to 5). The effects of ATQ (at 200 mg/kg) and Septrin, however, were not statistically significantly different. In additional studies (data not given), the effect of a subcurative dosage of ATQ (100 mg/kg/day p.o.) was discernible after 2 weeks of dosing, but when ATQ prophylaxis was withdrawn the infection rapidly recrudesced.

Prophylactic activity of ATQ in combinations with other drugs. On the basis of results obtained with ATQ alone, we selected 25 and 50 mg of ATQ per kg as appropriate drug levels at which to investigate drug combinations with ATQ. It was argued that in order to demonstrate synergy with ATQ we would need to evaluate ATQ at a dose which was subcurative and only partially effective in PCP prophylaxis. The antimicrobial agents to be combined with ATQ were used either at or very close to the maximum dose tolerated in SCID mice if no antipneumocystis effect was expected or at a dose at which the antipneumocystis effect was predicted (from the literature or earlier investigation) to be marginal. For the purpose of this study, synergy is defined as activity of the drug mixture being greater than the sum of the activities of the individual drugs on their own.

ATQ with COTRIM or DAP. The results of studies of ATQ in combination with COTRIM or DAP are presented in Table 2. COTRIM alone at 25 and 5 and at 5 and 1 mg of SMX and TMP, respectively, per kg had only a marginal effect on prophylaxis of PCP in SCID mice. COTRIM in combination with ATQ (groups 7, 8, 10, and 11) showed no evidence of synergy or antagonism. In all cases, the combinations were statistically no more efficacious than the ATQ (most active component). DAP alone was strongly active in the prophylaxis of mouse PCP at 25 mg/kg; the mean infection score was 0.70, and 4 of 10 mice were cleared of the infection (group 6). Combinations of DAP and ATQ (groups 9 and 12) showed no significant difference from the effect of DAP treatment alone, i.e., they were not synergistic.

ATQ with TMP. The results of studies of ATQ in combination with TMP are presented in Table 3. The range of concentrations of TMP evaluated was chosen to encompass both higher and lower concentrations than that encountered in the standard dose of COTRIM (i.e., 50 mg/kg). TMP alone (groups 3 through 5) had a slight effect which was statistically significant at 5 and 50 mg/kg. All combinations of ATQ and

TABLE 2. Effect of ATQ in combination with COTRIM or DAP on prophylaxis of PCP in SCID mice

Group no.	Drug(s)	Dose(s) (mg/kg/day p.o.)[a]	Infection score		No. of mice infected/total[b]	Statistical significance[c]
			Mean	SEM		
1	None (control)		3.70	0.14	10/10	
2	ATQ	50	2.60	0.15	10/11	
3	ATQ	25	3.36	0.19	11/11	
4	COTRIM	25/5	3.20	0.13	10/10	
5	COTRIM	5/1	3.40	0.21	10/10	A
6	DAP	25	0.70	0.20	6/10	
7	ATQ + COTRIM	50 + 25/5	2.80	0.13	10/10	C
8	ATQ + COTRIM	50 + 5/1	2.60	0.15	10/10	C
9	ATQ + DAP	50 + 25	0.90	0.09	9/10	B
10	ATQ + COTRIM	25 + 25/5	2.90	0.17	10/10	C
11	ATQ + COTRIM	25 + 5/1	3.20	0.19	10/10	C
12	ATQ + DAP	25 + 25	0.60	0.15	6/10	B
13	Septrin	250/50	0.10	0.09	1/10	

[a] Doses for COTRIM and Septrin are given as milligrams of SMX/milligrams of TMP.
[b] See Table 1, footnote *a*.
[c] A, not significantly different ($P > 0.05$) from results for the controls (group 1); B, not significantly different ($P > 0.05$) from values obtained with DAP alone (group 6); C, not significantly different ($P > 0.05$) from corresponding values obtained with ATQ (group 2 or 3).

TMP appear to be additive (groups 6 through 8), and a statistically significant difference between ATQ alone at 50 mg/kg (group 2) and ATQ plus TMP at 50 and 5 mg/kg, respectively (group 6), was noted.

ATQ with PROG or PS-15. The results of studies of ATQ in combination with PROG or PS-15 are presented in Table 4. PS-15 was tested at 5 and 2.5 mg/kg, doses which were derived from published rat prophylaxis results (12), selected with the aim of producing a slight antipneumocystis effect but not complete prevention. PS-15 was not evaluated to confirm the antipneumocystis activity reported for rats, as insufficient compound was synthesized for higher doses. PROG alone at 25 mg/kg/day administered p.o. was found to be without effect in the prophylaxis of PCP in SCID mice. PROG in combination with ATQ (groups 7 and 10) showed clear evidence of synergy, which was particularly evident at the higher dosage of ATQ (50 mg/kg/day p.o.; group 7), with the infection cleared in 5 of 10 mice examined. In contrast, PS-15 alone gave small but significant reductions in the mean infection scores (2.50 at 5 mg/kg [group 5] and 3.09 at 2.5 mg/kg [group 6] versus 3.70 for the control [group 1]). When PS-15 was combined with ATQ (groups 8, 9, 11, and 12), the prophylactic activities of the combinations were greater than the activity of either component alone, suggesting an additive effect between these two drugs.

ATQ with CLARI or AZITH. The results of studies of ATQ in combination with CLARI or AZITH are presented in Table 5. CLARI and AZITH at the doses administered both gave relatively minor reductions in the infection score when given alone. CLARI in combination with ATQ (groups 6 and 8) showed a modest additive effect, which was more evident at the higher dosage of ATQ (50 mg/kg/day p.o.; group 6). AZITH in combination with ATQ (groups 7 and 9) was also slightly additive, with no significant difference seen between the two doses of ATQ investigated.

ATQ with RIF. The results of studies of ATQ in combination with RIF are presented in Table 6. RIF alone at 200 mg/kg/day given p.o. (group 3) resulted in a small (nonsignificant) reduction in the infection score, while RIF alone at 100 mg/kg/day given p.o. (group 4) had no effect on PCP prophylaxis. All combinations of ATQ and RIF (groups 6 through 9) showed a pronounced synergistic effect. In all cases, the combination of ATQ and RIF was statistically more efficacious than ATQ or RIF alone. None of the results for the ATQ-plus-RIF combinations were significantly different from each other or from the results for Septrin (group 10).

DISCUSSION

ATQ was shown to have a significant dose-related effect on the prophylaxis of PCP in the SCID mouse model. Potent

TABLE 3. Effect of ATQ in combination with TMP on prophylaxis of PCP in SCID mice

Group no.	Drug(s)	Dose(s) (mg/kg/day p.o.)	Infection score		No. of mice infected/total[a]	Statistical significance[b]
			Mean	SEM		
1	None (control)		3.70	0.14	10/10	
2	ATQ	50	2.50	0.32	10/10	
3	TMP	5	3.09	0.20	11/11	
4	TMP	50	3.18	0.12	11/11	
5	TMP	100	3.36	0.15	11/11	A
6	ATQ + TMP	50 + 5	1.22	0.31	7/9	B, C
7	ATQ + TMP	50 + 50	1.73	0.34	9/11	C
8	ATQ + TMP	50 + 100	1.80	0.24	9/10	C
9	Septrin	250/50[c]	0.27	0.13	3/11	

[a] See Table 1, footnote *a*.
[b] A, not significantly different ($P > 0.05$) from results for the controls (group 1); B, significantly different ($P < 0.05$) from corresponding values obtained with ATQ (group 2); C, significantly different ($P < 0.05$) from corresponding values obtained with TMP (group 3, 4, or 5).
[c] 250 mg of SMX and 50 mg of TMP.

TABLE 4. Effect of ATQ in combination with PROG or PS-15 on prophylaxis of PCP in SCID mice

Group no.	Drug(s)	Dose(s) (mg/kg/day p.o.)	Infection score		No. of mice infected/total[a]	Statistical significance[b]
			Mean	SEM		
1	None (control)		3.70	0.14	10/10	
2	ATQ	50	2.50	0.16	10/10	
3	ATQ	25	3.10	0.17	10/10	
4	PROG	25	3.80	0.13	10/10	A
5	PS-15	5	2.50	0.16	10/10	
6	PS-15	2.5	3.09	0.16	11/11	
7	ATQ + PROG	50 + 25	0.50	0.21	5/10	B, C, D
8	ATQ + PS-15	50 + 5	1.44	0.28	8/9	B, C
9	ATQ + PS-15	50 + 2.5	2.20	0.13	10/10	C
10	ATQ + PROG	25 + 25	2.22	0.31	8/9	B, C
11	ATQ + PS-15	25 + 5	1.80	0.13	10/10	B, C
12	ATQ + PS-15	25 + 2.5	2.10	0.17	10/10	B, C
13	Septrin	250/50[c]	0.18	0.12	2/11	

[a] See Table 1, footnote *a*.
[b] A, not significantly different ($P > 0.05$) from results for the controls (group 1); B, significantly different ($P < 0.05$) from corresponding values obtained with ATQ (group 2 or 3); C, significantly different ($P < 0.05$) from corresponding values obtained with PROG or PS-15 (group 4, 5, or 6); D, not significantly different ($P > 0.05$) from values obtained with Septrin (group 13).
[c] 250 mg of SMX and 50 mg of TMP.

prophylactic activity, comparable to that of Septrin in this model, was seen at 200 mg/kg/day administered p.o., and at this dose ATQ cleared the infection in 64% of mice (7 of 11), with the remaining mice only slightly infected. In contrast, ATQ at a dose of 100 mg/kg/day p.o. was shown to be totally effective in the prevention of PCP in the rat model (11). This result probably reflects differences in the bioavailability and pharmacokinetics of ATQ between rats and mice. There also exists the possibility that mouse *P. carinii* may be intrinsically less susceptible to ATQ than rat- or human-derived *P. carinii*, although we are not aware of any other findings which would support this hypothesis. In the absence of a dose of ATQ that completely clears the infection in mice, we cannot confirm or reject earlier reports (11) that ATQ has a killing effect on *P. carinii*. We can, however, infer from prophylactic studies involving subcurative doses of ATQ that the drug acts rapidly (within 2 weeks), and when *Pneumocystis* organisms were not cleared by ATQ exposure, the remaining organisms were not permanently affected, since when drug pressure was withdrawn the infection very rapidly recrudesced.

When the drugs to be combined with ATQ were evaluated alone, only DAP and to a lesser extent PS-15 gave noteworthy antipneumocystis activity in our SCID mouse model. The potency of DAP was unexpected, as it was reported previously (14) to be only marginally effective in the treatment of rat PCP at 25 mg/kg/day p.o. PS-15 at 2.5 and 5 mg/kg/day p.o. was also moderately active against PCP in our SCID mouse model. The effect of PS-15 was dose related, suggesting that higher doses of PS-15 would have been more effective in the prevention of mouse PCP. In contrast, Hughes et al. (12) showed that PS-15 cleared rat PCP at 5 mg/kg, although activity was considerably reduced at 1 mg/kg. These differences between rat and mouse *Pneumocystis* models are probably due to different pharmacokinetic profiles, particularly as Hughes et al. (12) gave PS-15 as a component of the food pellets, which may have led to more sustained drug levels.

Of principal interest in this work are the results of the ATQ combination studies which clearly suggest that the prophylactic antipneumocystis effect of ATQ in SCID mice can be significantly enhanced when ATQ is used in combination with certain other drugs. The information obtained is of particular importance as we are aware of no other published preclinical studies of ATQ drug combinations for PCP. The drugs investigated as possible partners for ATQ were selected because they are either presently in use or undergoing clinical trials for the treatment or prophylaxis of PCP, toxoplasmosis, infection

TABLE 5. Effect of ATQ in combination with CLARI or AZITH on prophylaxis of PCP in SCID mice

Group no.	Drug(s)	Dose(s) (mg/kg/day p.o.)	Infection score		No. of mice infected/total[a]	Statistical significance[b]
			Mean	SEM		
1	None (control)		3.90	0.09	10/10	
2	ATQ	50	3.40	0.15	10/10	
3	ATQ	25	3.45	0.20	11/11	A
4	CLARI	200	3.30	0.14	10/10	
5	AZITH	200	3.70	0.14	10/10	A
6	ATQ + CLARI	50 + 200	2.27	0.19	11/11	B, C
7	ATQ + AZITH	50 + 200	2.64	0.23	11/11	B, C
8	ATQ + CLARI	25 + 200	3.09	0.20	11/11	
9	ATQ + AZITH	25 + 200	2.73	0.13	11/11	B, C
10	Septrin	250/50[c]	0.18	0.12	2/11	

[a] See Table 1, footnote *a*.
[b] A, not significantly different ($P > 0.05$) from results for the controls (group 1); B, significantly different ($P < 0.05$) from corresponding values obtained with ATQ (group 2 or 3); C, significantly different ($P < 0.05$) from corresponding values obtained with CLARI or AZITH (group 4 or 5).
[c] 250 mg of SMX and 50 mg of TMP.

TABLE 6. Effect of ATQ in combination with RIF on prophylaxis of PCP in SCID mice

Group no.	Drug(s)	Dose (mg/kg/day p.o.)	Infection score		No. of mice infected/total[a]	Statistical significance[b]
			Mean	SEM		
1	None (control)		3.73	0.19	11/11	
2	ATQ	50	2.30	0.14	10/10	
3	ATQ	25	2.82	0.28	11/11	
4	RIF	200	3.18	0.17	11/11	A
5	RIF	100	3.73	0.13	11/11	A
6	ATQ + RIF	50 + 200	0.40	0.15	4/10	B, C
7	ATQ + RIF	50 + 100	0.18	0.12	2/11	B, C
8	ATQ + RIF	25 + 200	0.64	0.23	5/11	B, C
9	ATQ + RIF	25 + 100	0.55	0.24	4/11	B, C
10	Septrin	250/50[c]	0.10	0.09	2/11	

[a] See Table 1, footnote *a*.
[b] A, not significantly different ($P > 0.05$) from results for the controls (group 1); B, not significantly different ($P > 0.05$) from values obtained with Septrin (group 10); C, not significantly different ($P > 0.05$) from values obtained with other ATQ-plus-RIF combinations (group 6, 7, 8, or 9).
[c] 250 mg of SMX and 50 mg of TMP.

by *Mycobacterium avium* complex, or malaria. Most noteworthy was the remarkable synergistic effect of RIF or PROG on ATQ, particularly as both PROG and RIF were ineffective against PCP when administered alone. If the results for RIF are extrapolated to humans, they suggest that the combination of ATQ plus RIF would be useful for prophylaxis of all the major microbial opportunistic infections in AIDS (i.e., *Toxoplasma*, *Pneumocystis*, and *Mycobacterium* infections). The combination of ATQ and RIF was recently shown to result in a significant enhancement of the activity of ATQ or RIF alone in the treatment of mice with disseminated acute toxoplasmosis (2). Additionally, the combination of ATQ plus PROG has also proved highly effective in the treatment of acute falciparum malaria in ongoing phase III trials and appears to reduce the rate of recrudesences associated with ATQ treatment alone (15). The basis for the observed marked synergy between ATQ and RIF or between ATQ and PROG against *P. carinii* and other parasites has yet to be established. Of the other drugs investigated, PS-15 and TMP were also shown to have a moderate additive effect on the antipneumocystis activity of ATQ in SCID mice. It is possible that the pharmacokinetics of ATQ may be altered in the drug combinations, but we cannot comment further as the levels of ATQ in plasma were not determined in the present investigations. However, since both PROG and PS-15 are believed to be metabolized in vivo to active components (cycloguanil and WR99120, respectively [12]) which have greater potency than their parents against *Pneumocystis* dihydrofolate reductase (15), there exists the possibility that the synergy seen with ATQ and PROG, PS-15, or TMP may be mediated via dihydrofolate reductase inhibition. It must be stressed that it has not been established whether PROG and PS-15 or their metabolites are responsible for any of the synergistic effects seen with ATQ. Interestingly, in vitro combination studies with rat *P. carinii* using a [^{3}H]-*p*-aminobenzoic acid incorporation assay (5) showed that both PROG and PS-15 antagonized the effect of ATQ (4).

The data obtained with the macrolides AZITH and CLARI combined with ATQ suggest a small additive effect against *P. carinii* infection. Our results support the findings of Romand et al. (13), who have observed prolonged survival with the combination of ATQ and CLARI in a murine model of acute toxoplasmosis. Hughes (10) found that these macrolides combined with sulfonamides had a synergistic effect on *P. carinii* infection in the rat model, but no data were reported for ATQ combinations. The lack of significant interaction between ATQ and COTRIM or DAP was surprising, as sulfadiazine has been shown to augment the activity of ATQ in murine toxoplasmosis (1, 12). Perhaps more important for any proposed clinical trials of these ATQ combinations is the fact that the results obtained clearly demonstrate that none of these drugs (AZITH, CLARI, COTRIM, and DAP) antagonize the prophylactic activity of ATQ against PCP.

In summary, in these investigations we have demonstrated the value of our SCID mouse model of PCP. Of particular note are the uniformity of control infections and the consistent antipneumocystis effects seen in studies with ATQ alone (at 50 and 25 mg/kg/day p.o.). The latter may also be attributed to the improved dispersion and small variation in particle size of the oral microparticulate suspension of ATQ used in these studies. As more and more drug combinations are being considered for the chemotherapy of opportunistic infections in AIDS, preclinical data in experimental animal models are proving valuable in the selection of appropriate drug combinations. Our results strongly support the further investigation of the ATQ-RIF and ATQ-PROG combinations. Clinical trials of ATQ with these and other synergistic drug combinations may now be justified, particularly if such drug combinations improve ATQ's efficacy and broaden its spectrum of activity.

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