Treatment of Human Babesiosis

Subjects: Infectious Diseases
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Babesiosis is an emerging tick-borne disease caused by apicomplexan parasites of the genus Babesia. With its increasing incidence worldwide and the risk of human-to-human transmission through blood transfusion, babesiosis is becoming a rising public health concern.

Keywords: babesiosis ; Babesia microti ; Babesia duncani ; parasite ; therapy ; atovaquone ; endochin-like quinolones (ELQs)

1. Introduction

Human babesiosis is a rapidly emerging tick-born infectious disease caused by intraerythrocytic parasites of the genus *Babesia*. Of several hundred *Babesia* species identified so far, only a few are known to infect humans. These include *Babesia microti*, *Babesia duncani*, *Babesia divergens* and *divergens*-like species, *Babesia crassa*-like, and *Babesia venatorum* [1]. In the United States, most cases of human babesiosis have been attributed to infection with *B. microti*, but sporadic cases due to infection with *B. duncani* and *B. divergens*-like MO1 have also been reported. In Europe, *B. divergens* used to be the main species responsible for infection in humans. However, recent studies suggest that *B. microti* and *B. venatorum* are now more prevalent than *B. divergens* [2]. In China, human babesiosis is mainly caused by *B. microti* and *B. venatorum*, and in the rest of the world, only a few sporadic cases have been reported and were mostly linked to *B. microti* infection [2].

Babesia spp. are apicomplexan parasites that infect the host red blood cells and are transmitted to mammals by tick vectors (**Figure 1**). The species of ticks involved in the transmission of *Babesia* pathogens vary depending on the geographical area and parasite species [1][2]. During the life cycle of *Babesia*, humans are typically accidental hosts, and most infections are linked to a tick route of transmission [1][2]. However, an increasing number of transfusion-transmitted babesiosis cases have been reported in the US over the past 2–3 decades, making *Babesia* infections a major public health concern [1][3][4][5][6]. In 2011, human babesiosis became a nationally notifiable disease in the US [5] and as one of the most common transfusion-transmitted pathogens in the US, *B. microti* was added to the list of significant threats to the blood supply [3][4]. In addition to human-to-human transmission through blood transfusion, several reports have also established the possibility of transplacental transmission from mother to child [1].

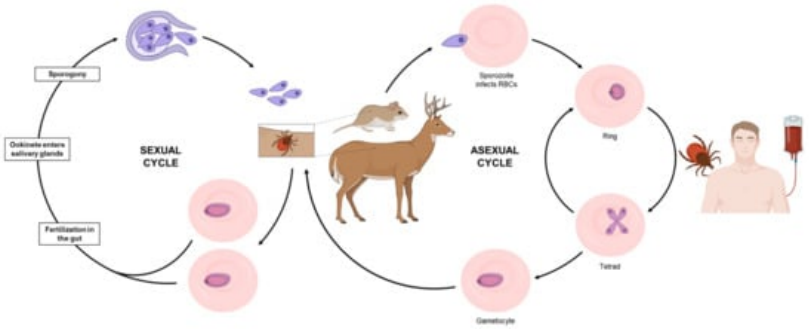

Figure 1. Cycle of transmission of the most common *Babesia* species, *B. microti*. During a blood meal, an infected tick introduces merozoites into the host (mouse or deer, for example). Free merozoites enter red blood cells and undergo asexual replication. While in the blood, some parasites differentiate into male and female gametocytes (not morphologically recognizable by light microscopy). These gametocytes are then taken up by a tick during a blood meal and differentiate into gametes. While in the gut, gametes fuse to form a zygote, that will subsequently undergo meiotic and several mitotic divisions to form sporozoites that are then transmitted to a mammalian host. Humans are typically accidental hosts and become infected through the bite of an infected tick. Human to human transmission is also possible via blood transfusion.

In most individuals, babesiosis remains asymptomatic or presents with mild flu-like symptoms [1][2]. However, in more susceptible populations, such as the elderly, asplenic, or immunocompromised individuals, the disease can become severe and even life-threatening, with symptoms such as severe anemia, acute respiratory distress, organ failure, and death [1][2].

2. Current Treatments against Human Babesiosis

The current arsenal for the treatment of human babesiosis relies principally on four drugs: atovaquone, azithromycin, clindamycin, and quinine. Atovaquone is used to treat several human diseases, including *Pneumocystis jirovecii* pneumonia [7], toxoplasmosis [8], and malaria (in combination with proguanil (Malarone) [9]. In apicomplexan parasites, atovaquone targets the cytochrome bc_1 complex of the mitochondrial electron transport chain (**Figure 2** and **Figure 3**) [10][11][12][13]. Azithromycin is a relatively broad-spectrum antibiotic indicated for the treatment of numerous bacterial infections, such as those caused by *Staphylococcus* spp. [14][15][16] and *Legionella* spp. [17]. The antibiotic is also used for the treatment of toxoplasmosis [12] and, in combination with other drugs, for the treatment of malaria [18]. Azithromycin is a well-characterized protein synthesis inhibitor, which in apicomplexan parasites targets the translation machinery in the apicoplast (**Figure 2**) [19][20][21]. It is worth noting that azithromycin was found to have a “delayed death” effect, in which parasite division produces viable daughter cells that are subsequently unable to divide in the following cycle [19][21][22]. Clindamycin is another antibiotic commonly used for the treatment of various bacterial infections [23] and repurposed for the treatment of parasitic infections. In combination with quinine, clindamycin is used for the treatment of both malaria and babesiosis [24][25][26]. Several reports have suggested that clindamycin acts in a similar way as azithromycin and targets protein synthesis in the apicoplast (**Figure 2**) [19][21][22]. Furthermore, selection of clindamycin-resistant *T. gondii* parasites showed cross-resistance to azithromycin, further suggesting a common target [27]. Quinine is a widely used antimalarial agent, typically administered in combination with an antibiotic such as clindamycin or doxycycline [28]. However, the drug is poorly tolerated and, as such, tends to be replaced by alternative drugs with fewer side-effects [28][29]. In malaria parasites, several modes of action for quinine have been proposed. The most commonly reported mechanism of action involves the disruption of hemozoin formation, resulting in accumulation of free ferriprotoporphyrin IX, a by-product of hemoglobin degradation, which is deleterious to parasite growth [30][31][32]. Confocal imaging using fluorescent derivatives of quinine and its structural analogues, quinidine and chloroquine, have shown accumulation of the probes in the digestive vacuole, consistent with the activity of this compound in this organelle [32][33]. Unlike *Plasmodium* parasites, *Babesia* species lack a digestive vacuole, do not degrade hemoglobin, and do not produce hemozoin. Therefore, the mode of action of quinine against *Babesia* parasites is likely to be different from that in *Plasmodium*. Interestingly, fluorescent probes were found to bind to phospholipids and to accumulate in membranous structures, including the parasite plasma membrane, the endoplasmic reticulum, and the mitochondrion, suggesting that quinine may inactivate specific biological functions in these organelles [32][33]. Another proposed hypothesis is that quinine acts as a DNA intercalator [34][35][36]. However, the lack of fluorescence in the nucleus reported by Woodland et al. seem to refute interactions with DNA as a potential mode of action [32][33]. More recently, a study in *P. falciparum* using thermal shift assays suggested that the purine nucleoside phosphorylase (PfPNP) might also be a target of quinine [37].

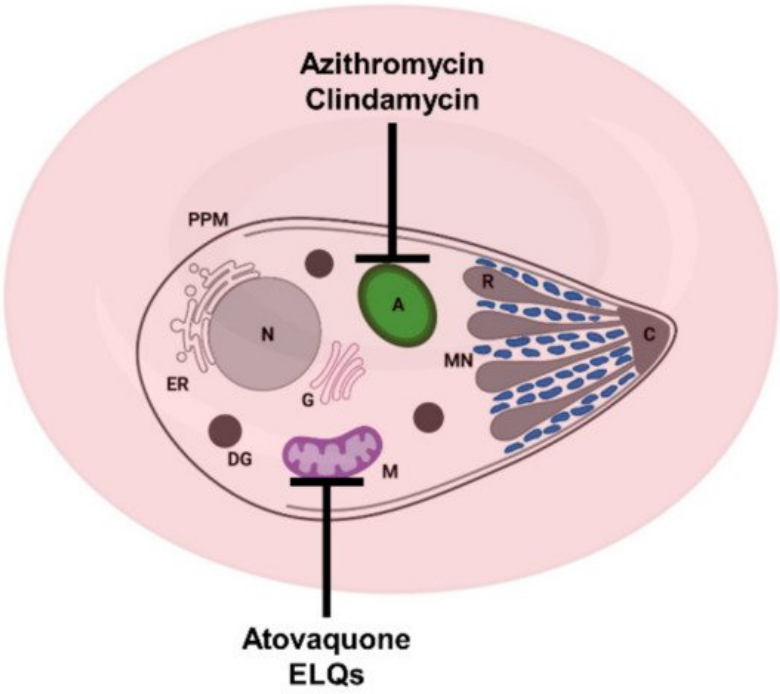

Figure 2. Schematic representation of a *Babesia*-infected red blood cell and sites of action of some approved and experimental drugs. Azithromycin and clindamycin target the apicoplast; atovaquone and ELQs target the mitochondrion. A: apicoplast, C: conoid + polar rings, DG: dense granule, ER: endoplasmic reticulum, G: Golgi apparatus, M: mitochondrion, MN: microneme, PPM: parasite plasma membrane and R: rhoptry.

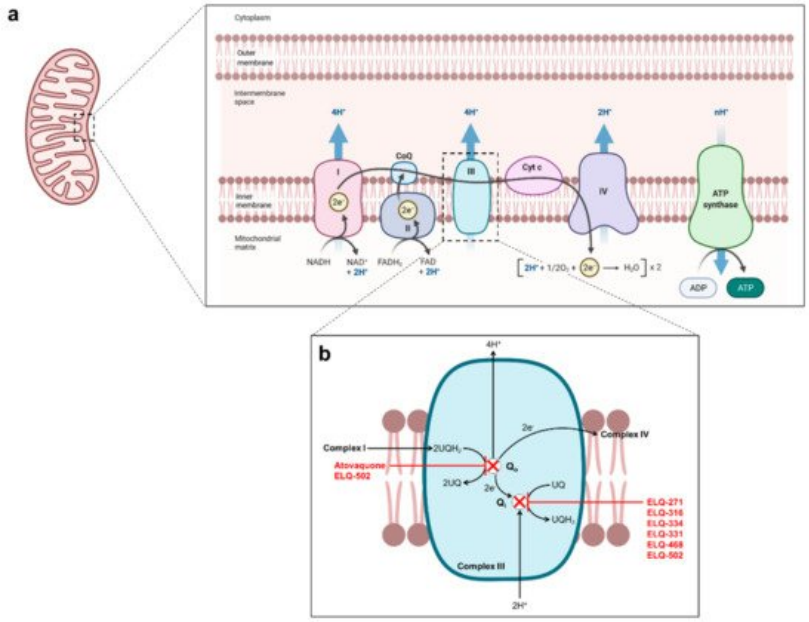

Figure 3. Proposed mechanism of action of atovaquone and endochin-like quinolones in *Babesia* mitochondrion. (**a**) Schematic representation the mitochondrial electron transfer chain. (**b**) Schematic representation of the parasite *bc*$_1$ complex with proposed mode of action of atovaquone and ELQs.

The severity of babesiosis depends mainly on the host's immune status, the presence of risk factors and the *Babesia* species responsible for the infection. In symptomatic patients, babesiosis usually manifests with flu-like symptoms such as fever, fatigue, chills, sweats, and headache [38]. For this moderate form of the disease, typically associated with a low parasitemia level (<4%) [26], no hospital admission is required and a 7–10-day treatment course of oral atovaquone + azithromycin (500 mg azithromycin on day 1, followed by 250 mg on subsequent days + 750 mg b.i.d. atovaquone) is recommended [26][38]. Babesiosis typically resolves within seven days from the start of the treatment, but asymptomatic, low level parasitemia may persist for up to one year [26]. Monitoring of persistent parasitemia in immunocompetent individuals following treatment is usually not necessary. However, given the risk of transmission of *Babesia* parasites through blood transfusion, these patients are excluded as blood donors [3]. Immunocompromised individuals are more at risk of developing a severe form of babesiosis, resulting in complications such as acute respiratory distress syndrome, disseminated intravascular coagulation, severe hemolytic anemia, organ failure, splenic rupture, relapse, and death [2][26]. A combination of oral clindamycin + quinine (600 mg + 650 mg, every 8 h) is the standard of care for the treatment of severe babesiosis [26][38]. However, this treatment regimen is frequently associated with serious side effects, such as hearing loss, vertigo, and tinnitus. In some cases, these side effects can be so severe that dose reduction or discontinuation of treatment is required [38]. Recently, it has been demonstrated that a combination of atovaquone + azithromycin is also suitable for the treatment of severe babesiosis, displaying comparable efficacy to clindamycin + quinine with fewer side effects [39]. Although atovaquone + azithromycin is now the preferred course of treatment for severe babesiosis, the standard 7–10-day treatment regimen of oral atovaquone + azithromycin is usually not enough to eliminate *Babesia* infection. Higher doses, longer treatment duration, and in some cases intravenous administration is required to clear the infection [26]. It is also worth noting that the use of immunosuppressive agents such as Rituximab to treat prior illnesses (B cell lymphoid malignancies, rheumatoid arthritis, etc.) may lead to babesiosis relapse and extended persistence of *Babesia* parasites [40][41][42].

One downside of a prolonged treatment regimen and dose escalation is the risk of developing drug resistance. Previous reports have established the emergence of mutations in the cytochrome b (Cytb) of *Babesia* parasites in humans and animal models following treatment with atovaquone [11][42][43]. In 2016, Lemieux et al. examined clinical isolates of relapsing babesiosis and identified a methionine to isoleucine mutation (M134I) in the Q_o site (atovaquone-binding site) of the BmCytb [43]. This same mutation was observed in a murine model of *B. microti* infection [11], as well as in other apicomplexan parasites, such as *P. falciparum* and *T. gondii* [43]. Later, Simon et al. reported a Y272C mutation in the BmCytb Q_o site in a patient presenting with relapsed *B. microti* infection following an atovaquone + azithromycin treatment course [42]. In both cases, these mutations have been shown to impact the atovaquone-binding domain [44] and appear to

be associated with decreased sensitivity to the drug [42][43]. With regard to azithromycin resistance, sequencing of clinical isolates obtained from patients with relapsing babesiosis identified mutations in the ribosomal protein subunit L4 (RPL4) encoded by the apicoplast genome [42][43]. Lemieux et al. identified three substitutions in the RPL4: R86H, R86C and S73L [43]. Simon et al. observed the same R86C mutation in a patient presenting with relapsing babesiosis following atovaquone + azithromycin treatment [42]. Similar mutations associated with azithromycin resistance have been reported in *P. falciparum* [20] and *S. pneumoniae* [45] RPL4. Alternative management strategies for human babesiosis in the case of persistent relapse include the use of different drug combinations such as atovaquone + azithromycin + clindamycin, atovaquone + clindamycin, atovaquone + proguanil, or atovaquone + azithromycin + clindamycin + quinine [26][41][46][47]. The introduction of other drugs such as doxycycline, moxifloxacin, pentamidine, trimethoprim-sulfamethoxazole or artemisinin to treatment regimens with the standard therapies was also reported [40][48]. A recent study in a small cohort of patients suffering from Lyme disease and babesiosis co-infection suggested improvement, and in some cases remission, following one course of disulfiram monotherapy [49]. In patients with high parasitemia (>10%), exchange transfusion is recommended and often results in a rapid reduction of the parasite load [26][50].

Despite clinical evidence that atovaquone, azithromycin, clindamycin and quinine can be used to manage human babesiosis, preclinical evaluation of these drugs in different models of *Babesia* infection has not demonstrated unanimous results with regards to their efficacy. Clindamycin showed only limited activity at a dose of 300 mg/kg (p.o.) in *B. microti*-infected Mongolian jirds [51]. When evaluated in *B. microti*-infected hamster, a course of 150 mg/kg (i.m. or p.o.) of clindamycin resulted in a two-fold decrease in peak parasitemia. Similar results were obtained when clindamycin was administered in combination with quinine [52]. AbouLaila et al. reported a ~three-fold decrease in peak parasitemia following i.p. injection of 500 mg/kg of clindamycin in *B. microti*-infected Balb/c mice [53]. Another study using the same Balb/c model of *B. microti* infection showed that oral administration of clindamycin at 25, 50, and 100 mg/kg did not lead to reduction of parasite burden [54]. Similar results were obtained by Lawres et al. following oral administration of 10 or 50 mg/kg of clindamycin to immunocomprimized mice infected with *B. microti* [11]. The consensus seems to be more apparent in the case of quinine, where most studies report no effect on parasitemia following administration of quinine as a single drug [11][52][54]. Interestingly, a combination of clindamycin + quinine was reported to achieve up to 70% suppression of parasitemia [55] and result in a faster resolution of parasitemia compared to clindamycin alone [52], suggesting a potential synergy between the two drugs. Preclinical investigation of azithromycin efficiency against *Babesia* parasites also yielded inconsistent results. In *B. microti*-infected Balb/c mice, a four-day treatment course with azithromycin at 25, 50, and 100 mg/kg was found to be potent, resulting in 75–96% suppression of parasitemia [54]. In contrast, the evaluation of azithromycin in *B. microti*-infected SCID mice showed no effect on parasitemia at 10 and 50 mg/kg after a seven-day treatment course [11]. Similar results were obtained in *B. microti*-infected hamsters, where 150 mg/kg azithromycin treatment regimen, administered daily for almost two weeks, showed no apparent effect on parasitemia [56]. Out of the four clinically used drugs in the treatment of babesiosis, only atovaquone seems to consistently show high potency against *Babesia* parasites [11][56][57][58][59]. Studies carried out in *B. microti*-infected hamsters and SCID mice reported fast clearance of parasitemia following treatment with atovaquone [11][56]. However, recrudescence due to atovaquone-resistant parasites was observed [11][56]. In *B. microti*-infected hamsters, a combination therapy of atovaquone + azithromycin resulted in rapid clearance of parasitemia without recrudescence [56]. In a lethal model of *B. microti* infection in hamsters, atovaquone monotherapy was found to be superior to a combination of clindamycin + quinine, resulting in low to undetectable parasitemia and extended survival [58]. Potency of atovaquone was also demonstrated in *B. divergens* [59] and *B. duncani* [57] models, with IC_{50} values in the low nanomolar range. In gerbils, although prophylaxis experiments were not successful, a dose of atovaquone as low as 0.5 mg/kg was found to efficiently prevent *B. divergens* infection, so long as daily treatment was maintained several days post-infection [59]. In the case of *B. duncani*, a treatment course of 10 mg/kg atovaquone resulted in a clear reduction of parasitemia and 80% survival using a mouse model of lethal infection [57]. The results derived from the evaluation of atovaquone, azithromycin, clindamycin, and quinine in preclinical models of babesiosis are summarized in **Table 1**.

While combinations of atovaquone + azithromycin and clindamycin + quinine have been used for more than 20 years for the treatment of human babesiosis [60], the efficacy of these drugs and their primary modes of action in *Babesia* parasites have only recently started to be elucidated.

Table 1. Reported efficacy of atovaquone, azithromycin, clindamycin and quinine in animal models of babesiosis.

Drug	Treatment Regimen	Model	Effect	Ref.
Atovaquone	20 mg/kg (p.o.), 5 d	*B. microti* Balb/c mice	~5.7 × reduction in peak parasitemia.	[61]
	25 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	77% suppression of parasitemia at DPI 9.	[54]
	50 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	87% suppression of parasitemia at DPI 9.	[54]
	100 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	93% suppression of parasitemia at DPI 9.	[54]
	10 mg/kg (p.o.), 7 d	*B. microti* SCID mice	Parasitemia clearance followed by recrudescence by D5-9 post-treatment.	[11]
	10 mg/kg (p.o.), 10 d	*B. microti* SCID mice	Parasitemia clearance followed by recrudescence by D14 post-treatment.	[57]
	10 mg/kg (p.o.), 10 d	*B. duncani* C3H/HeJ mice	Parasitemia clearance followed by recrudescence by D10 post-treatment. 80% survival.	[57]
Azithromycin	25 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	75% suppression of parasitemia at DPI 9.	[54]
	50 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	96% suppression of parasitemia at DPI 9.	[54]
	100 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	95% suppression of parasitemia at DPI 9.	[54]
	10 mg/kg (p.o.), 7 d	*B. microti* SCID mice	No effect.	[11]
	50 mg/kg (p.o.), 7 d	*B. microti* SCID mice	No effect.	[11]
Clindamycin	300 mg/kg (p.o.), 5d	*B. microti* Mongolian jirds	9.4% suppression of parasitemia at DPI 9.	[51]
	150 mg/kg (i.m.), 8d	*B. microti* Golden hamsters	~2× reduction in peak parasitemia.	[52]
	150 mg/kg (p.o.), 8d	*B. microti* Golden hamsters	~2× reduction in peak parasitemia.	[52]
	500 mg/kg (i.p.), 5d	*B. microti* Balb/c mice	~3.2× reduction in peak parasitemia.	[53]
	25 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	No effect.	[54]
	50 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	No effect.	[54]
	100 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	No effect.	[54]
	10 mg/kg (p.o.), 7 d	*B. microti* SCID mice	No effect.	[11]
	50 mg/kg (p.o.), 7 d	*B. microti* SCID mice	No effect.	[11]

Drug	Treatment Regimen	Model	Effect	Ref.
Quinine	125 mg/kg (s.c.), 8d	*B. microti* Golden hamsters	No effect.	[52]
	250 mg/kg (p.o.), 8d	*B. microti* Golden hamsters	No effect.	[52]
	25 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	No effect.	[54]
	50 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	No effect.	[54]
	100 mg/kg (p.o.), 4 d	*B. microti* Balb/c mice	No effect.	[54]
	10 mg/kg (p.o.), 7 d	*B. microti* SCID mice	No effect.	[11]
	50 mg/kg (p.o.), 7 d	*B. microti* SCID mice	No effect.	[11]
	100 mg/kg (p.o.), 7 d	*B. microti* SCID mice	No effect.	[11]

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