

ABILITY OF AZITHROMYCIN IN COMBINATION WITH QUININE FOR THE ELIMINATION OF BABESIAL INFECTION IN HUMANS

CHIEN-MING SHIH AND CHIH-CHIEN WANG

Department of Parasitology and Tropical Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China;
Department of Pediatrics, Tri-Service General Hospital, Taipei, Taiwan, Republic of China

Abstract. We report the ability of azithromycin in combination with quinine to eliminate the *Babesia* infection in a native Taiwanese woman. Failure of elimination of the babesial infection was observed two weeks after treating with standard regimen of oral quinine plus intravenous clindamycin for a 10-day course of therapy. Azithromycin in place of clindamycin was administered for another 10-day course of therapy two months following initial treatment. Clearance of *Babesia* parasites was observed and verified by hamster inoculation. These results suggest that azithromycin plus quinine should be considered as an alternative therapy for human babesiosis, especially in the failure of treatment with standard regimens.

Babesiosis is a tick-transmitted protozoan infection caused by an intraerythrocytic malaria-like organism that infects a wide variety of wild and domestic animals.¹ Human infection with *Babesia* parasite is usually acquired via the bite of an infective *Ixodes* tick^{2,3} or accidentally acquired via blood transfusion.^{4–6} Although numerous cases of human babesial infections have been reported in Europe and the United States,^{7,8} reported cases of human babesiosis are rare in eastern Asia and the possible vector responsible for transmission remains undefined.^{9,10}

Based on numerous clinical observations and an experimental hamster model,^{11–13} the drug combination of clindamycin plus quinine has been recommended as the standard regimen for human babesial infection. However, failure of elimination of babesial infection by the standard regimen has been reported in some immunocompromised and human immunodeficiency virus (HIV)-infected persons,^{14–16} and relapses of *Babesia* infection following therapy with chloroquine and other combination regimens are well documented.^{12,17}

Azithromycin, a new azalide analog of erythromycin, has been proven effective against various protozoal infections,^{18–21} especially its therapeutic and prophylactic effects on chloroquine-resistant malaria infections of humans.^{22–24} Although the suppression of *Babesia* parasitemia by azithromycin plus quinine has been reported in the hamster model,²¹ the ability of this combination regimen to eliminate *Babesia* infection in human remains unclear. In this paper, we report the efficacy of a drug combination of azithromycin plus quinine for the clearance of *Babesia* infection in humans.

MATERIALS AND METHODS

Case history. A suspected case of *Babesia* infection was diagnosed in a 51-year-old Taiwanese woman who presented to the local hospital of Chia-i Country in March 1994 with the chief complaints of headache, malaise, fatigue, and frequently mild fever (38.3°C) during the past few months. She was transferred to the Medical Center in Taipei and was hospitalized from April 15 to May 18, 1994. Routine hematologic examinations revealed that the patient had a normal leukocyte count ($5.1 \times 10^3/\mu\text{l}$) and platelet count ($200 \times 10^3/\mu\text{l}$), but an abnormal erythrocyte count ($3.95 \times 10^6/\mu\text{l}$) and hematocrit (27.8%). She also had a decreased he-

moglobin level (8.9 g/dL) and was suspected of having hemolytic anemia. In addition, the patient had not traveled abroad during the previous 10 years.

Diagnosis of infection. Blood specimens were collected from the patient into EDTA-coated blood collection tubes (Vacutainer 6457; Becton Dickinson, Taipei, Taiwan) and Giemsa-stained thin smears were made and examined for the evidence of babesial infection by oil-immersion microscopy (Model BX60; Olympus Optical, Ltd., Tokyo, Japan). The parasitemia of the patient was examined two weeks after antibabesial treatment and followed every month thereafter by examining blood smears. In addition, serologic evidence of infection was also confirmed by antibody reactivity of the patient's serum to *Babesia microti* and by seroreactivity of *Babesia*-infected human serum (courtesy of Dr. Sam R. Telford III, Department of Tropical Public Health, Harvard School of Public Health, Boston, MA) against the patient's isolate.

Hamster inoculation. To verify the clearance of *Babesia* infection, golden Syrian hamsters (*Mesocricetus auratus*; weight = 50–60 g) obtained from the animal supply center of the National Taiwan University were inoculated intraperitoneally with 1-ml whole blood specimens from the patient, and Giemsa-stained thin smears of blood from the inoculated hamsters were examined (at least 25 random fields per smear) weekly by oil-immersion microscopy for three months.

Drug treatment. Initially, the patient was treated with a standard regimen of oral quinine (650 mg, three times a day) plus intravenous clindamycin (300 mg, four times a day) for 10 consecutive days. In an attempt to eradicate the parasite, the patient was re-treated with a drug combination of oral quinine (650 mg, three times a day) and azithromycin (500 mg, twice a day) for another 10-day course of therapy. Giemsa-stained blood smears and hamster inoculation of the patient's blood were performed to verify the clearance of *Babesia* infection.

RESULTS

The *Babesia* parasites observed in the Giemsa-stained thin blood smears of the patient's blood appeared to be morphologically consistent with small-form piroplasm measuring about 1.0–2.0 μm in diameter. The typical feature of the

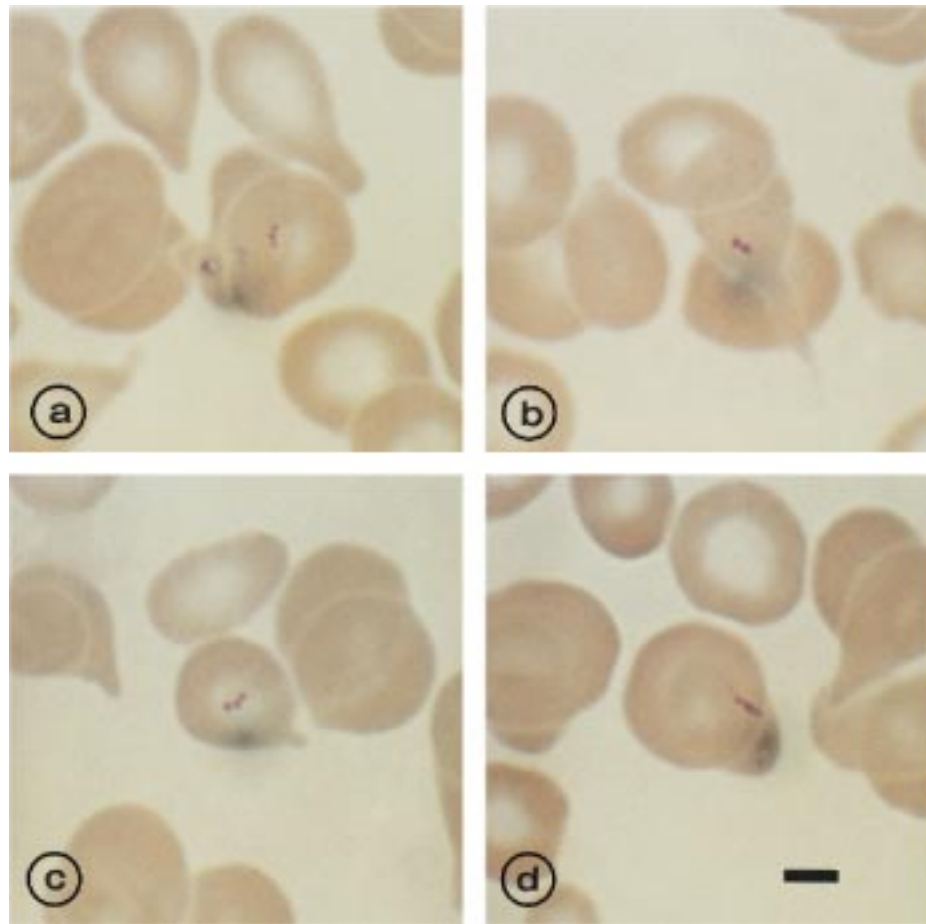


FIGURE 1. Photomicrographs of Giemsa-stained thin blood films from the patient at two months following treatment with the drug combination of clindamycin and quinine. **a**, a ring-form trophozoite and a trophozoite with three chromatin dots. **b**, a binucleate trophozoite and **c**, a trophozoite with three chromatin dots. **d**, a trophozoite with a banded chromatin mass. (Magnification $\times 2,000$, bar = $3.5 \mu\text{m}$.)

parasite resembled closely the ring-form stage of *B. microti* (Figure 1a). More mature forms of the parasites with two or three red dots of chromatin was also observed in the blood smears (Figure 1b and c), and a parasite with a banded chromatin mass was occasionally seen in the blood smear (Figure

1d). No parasite with the typical tetrad form was observed in the infected erythrocytes of this patient after antibiotic treatment.

After initial treatment with the standard regimen of clindamycin plus quinine, failure of clearance of the parasites from the peripheral blood was observed two weeks following treatment, and the parasitemia was 0.5% by blood smears (Table 1). Thus, the patient was re-treated with a combination regimen of azithromycin plus quinine for 10 consecutive days, and the clearance of *Babesia* infection was observed two weeks following re-treatment and was verified by hamster inoculation with the patient's blood. These results indicate that the relapsed *Babesia* infection can be eliminated by treating with a drug combination of azithromycin and quinine.

DISCUSSION

Although the efficacy of azithromycin in combination with quinine in treating *Babesia* infection of human remains to be defined, the present study demonstrates the effectiveness of this combination regimen in eliminating a persistent *Babesia* infection in an antibiotic-treated patient. The relapses following treatment with the standard regimen of clindamycin plus quinine may account for the persistent *Babesia*

TABLE 1

Differential efficacy of drug combinations in the elimination of *Babesia* infection in humans

Combination of drugs*	Daily dosage (mg)	Weeks after treatment	<i>Babesia</i> detected in patient by	
			Giemsa-stained blood smears	Hamster inoculation†
Combination A				
Clindamycin plus quinine	1,200	2	Positive	ND
	1,950	4	Positive	ND
		8	Positive	ND
		12	Positive	ND
Combination B				
Azithromycin plus quinine	1,000	2	Negative	Negative
		4	Negative	Negative
		8	Negative	Negative
		12	Negative	Negative

* A 10-day course of therapy with combination A was given and combination B was administered for another 10 consecutive days two months after initial treatment, and hamsters inoculated with 1 ml of the patient's blood were used to verify the clearance of babesial infection.

† ND = not done.

infection in our patient, and the possible mechanism responsible for the relapses of *Babesia* infection could be attributed to the induction of drug resistance of *Babesia* parasites during the course of therapy. On the basis of these observations, we suggest that the combination regimen of azithromycin plus quinine should be considered as an alternative therapy for treating *Babesia* infection in humans.

Another possible mechanism that may account for the relapses of *Babesia* infection in treated patients could be correlated with tissue invasion by *Babesia* parasites. Although the natural cycle of the pre- or exoerythrocytic stages of *Babesia* parasites remains unclear, the exoerythrocytic forms of *B. equi* in host lymphocytes have been documented and were suggested as the other small-form piroplasm of *B. microti*.²⁵ Thus, the possibility of immune evasion of *Babesia* parasites by invading specific tissues, such as the liver, needs to be determined.

The mechanism responsible for the clearance of *Babesia* infection in immunocompetent patient remains undefined. Although the spleen appears to play an important role in limiting the intensity of parasitemia in the early stage of *Babesia* infection,²⁶ the critical role of cellular immunity has been shown by persistent higher levels of parasitemia in athymic (nude) mice.²⁷ Indeed, human babesial infection was reported more severe in splenectomized and HIV-infected patients,¹⁴⁻¹⁶ and eradication of *Babesia* infection in HIV-infected patients with severe immunosuppression is unlikely.¹⁶ However, clearance of *Babesia* infection after appropriate antibiotic treatment was observed in our asymptomatic immunocompetent human case. These observations suggest that the ability of clearing *Babesia* parasites after treatment may depend on the severity of babesial infection and cellular immunity of infected humans.

The greater tissue penetration and prolonged elimination half-life of azithromycin may contribute to its effectiveness in eliminating relapsed or persistent *Babesia* infection. In our case, the *Babesia* parasitemia was suppressed by the standard treatment regimen but was eradicated by a subsequent regimen of azithromycin plus quinine. Indeed, pharmacokinetic studies have indicated that azithromycin achieves a higher and prolonged level in blood cells and hepatic tissue.²⁸⁻³⁰ This tissue-directed pharmacokinetics of azithromycin may actually be beneficial to the elimination of relapsing parasites that remain in the circulation or persist in the tissues after treatment with the standard regimen of clindamycin plus quinine.

In conclusion, the present report demonstrates the additive effect of azithromycin in the clearance of persistent *Babesia* infection in a relapsed patient. Because azithromycin plus quinine may have few adverse effects on pregnant women and children, this drug combination should be further evaluated for treatment and prophylaxis of human babesiosis.

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Authors' addresses: Chien-Ming Shih, Department of Parasitology and Tropical Medicine, National Defense Medical Center, PO Box

90048, Taipei, Taiwan, Republic of China. Chih-Chien Wang, Department of Pediatrics, Tri-Service General Hospital, Taipei, Taiwan, Republic of China.

Reprint requests: Chien-Ming Shih, Department of Parasitology and Tropical Medicine, National Defense Medical Center, P. O. Box 90048-506, Taipei, Taiwan, Republic of China.

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