Babesiosis is a tick-borne infection caused by intraerythrocytic protozoan parasites of the genus *Babesia* and affects wild and domestic animals worldwide. It is a well-known disease of veterinary importance in cattle, horses, and dogs which causes considerable economic losses in livestock industry and is gaining interest as an emerging zoonosis. Bovine disease is most common in tropical and subtropical regions (*Babesia bovis, Babesia bigemina*), but infections are also seen in Europe (*Babesia divergens, Babesia major*). Clinical manifestations like fever, malaise, hemolytic anemia, and hemoglobinuria may be absent, but babesiosis can develop into a severe, rapidly fatal disease. Even though a number of effective antibabesial drugs exist, they are not readily available in all areas due to safety, residue, or marketing issues. The most widely used babesicidacides are imidocarb dipropionate and diminazene aceturate; however, only imidocarb is able to consistently clear the host of parasites and has chemoprophylactic properties (29). Thus, concerns about development of resistance are growing, and the need for alternative compounds for the veterinary market is evident.

Human infections with *Babesia* species have been known since the late 1950s. While in Europe the causative agent in cases of babesiosis in splenectomized individuals was identified as the cattle species *B. divergens*, the rodent species *Babesia microti* was reported in most cases of human babesiosis in North America (8, 14, 28), where the disease is endemic and transmitted by the tick *Ixodes damiani* (also known as *I. scapularis*). *Babesia duncanii* and *B. divergens*-like organisms later attracted attention in the west and midwest of the United States. Sporadic cases of human babesiosis have also been identified in Asia, Africa, and South America (28).

Diamidines have a long history as chemotherapeutic agents against protozoan infections, and their synthetic productivity, as well as their low molecular weight, makes them an attractive drug class. The activity is due mostly to the selective accumulation by the parasite rather than the host cell. Aromatic diamidonic molecules are thought to act by binding to the minor groove of DNA at AT-rich sites, which are present in many parasitic organisms, and thus they may possibly inhibit DNA-dependent enzymes or cause direct inhibition of transcription (26). In previous studies, the significant antiparasitic activity of novel diamidines against *Trypanosoma brucei* and *Plasmodium* spp. has been shown (2, 15, 25, 26).

In the present study, we selected new diamidine compounds according to their *in vitro* activities against *Plasmodium falciparum* and demonstrated their *in vitro* potential against the two *B. divergens* strains 1903B and 4201. Subsequently, a selection of compounds was evaluated in a *B. microti* mouse model.

**MATERIALS AND METHODS**

*Parasite strains and cultivation.* The two bovine *B. divergens* strains 1903B and 4201 were kindly provided by Laurence Malandrin of the Ecole Nationale Vétérinaire de Nantes, Nantes, France. Continuous *in vitro* cultures were maintained in human red blood cells (RBC) diluted to 5% hematocrit in RPMI 1640 with 25 mM HEPES and 2 mM glutamine (BioConcept, Allschwil, Switzerland) supplemented with 5 g/liter Albumax I (Gibco/BRL Life Technologies, Belgium) and 10 μg/liter gentamicin (Sigma, Steinheim, Germany). All cultures were kept in 25-ml flasks at 37°C in a 4% CO₂, 3% O₂, 93% N₂ gas mix. The medium was changed daily, and subpassages were performed every 2 to 3 days when the parasitemia reached 20%.

The strain of *B. microti* was kindly donated by Lise Gern (University of Neuchatel, Neuchatel, Switzerland). It was isolated from a bank vole (*Myodes glareolus*) in Central Switzerland and finally maintained by passages in female Swiss NMRI mice. The animals were sacrificed when the parasitemia reached 20%, and blood was collected by cardiac puncture. The course of infection was investigated using different infective doses. After intravenous inoculation with 2 × 10⁷ *B. microti*-infected RBCs, mice typically showed a peak parasitemia of 70 to 80% by day 7 and a subsequent decrease to low or undetectable values by day 30.

**Antibabesial agents.** The diamidines were synthesized in the laboratories of David Boykin and Richard Tidwell as previously described (5, 11, 13). The synthesis of the unpublished compounds were achieved in an analogous manner. For *in vitro* studies, stocks of 10 mg/ml were prepared in dimethyl sulfoxide (DMSO) and subsequently diluted in RPMI cultivation medium, whereas for *in vivo* studies, compounds were dissolved in a 10% DMSO-water solution. Dimi-
nizene acurate (Berenil; Sigma, Steinheim, Germany), imidocarb dipropionate
(Carbosia; Schering-Plough, kindly donated by Pierre Bonnemain), and atova-
quone (GSK, Muenchenbuchsee, Switzerland) served as standard drugs. Stan-
dards were prepared as described above except for imidocarb dipropionate, a
sterile solution that was diluted directly in RPMI or sterile deionized water.

Cytotoxicity determination. Cytotoxicity for L6 rat skeletal myoblasts was
determined using the Alamar blue assay as described earlier (7, 21, 23).

In vitro growth-inhibitory assay. Growth inhibition was determined by mea-
suring the incorporation of radiolabeled [8-3H]hypoxanthine (GE Healthcare,
Amersham, United Kingdom) as described before (4). Twofold serial drug di-
lutions were prepared in 96-well microtiter plates in order to test seven drug
concentrations to determine the 50% inhibitory concentrations (IC50s). Dupli-
cate wells received 100 μl of drug dilution and 100 μl of human RBC (2% parasitemia, 2.5% hematocrit). Controls consisted of infected RBCs without
drug and noninfected RBCs. Plates were incubated at 37°C in a 4% CO2, 3% O2,
95% N2 atmosphere for 48 h. Then 50 μl [8-3H]hypoxanthine was added (0.5
μCi/well), and plates were incubated for another 24 h. Cells were then harvested
on glass fiber filters with a cell harvester (Betaplate; Wallac PerkinElmer, Swit-
zerland), the incorporated radioactivity was counted in a liquid scintillation
counter (Betaplate; Wallac PerkinElmer, Switzerland), and IC50 were calcu-
lated.

In vivo drug susceptibility test. Female Swiss NMRI mice (18 to 20 g; RCC,
Switzerland) were used for in vivo drug tests. On day 0, groups of three mice each
were inoculated intravenously with 2 × 107 B. microti-infected RBCs. Diamidine
compounds and standard drugs were administered subcutaneously or orally
(atovaquone) in intervals of 4, 24, 48, and 72 h postinfection. Doses ranged from
50 to 12.5 mg/kg of body weight in a volume of 10 ml/kg. All experiments
included a group of untreated controls. Tail blood was collected at least twice a
week starting on day 7. Blood smears were stained with Giemsa, and parasitemia
was determined microscopically with a detection limit of 1 parasite in 10,000
erythrocytes. A compound was defined as curative if no parasites were detectable
up to day 60.

RESULTS

Diff erent chemical classes of diamidines were screened for activity against B. divergens in vitro and B. microti in vivo
and compared to the standard drugs diminazene acurate, imido-
carb dipropionate, and atovaquone. The results are summa-
rized in Tables 1 and 2.

In vitro activity against B. divergens. The dicatonic mole-
ecules demonstrated a high selectivity for B. divergens compared
to their cytotoxicity for mammalian cells, which was tested
using L6 rat myoblast cells in an Alamar blue assay (7, 21, 23).
Of the 214 diamidine compounds, 80 showed excellent IC50 values (Fig. 1). The standard drugs diminazene at 25 mg/kg
and imidocarb at 12.5 mg/kg administered on four consecutive
days did not cure the mice but resulted in a delayed para-
sitemia peak of approximately 50% around day 23 followed by
a decrease to 5% or less by day 30 postinfection. Most of the
compounds tested in vivo were potent enough to cause rapid
suppression and initial clearance of parasites from the blood by
day 10 at a dosage of 25 mg/kg subcutaneously for the fi rst 4
days. Failure to cure resulted in recrudescence by day 30, and
peak parasitemia was generally seen 5 to 10 days after the
relapse.

The best antibabesial properties were exhibited by the ter-
phenyls, benzimidazoles, diphenyl furans, and pentamidine
and its analogues as well as a diaryldiamidine (Table 2).

Three terphenyls provided cures of 2/3 (23DAP055, 19DAP085) or 3/3 (19DAP025) mice at a dosage of 25 mg/kg
administered for 4 days. At a lower dose of 12.5 mg/kg, 19DAP025 still showed excellent activity and yielded a 3/3
cure.

The tested benzimidazoles were either very effi cient in elimi-
inating the parasites or could not reduce the parasite load
signifi cantly. DB818 and 6KXR030 cured 3/3 mice at a dosage of
25 mg/kg subcutaneously for 4 days. Administration of 12.5
mg/kg for 4 days resulted in a 2/3 cure for 6KXR030; however,
in the case of DB818, all mice showed recrudescence around
day 20 postinfection. DB942 and DB921 provided 100% suppression
on day 10 but did not suppress parasitemia after day 18.

The diphenyl furan DB75, also known as furamidine, had
excellent antibabesial activity when given subcutaneously. Four
doses at 25 mg/kg cured all mice; at 20 mg/kg, 2/3 mice were
cured, and at 12.5 mg/kg, 1/3 was cured. Even at lower doses,
DB75 suppressed parasitemia completely through day 13 (6.25
mg/kg) or reduced parasitemia by >99% by day 7 (3.125 mg/
kg). Mice treated with other diphenyl furans (DB530, DB555,
and DB930) showed no detectable parasitemia until 10 days
after the last of four injections of 25 mg/kg, but then parasites
gradually reemerged.

Pentamidine is well known for its broad-spectrum antimicro-
bial activity, including antibabesial properties (24). In the
series of pentamidine and pentamidine analogues, two com-
ponds provided cures at 25 mg/kg: the pentamidine HCI
3SLT057 (1/3 cure) and the analogue 3KEG083 (3/3 cure).

The isoxazoles and thiophenes did not show very good po-
tency against B. microti. Although most of them caused a
decrease in parasitemias to <1% by day 7, only one compound
in each series could initially clear the parasites, and all mice
had relapsed by day 17.

Further chemical groups tested only in small numbers in-
cluded guanidino indenes, aza-furans, and biphensyl. All three
groups seemed to bear considerable antibabesial potential.
When administered subcutaneously on four consecutive days
at 25 mg/kg, DB905A, DB820C, and DB986 cured 3/3, 2/3, and
1/3 mice, respectively.

In comparison with the diamidine compounds, the standard
drugs were not particularly eff ective (Fig. 1). Even at a dosage
of 50 mg/kg for 4 days, diminazene treatment resulted in a
pronounced relapse. Atovaquone only reduced the parasite
burden and slightly delayed the peak parasitemia. Only imido-
carb dipropionate provided a 2/3 cure at a dosage of 25 mg/kg
for 4 days.

Correlation of in vitro activity and in vivo efficacy. Of the 25
compounds with low IC50 (<20 ng/ml) tested in vivo against B. microti, 6 caused clearance of the parasites in one or more
mice. A comparable number (7/30) of compounds with IC50 in
the middle range (20 to 85 ng/ml) could also eliminate B. microti from the blood.
TABLE 1. In vitro antibabesial and cytotoxic activities of diamidines

<table>
<thead>
<tr>
<th>Compound (reference)</th>
<th>Structure</th>
<th>Chemical group</th>
<th>Mol wt</th>
<th>IC50 (μg/ml)</th>
<th>B. divergens strain 1903</th>
<th>B. divergens strain 4201</th>
<th>L6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB 0103 (5)</td>
<td>Diphenyl furan</td>
<td>457.00</td>
<td>0.0015</td>
<td>0.0017</td>
<td>&gt;82.27</td>
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<td></td>
</tr>
<tr>
<td>DB 1193b</td>
<td>Diaryl diamidine</td>
<td>553.00</td>
<td>0.0016</td>
<td>0.0026</td>
<td>19.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48–702</td>
<td>Benzimidazole</td>
<td>654.64</td>
<td>0.0017</td>
<td>0.0018</td>
<td>26.7</td>
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<td></td>
</tr>
<tr>
<td>DB 0940 (1)</td>
<td>Benzimidazole</td>
<td>583.40</td>
<td>0.0020</td>
<td>0.0021</td>
<td>65.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB 1191b</td>
<td>Indole</td>
<td>425.35</td>
<td>0.0021</td>
<td>0.0020</td>
<td>6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB 1172b</td>
<td>Indole</td>
<td>402.30</td>
<td>0.0022</td>
<td>0.0021</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB 0921 (11)</td>
<td>Benzimidazole</td>
<td>566.00</td>
<td>0.0023</td>
<td>0.0027</td>
<td>9.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB 1250 (11)</td>
<td>Benzimidazole</td>
<td>581.80</td>
<td>0.0025</td>
<td>0.0020</td>
<td>15.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB 1236 (11)</td>
<td>Benzimidazole</td>
<td>575.00</td>
<td>0.0027</td>
<td>0.0034</td>
<td>17.71</td>
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</tr>
<tr>
<td>DB 0922 (11)</td>
<td>Benzimidazole</td>
<td>541.00</td>
<td>0.0030</td>
<td>0.0035</td>
<td>13.8</td>
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<td></td>
</tr>
<tr>
<td>DB 1190b</td>
<td>Indole</td>
<td>489.4</td>
<td>0.0030</td>
<td>0.0039</td>
<td>64.6</td>
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<td></td>
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<tr>
<td>DB 0942 (11)</td>
<td>Benzimidazole</td>
<td>575.90</td>
<td>0.0035</td>
<td>0.0026</td>
<td>12.6</td>
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</tr>
<tr>
<td>DB 1197b</td>
<td>Benzimidazole</td>
<td>581.50</td>
<td>0.0037</td>
<td>0.0054</td>
<td>42.7</td>
<td></td>
<td></td>
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<tr>
<td>DB 1171 (27)</td>
<td>Indole</td>
<td>354.75</td>
<td>0.0042</td>
<td>0.0038</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued on following page
The results presented in this study demonstrate that a variety of diamidines possess in vitro and in vivo antibabesial activities equal or superior to those of the existing drugs (diminazene aceturate, imidocarb dipropionate, and atovaquone).

The in vitro data with IC50s of less than 10 ng/ml reflect the great antiprotozoal potential of the dications that had already been found against other protozoan parasites. A clear correlation between in vitro activities of the diamidines against B. divergens, P. falciparum, and T. brucei rhodesiense, however, was not evident. A fourth of the tested compounds showed IC50s that were very similar to those of P. falciparum, and another fourth showed IC50s that were very similar to those of T. brucei rhodesiense. Nevertheless, only a very limited number demonstrated comparable activity against all three parasites.

### TABLE 1—Continued

<table>
<thead>
<tr>
<th>Compound (reference)</th>
<th>Structure</th>
<th>Chemical group</th>
<th>Mol wt</th>
<th>R. divergens strain 1903</th>
<th>R. divergens strain 4201</th>
<th>L6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>40–278</td>
<td><img src="image" alt="Structure" /></td>
<td>Benimidazole</td>
<td>677.42</td>
<td>0.0048</td>
<td>0.0046</td>
<td>57.1</td>
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<tr>
<td>DB 0755&lt;sup&gt;a&lt;/sup&gt;</td>
<td><img src="image" alt="Structure" /></td>
<td>Benimidazole</td>
<td>636.10</td>
<td>0.0049</td>
<td>0.0051</td>
<td>13.2</td>
</tr>
<tr>
<td>DB 0818 (19)</td>
<td><img src="image" alt="Structure" /></td>
<td>Benimidazole</td>
<td>474.00</td>
<td>0.0049</td>
<td>0.0051</td>
<td>10.6</td>
</tr>
<tr>
<td>DB 0192 (6)</td>
<td><img src="image" alt="Structure" /></td>
<td>Benimidazole</td>
<td>546.80</td>
<td>0.0052</td>
<td>0.0159</td>
<td>&gt;90</td>
</tr>
<tr>
<td>DB 0988 (11)</td>
<td><img src="image" alt="Structure" /></td>
<td>Benimidazole</td>
<td>553.00</td>
<td>0.0054</td>
<td>0.0058</td>
<td>14.6</td>
</tr>
<tr>
<td>DB 0558 (3)</td>
<td><img src="image" alt="Structure" /></td>
<td>Diphenyl furan</td>
<td>474.50</td>
<td>0.0055</td>
<td>0.0050</td>
<td>87.1</td>
</tr>
<tr>
<td>Imidocarb</td>
<td><img src="image" alt="Structure" /></td>
<td>Standard drug</td>
<td>348.4</td>
<td>0.0106</td>
<td>0.0112</td>
<td>ND</td>
</tr>
<tr>
<td>Diminazene (diaceturate)</td>
<td><img src="image" alt="Structure" /></td>
<td>Standard drug</td>
<td>515.5</td>
<td>0.0158</td>
<td>0.0149</td>
<td>ND</td>
</tr>
<tr>
<td>Atovaquone</td>
<td><img src="image" alt="Structure" /></td>
<td>Standard drug</td>
<td>366.8</td>
<td>0.0091</td>
<td>0.0087</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> The IC50s are mean values for at least two replicates. ND, not done.

<sup>b</sup> Not published.
### TABLE 2. Activities of diamidine compounds and standard drugs against *B. microti* in NMRI mice and *B. divergens* in vitro

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>Compound (reference)</th>
<th>Structure</th>
<th>Dosage(^a) (mg/kg)</th>
<th><em>B. microti</em> cures(^b)</th>
<th>IC(_{50}) (µg/ml)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terphenyls</td>
<td>19DAP025</td>
<td><img src="image1" alt="Structure" /></td>
<td>25, 12.5</td>
<td>3/3, 3/3</td>
<td>0.0331</td>
</tr>
<tr>
<td></td>
<td>23DAP055</td>
<td><img src="image2" alt="Structure" /></td>
<td>25</td>
<td>2/3</td>
<td>0.0633</td>
</tr>
<tr>
<td></td>
<td>19DAP085</td>
<td><img src="image3" alt="Structure" /></td>
<td>25</td>
<td>2/3</td>
<td>0.0457</td>
</tr>
<tr>
<td>Benzimidazoles</td>
<td>6KXR030</td>
<td><img src="image4" alt="Structure" /></td>
<td>25, 12.5</td>
<td>3/3, 2/3</td>
<td>0.0081</td>
</tr>
<tr>
<td></td>
<td>DB818 (19)</td>
<td><img src="image5" alt="Structure" /></td>
<td>25</td>
<td>3/3</td>
<td>0.0049</td>
</tr>
<tr>
<td></td>
<td>DB942 (11)</td>
<td><img src="image6" alt="Structure" /></td>
<td>50</td>
<td>2/2</td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td>DB988 (11)</td>
<td><img src="image7" alt="Structure" /></td>
<td>50</td>
<td>1/2</td>
<td>0.0054</td>
</tr>
<tr>
<td>Diphenyl furan</td>
<td>DB75 (5)</td>
<td><img src="image8" alt="Structure" /></td>
<td>25, 20, 15, 12.5</td>
<td>3/3, 2/3, 1/3, 1/3</td>
<td>0.0112</td>
</tr>
<tr>
<td>Pentamidine/analouges</td>
<td>3KEG083</td>
<td><img src="image9" alt="Structure" /></td>
<td>25</td>
<td>3/3</td>
<td>0.0101</td>
</tr>
<tr>
<td></td>
<td>3SLT057</td>
<td><img src="image10" alt="Structure" /></td>
<td>25</td>
<td>1/3</td>
<td>0.0329</td>
</tr>
<tr>
<td>Guanidino indene</td>
<td>DB905A (3)</td>
<td><img src="image11" alt="Structure" /></td>
<td>25</td>
<td>3/3</td>
<td>0.0289</td>
</tr>
<tr>
<td>Aza-furan</td>
<td>DB820C (13)</td>
<td><img src="image12" alt="Structure" /></td>
<td>25</td>
<td>2/3</td>
<td>0.0410</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>DB986 (12)</td>
<td><img src="image13" alt="Structure" /></td>
<td>25</td>
<td>1/3</td>
<td>0.0844</td>
</tr>
</tbody>
</table>

*Continued on following page*
Interestingly, most of those compounds belonged to the benzimidazoles. This result could suggest that these parasites have common transporters which recognize the amidino-benzimidazole motif.

In the *B. microti* mouse model, the test compounds were considerably more active than the standard drugs. Diminazene aceturate and imidocarb dipropionate are used for treatment and prophylaxis of bovine, equine, canine, and ovine babesiosis in the field, usually as an intramuscular or subcutaneous injection of 3 to 5 mg/kg. In our *B. microti* mouse model, diminazene could not provide a cure at a 10-fold-higher dosage. In previous studies, where different antiprotozoal drugs had been screened against *B. microti* in Mongolian gerbils (24) and hamsters (20), diminazene was one of the most active drugs tested. Imidocarb, though more effective than diminazene, yielded only 2/3 cures at 25 mg/kg for 4 days, while several of the diamidines provided 3/3 cures. To our knowledge, there are no similar studies published where the efficacy of imidocarb was tested in *B. microti* mouse models, which is probably due to the fact that imidocarb is not licensed for human use. With atovaquone we expected to see a higher efficacy, since the drug had been reported to be very effective in *B. microti*-infected hamsters (10, 31) as well as in Mongolian gerbils infected with *B. microti* (9) or *B. divergens* (22). It was suggested, though, that *B. divergens* might be more sensitive than *B. microti*. In comparing our results with previous ones, it has to be considered that we used a different animal species and different treatment regimens. While in our study diminazene was administered at 50 mg/kg on four consecutive days, the Mongolian gerbils were treated at 20 mg/kg over a period of 14 days to achieve a complete eradication of the infection (24). Hughes and Oz administered atovaquone at doses up to 300 mg/kg over 2 weeks to hamsters and reported survival at day 24, but parasites were not cleared at all doses. In a further study in hamsters where atovaquone was used as monotherapy, recrudescences with the emergence of resistant organisms were described. Resistance could be prevented by the addition of

![Chemical structures of compounds](image)

**TABLE 2—Continued**

<table>
<thead>
<tr>
<th>Chemical group (reference)</th>
<th>Compound</th>
<th>Structure</th>
<th>Dosage &lt;sup&gt;a&lt;/sup&gt; (mg/kg)</th>
<th>B. microti &lt;sup&gt;b&lt;/sup&gt; cures&lt;sup&gt;c&lt;/sup&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td>Imidocarb</td>
<td><img src="image" alt="Imidocarb structure" /></td>
<td>25, 12.5</td>
<td>2/3, 0/3</td>
<td>0.0106, 0.0112</td>
</tr>
<tr>
<td>Diminazene</td>
<td><img src="image" alt="Diminazene structure" /></td>
<td>50</td>
<td>0/0</td>
<td>0.0158, 0.0149</td>
<td></td>
</tr>
<tr>
<td>Atovaquone</td>
<td><img src="image" alt="Atovaquone structure" /></td>
<td>25</td>
<td>0/0</td>
<td>0.0091, 0.0087</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All doses were given subcutaneously for 4 days.  
<sup>b</sup> Results are given as number of cured animals/number of infected animals.  
<sup>c</sup> The IC<sub>50</sub> values are mean values for at least two replicates.

FIG. 1. Parasitemia in mice infected with *B. microti* in an untreated control group and after treatment with the standard drugs diminazene (25 mg/kg) and imidocarb (12.5 mg/kg) and with diamidine 19DAP025 (12.5 mg/kg). The standard drugs led to a delayed parasitemia of shorter duration than that for the untreated control, while the diamidine-treated mice were cured.
azithromycin (31). The combination of atovaquone and azithromycin is also used for the treatment of human babesiosis (17, 30). However, unlike the situation with P. falciparum (16), there has been no evidence of treatment failure due to resistant strains of B. microti (18) in humans. Nevertheless, for further in vivo experiments in our B. microti mouse model, combination treatments could provide valuable information.

The IC50 values of the in vivo active diamidines varied from 3.5 ng/ml to >60 ng/ml. This finding suggests that a low IC50 should not be the only selection criterion for in vivo testing, as pharmacological parameters are as important as the antiparasitic activity. The ability of IC50s to predict in vivo activity is problematic, and thresholds should not be set too low when compounds are being selected for in vivo trials.

In conclusion, the data presented in this report indicate the potential of dicationic aromatic molecules as antibabesial agents. It is evident that further studies of structure-activity relationships, mode of action, toxicity, and in vivo efficacy of these compounds are needed before a clinical candidate can be selected.

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