
Copyright © 2015 The Authors.

This work is made available under the Creative Commons Attribution 3.0 Unported License (CC BY 3.0)

Version: Published

http://eprints.gla.ac.uk/99300/

Deposited on: 30 Mar 2015

Enlighten – Research publications by members of the University of Glasgow http://eprints.gla.ac.uk
To achieve malarial elimination, we must employ interventions that reduce the exposure of human populations to infectious mosquitoes. To this end, numerous antimalarial drugs are under assessment in a variety of transmission-blocking assays which fail to measure the single crucial criteria of a successful intervention, namely impact on case incidence within a vertebrate population (reduction in reproductive number/effect size). Consequently, any reduction in new infections due to drug treatment (and how this may be influenced by differing transmission settings) is not currently examined, limiting the translation of any findings. We describe the use of a laboratory population model to assess how individual antimalarial drugs can impact the number of secondary Plasmodium berghei infections over a cycle of transmission. We examine the impact of multiple clinical and preclinical drugs on both insect and vertebrate populations at multiple transmission settings. Both primaquine (>6 mg/kg of body weight) and NITD609 (8.1 mg/kg) have significant impacts across multiple transmission settings, but artemether and lumefantrine (57 and 11.8 mg/kg), OZ439 (6.5 mg/kg), and primaquine (<1.25 mg/kg) demonstrated potent efficacy only at lower-transmission settings. While directly demonstrating the impact of antimalarial drug treatment on vertebrate populations, we additionally calculate effect size for each treatment, allowing for head-to-head comparison of the potential impact of individual drugs within epidemiologically relevant settings, supporting their usage within elimination campaigns.
transmission (5, 13), a wide range of novel potential TBDs are demonstrated in Fig. S2 in the supplemental material. All treatments were administered in Fig. S2 in the supplemental material. All treatments were delivered by oral gavage except sulfadiazine and ATV, which were administered by intraperitoneal (i.p.) injection. Table 1 summarizes the treatments and doses used.

**Mouse-to-mouse transmission model.** For each treatment regime, mouse-to-mouse transmission was performed in duplicate (except primaquine, where individual doses were tested in single experiments) to maximize statistical significance in balance with cost, with general parasite maintenance carried out as previously described (28). A schematic of the model is shown in Fig. 1. Briefly, for each treatment group, five female TO mice (6 to 8 weeks old) were infected with Plasmodium berghei clone 507cl1 (31) by syringe inoculation (i.p.). All care and handling of animals was in accordance with the Guidelines for Animal Care and Use prepared by Imperial College London. At day 9 postinfection, tail blood drops were examined for the presence of exflagellation, and mice were given the appropriate drug/control treatment by the appropriate route (see Table 1). Blood stage infections were monitored on Giemsa-stained tail blood smears, before and 24 h after treatment, as previously described (28).

Ten days postinfection and 24 h posttreatment, the mice were anesthetized and exposed to 500 starved female *Anopheles stephensi* (line sd 500) mosquitoes. Mosquitoes that did not take a blood meal were dis-
5 mice were infected with *P. berghei*. Smears were taken daily starting from day 4-10 post-bite. Record: 1) Presence of infection 2) Parasitemia 3) Gametocytemia. 4 days after feeding, individual mice were exposed to pre-set number of mosquitoes (MBR). Sporozoites were infective. To calculate classical inhibition of transmission, the percentage of inhibited mosquitoes and mean sporozoite intensity were compared to the no-drug control group. The mouse-to-mouse model was used to estimate the overall effectiveness of the different interventions combining data from all repeat replicates. Efficacy of the treatment was assessed by fitting a binomial model. A full description of the methodology is given in reference 28. The models were fitted to the data using maximum likelihood methods and the 95% confidence interval estimates obtained from the likelihood profile.

**RESULTS**

Mouse-to-mouse model and drug treatments. Antimalarials and clinical clinical TBD candidates were screened for malaria transmission-blocking efficacy using the mouse-to-mouse model. Further details regarding treatment and controls are described in Materials and Methods. Raw data generated from individual experiments are included in Dataset S1 in the supplemental material.

**Effect on parasite transmission to, and within, the mosquito vector.** The impact of drugs administered to groups of *P. berghei*-infected mice on the development of oocysts and sporozoites in the salivary glands of *Anopheles stephensi* mosquitoes was recorded. The percentages of inhibition of oocyst and sporozoite intensity and prevalence for each treatment regime compared to those of the no-drug control were calculated (Fig. 2). For the no-drug control, overall oocyst intensity was 47.7 (standard error of the mean [SEM] = 4.0) and oocyst prevalence was 77.7%.

Compared to the no-drug control, A-L at 57 mg/kg-11.8 mg/kg significantly reduced parasite intensity and prevalence in the mosquito. Oocyst intensity was inhibited by 94% (95% confidence interval [CI] of 88 to 97), oocyst prevalence by 41% (95% CI of 22 to 58), and ensuing sporozoite intensity and prevalence by 68% (95% CI of 36 to 77) and 61% (95% CI of 42 to 74), respectively. Overall oocyst intensity for A-L was 6.78 (SEM = 3.2), and oocyst to 100 sporozoites visible; 3 = 100 to 1,000 sporozoites visible; 4 = 1,000+ sporozoites visible). For each treatment group, sporozoite prevalence and mean sporozoite intensity were compared to the no-drug control group to calculate inhibition. The "bitten" mice were allowed to recover and maintained for 10 days postfeeding. Daily tail blood smears were performed from days 4 to 10 to establish parasitemia, and gametocytemia.

Data reporting proportion of biting mosquitoes with salivary gland sporozoites, oocyst intensity, and oocyst prevalence from all experiments are included in Dataset S1 in the supplemental material.
prevalence was 69.5%. The impact of primaquine was clearly dose dependent. At 12 mg/kg (independently or in combination with A-L) and 6 mg/kg, oocyst and sporozoite development was completely blocked, whereas the drug had no significant effect at 1.25 and 0.25 mg/kg (1.25 mg/kg, oocyst intensity \(39.1 \pm 6.1\) and oocyst prevalence 80.6%; 0.25 mg/kg, oocyst intensity \(42.9 \pm 6.1\) and oocyst prevalence 79.1%). In this model, the triple combination, containing primaquine (0.25 mg/kg), was marginally but significantly \((P = 0.0014)\) less efficacious than A-L alone (inhibition in oocyst intensity of 71% [95% CI of 43 to 86] compared to 94% [95% CI of 88 to 97], mean oocyst intensity = 16.6 [SEM = 3.2], oocyst prevalence = 69.5%). OZ439 significantly inhibited both oocyst and sporozoite intensity and prevalence, though the efficacy was relatively modest (46% against oocyst intensity [95% CI of 13 to 67], 29% against oocyst prevalence [95% CI of 13 to 46], mean oocyst intensity = 26.6 [SEM = 4.1], oocyst prevalence = 56.3%). NITD609 significantly inhibited both oocyst and sporozoite intensity and prevalence by \(\geq 85\%\) (mean oocyst intensity = 0.1 [SEM = 0.03], oocyst prevalence = 8.3%). The atovaquone (ATV) positive control completely blocked the mosquito stages of infection, whereas the sulfadiazine negative control, compared to the no-drug control, had no detectable effect on vertebrate-mosquito transmission.

**Effect on transmission to secondary vertebrate populations at different mosquito biting rates.** The impact of individual drug regimens on malarial transmission from infected mosquitoes to a secondary population of naive mice at different transmission intensities (MBRs of 2, 5, and 10 bites) was assessed (the use of differing MBRs permits the estimation of the key output, namely, effect size). Table 2 presents the impact of each compound on mouse infection, compared to that of the drug-free controls, illustrating the prepatent period between sporozoite inoculation and observation of asexual parasites and the percentage reduction in parasitemia and gametocytemia at day 10 postbite. The number of secondary infections (infection prevalence of blood stage infec-

### TABLE 2 Effect of drug treatments on transmission to secondary mouse populations

<table>
<thead>
<tr>
<th>Drug(s) (dose)</th>
<th>Prepatent period in days (±SEM)</th>
<th>% inhibition</th>
<th>Infection prevalence</th>
<th>Parasitemia day 10</th>
<th>Gametocytemia day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>5.7 (±0.31)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>A-L (57 and 11.8 mg/kg)</td>
<td>6.9 (±0.65)</td>
<td>57.9(^b)</td>
<td>50.4(^b)</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>PQ (12 mg/kg)</td>
<td>Not infected</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td></td>
</tr>
<tr>
<td>PQ (6 mg/kg)</td>
<td>Not infected</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td></td>
</tr>
<tr>
<td>PQ (1.25 mg/kg)</td>
<td>5.4 (±0.13)</td>
<td>0.0</td>
<td>4.4</td>
<td>37.4</td>
<td></td>
</tr>
<tr>
<td>PQ (0.25 mg/kg)</td>
<td>5.9 (±0.19)</td>
<td>-9.1</td>
<td>34.2(^a)</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>A-L and PQ (12 mg/kg)</td>
<td>Not infected</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td></td>
</tr>
<tr>
<td>A-L and PQ (0.25 mg/kg)</td>
<td>27.3</td>
<td>34.5</td>
<td>54.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OZ439 (6.5 mg/kg)</td>
<td>6.1 (±0.45)</td>
<td>12.0</td>
<td>13.1</td>
<td>-12.4</td>
<td></td>
</tr>
<tr>
<td>NITD609 (8.1 mg/kg)</td>
<td>Not infected</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td>100.0(^b)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The prepatent period reduction in infection prevalence and reduction in asexual and sexual infection intensity (in infected mice) are illustrated. Percentage of inhibition was calculated relative to the no-drug control. NA, not applicable. Results from all biting rates are included.

\(^b\) Statistical significance (calculated using Fisher’s exact test for percent inhibition in infection prevalence and using bootstrapping, 10,000 replicates, for percent inhibition in parasitemia and gametocytemia at day 10).
indicating primaquine (alone) at 0.25 mg/kg was comparatively low (8%).

As with the ATV positive control, we estimated a 100% effect size for NITD609 (despite a lower number of oocysts and sporozoites being observed in mosquito populations [Fig. 2]) and for primaquine at 12 and 6 mg/kg, indicating that at the maximum exposure considered (MBR = 10), these drugs would result in elimination from these laboratory populations. Drugs with a moderate estimated effect size included A-L, OZ439, and primaquine at 1.25 mg/kg (58%, 57%, and 29%, respectively, at the stated doses), while the estimated effect size for primaquine (alone) at 0.25 mg/kg was comparatively low (8%).

**DISCUSSION**

Using a mouse-to-mosquito-to-mouse population transmission model, we have analyzed five clinical and preclinical antimalarial drug candidates for transmission-blocking activity, individually assessing impact upon both mosquito (oocyst and sporozoite) and vertebrate (asexual and sexual blood stage) phases of development. This allows for the head-to-head comparison of efficacy and the estimation of effect size, thereby measuring the potential ability of TBDs to reduce R<sub>p</sub>. In this manner, we can simply triage individual drugs by their potential impact on vertebrate populations. The TBDs examined can be broadly divided into three categories: those with total (100%), moderate (25% to 60%), or low (<10%) estimated effect sizes.

A 100% effect size was estimated when examining the ability of the novel antimalarial drug candidate NITD609 to block malarial transmission. As an inhibitor of *Plasmodium* protein synthesis, NITD609 is known to act against multiple parasite life stages (7, 34) and has previously demonstrated excellent potency against *P. falciparum* and *P. berghei*. In previous studies, examining the asexual form of the parasite, a single oral dose of NITD609 at 100 mg/kg was reported to completely cure *P. berghei*-infected mice, whereas 30 mg/kg cured 50% of mice and 10 mg/kg could not clear infection (34). In terms of activity as a TBD, it has previously been shown to inhibit both early and late gametocyte development in a dose-dependent manner between 5 and 500 nM (7). NITD609 demonstrates efficacy in an *in vitro* *P. berghei* ookinete conversion assay, with a 50% inhibitory concentration (IC<sub>50</sub>) of 3 μM, and strongly inhibits transmission to mosquitoes in the SMFA (7). Here, NITD609 showed excellent potential as a TBD. At a dose of 8.1 mg/kg, NITD609 exhibited a potent (but not total) ability to inhibit transmission from mouse to mosquito and completely blocked progression of the life cycle to subsequent mice with a single dose. As NITD609 belongs to a novel, synthetic class of antimalarials, the spiroindolones, if approved, it has strong prospects for future use when targeting artemisinin-resistant parasites. It is the first antimalarial with a novel mode of action to enter phase Ia trials in the last 20 years. Our results, combined with impressive data reported previously, clearly demonstrate that NITD609 has exciting potential to be used as a potent single-dose TBD in the near future. A 100% effect size was also observed when examining the transmission-blocking efficacy of the control drug ATV, supporting previous studies showing potency (28). ATV is commonly administered with proguanil (Malarone) for treatment and prevention of malaria; however, resistance against the cytochrome bc<sub>1</sub> complex that ATV targets is considered to be widespread and comparatively easily generated (35).

Primaquine, a widely utilized antimalarial, additionally generated a 100% estimated effect size at higher dosages (12 and 6 mg/kg). Its potent impact is strongly dose dependent; any induced infection (34). In previous studies, primaquine has been reported to reduce gametocyte carriage in combination with an ACT, with a wide range of studies reporting a significant reduction compared to that of the ACT alone (10, 36–41). The infectiousness of treated gametocytes to mosquitoes, or onward transmission and impact on new vertebrate infections, was not examined within any of these studies.

**TABLE 3 Effect size of individual drug treatments**

<table>
<thead>
<tr>
<th>Effect size</th>
<th>Drug(s) (dose)</th>
<th>Effect size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>ATV (0.3 mg/kg)</td>
<td>100 (96–100)</td>
</tr>
<tr>
<td></td>
<td>PQ (6 mg/kg)</td>
<td>100 (96–100)</td>
</tr>
<tr>
<td></td>
<td>NITD609 (8.1 mg/kg)</td>
<td>100 (96–100)</td>
</tr>
<tr>
<td></td>
<td>PQ (12 mg/kg)</td>
<td>100 (58–100)</td>
</tr>
<tr>
<td></td>
<td>A-L and PQ (12 mg/kg)</td>
<td>100 (58–100)</td>
</tr>
<tr>
<td>Moderate</td>
<td>A-L (57 and 11.8 mg/kg)</td>
<td>58 (19–86)</td>
</tr>
<tr>
<td></td>
<td>OZ439 (6.5 mg/kg)</td>
<td>57 (31–75)</td>
</tr>
<tr>
<td></td>
<td>A-L and PQ (0.25 mg/kg)</td>
<td>42 (20–99)</td>
</tr>
<tr>
<td></td>
<td>PQ (1.25 mg/kg)</td>
<td>29 (–29–69)</td>
</tr>
<tr>
<td>Low</td>
<td>PQ (0.25 mg/kg)</td>
<td>8 (–18–48)</td>
</tr>
<tr>
<td></td>
<td>SD (8.4 mg/kg)</td>
<td>−0.1 (–0.8–0.3)</td>
</tr>
</tbody>
</table>

* Drug treatments have been ranked in order of effect size and transmission-blocking efficacy. Treatments have been broadly divided into three groups: drugs with total (100%) effect size, drugs with a moderate effect size, and drugs with a low effect size. ATV, atovaquone; PQ, primaquine; A-L, artemether-lumefantrine; SD, sulfadiazine.
Two older studies have directly examined the ability of primaquine to directly inhibit transmission in a small number of patients ($n = 2$) (42, 43). In these studies, a single dose of 45 mg of primaquine base proved to inhibit onward transmission from human to human (1 from a group of 4 individuals developed patent P. falciparum infection posttreatment at a single MBR of 75). Despite this raft of largely positive findings, the transmission-blocking efficacy of primaquine, particularly at lower doses and in combination with an ACT, remains poorly understood (1, 12, 44, 45). Our results corroborate previously observed dose dependency, although the doses at which we identified a significant effect were considerably higher. We observe no additional benefit of adding primaquine (at 0.25 mg/kg) to the A-L combination, consistent with two recent Cochrane reviews which concluded that there was no clear evidence as to whether primaquine (in combination with an ACT) can directly reduce onward transmission in an area where malaria is endemic, even if it significantly reduces gametocyte prevalence (44, 45). In light of these, and our, findings, the role of primaquine as a TBD in malaria treatment should continue to be examined carefully in subsequent studies, especially at low dosages. Several valuable clinical trials to determine the most effective dosing are under way (10).

A moderate effect size was observed when examining two further antimalarials, A-L and OZ439. A-L is a common ACT currently approved for treatment of uncomplicated P. falciparum malaria. Our data demonstrated only a moderate transmission-blocking effect of A-L at the tested dosages on P. berghei transmission to mosquitoes, which translated into a significant reduction in secondary malaria infections in mice. This is consistent with previous studies of P. falciparum in humans given this ACT as first-line therapy, which have demonstrated reduction in both gametocyte carriage posttreatment and onward transmission to mosquitoes following A-L treatment (11, 36, 46–50). The data described here further demonstrate the potential impact of A-L to achieve a substantial reduction in malaria infection within vertebrate populations at the dosages tested. Previous studies have discussed the translation of laboratory-based transmission of Plasmodium compared to transmission in the field (28, 51, 52). Translation of an effect size into impact in epidemiological settings requires further consideration of the mode of delivery, coverage, and the balance between reducing onward transmission and protection from reinfection (53). Nevertheless, an estimated effect size of 58% suggests that use of such drugs could lead to significant reductions in transmission if deployed appropriately in low-transmission settings.

Our results confirm that the endoperoxide, OZ439, has potential as a transmission-reducing drug. OZ439 has several advantages over artemisinin derivatives, including prolonged plasma exposure, higher potency, and its stable, synthetic nature (6). It has shown comparable efficacy in the treatment of P. falciparum and Plasmodium vivax patients (6), is undergoing phase Ia clinical trials, and is intended for use as a single-dose combination therapy for acute malaria. It has previously been demonstrated to completely cure P. berghei-infected mice (blood stage infection) with a single oral dose of 20 mg/kg (6) but has no effect on P. berghei ookinete development in in vitro assays at 10 μM, suggesting it functions prior to the ookinete stage (20). It has additionally demonstrated a potent effect in the SMFA with P. falciparum at 10 μM (20). Here, when administered 24 h prefeed at the tested dose of 6.5 mg/kg, OZ439 significantly reduced oocyst and sporozoite development in the mosquito but did not significantly reduce the number of secondary mouse infections. Despite this, when mosquito and mouse data were collated and examined using a chain-binomial model, a moderate effect size was estimated, comparable to that observed when using A-L at 3 × ED90. Recent studies using SCID mice have suggested promising results against asexual blood stage infection using a dose of 19.5 mg/kg. Future studies should aim to complement these results by assessing the transmission-blocking efficacy of OZ439 at this higher dose. Additionally, evaluation of potential partner drugs for OZ439 is under way, with the aim of increasing the transmission-blocking potency of treatment and thus combating potential emerging resistance to the compound in the future.

The use of the mouse-to-mouse model addresses key gaps in our existing knowledge (54) that other transmission-blocking assays cannot, being the only fully in vivo model that measures the impact of an intervention on mosquito-to-vertebrate and vertebrate-to-vertebrate transmission. The resulting generation of effect size can be subsequently used within mathematical models of malaria transmission to predict the public health significance of individual TBDs in different settings and in combination with different interventions. These studies will, as data accumulate, also allow us to correlate “standard” transmission-blocking assay outputs (e.g., reduction in oocyst intensity/prevalence) to reduction in parasite prevalence/intensity in subsequent vertebrate populations. The use of the tractable rodent malaria parasite P. berghei provides a safe, cost-effective, and robust population model to examine these parameters. We are fully aware that any findings using rodent parasites require validation with respect to human malaria parasites; however, early-stage efficacy trials of this type using human volunteers are currently technically and ethically impossible (13). We are additionally aware that differences in drug pharmacokinetics (PK) between mice and humans are critical to any field extrapolation of the data (55, 56). Recognizing that the rate of drug clearance in rodents is approximately three times faster than that in humans (55–57), drugs given to rodents at 3 × ED90 in comparison with an equivalent allometric dose in humans, will potentially result in lower levels of active circulating TBD 24 h posttreatment, potentially underpredicting the impact of compounds. To enhance our understanding of the biological process of metabolism and clearance of specific drugs, it would be advantageous for future studies to compare PK data for individual TBDs in rodents and humans at multiple time points posttreatment.

The systematic development, examination, approval, and widespread use of new antimalarial TBDs will require phase 3 trials, with successful outcome determined as the reduction in transmission measured by decreased incidence of malarial infection. Given the exceptionally high costs of such studies, and the subsequent ethical considerations, it is both important and logical to set realistic go/no-go criteria for efficacy before such trials. It is additionally highly advantageous to triage potentially effective drugs by head-to-head comparison prior to development to this level. The studies described here assist this comparison in a cost-effective, ethical, safe, and robust manner. Our results demonstrate that different TBDs have various transmission-blocking potentials at both the mosquito, vertebrate, and population levels, and hence successful formulation for their field utilization will differ in various transmission settings. The intelligent use of specific antimalarial drugs will require careful consideration of TBD efficacy, effect size, and transmission intensity/EIR in targeted areas. Even drugs with high transmission-blocking levels are likely to
have the greatest impact if delivered as part of focal case detection strategies in low-transmission settings in which a high proportion of the infectious reservoir is treated (58, 59). We directly compare the ability of currently utilized (A-L and PQ) and novel preclinical (OZA439 and NITD609) antimalarials to reduce parasite intensity and prevalence at multiple transmission settings. We examine these crucial outputs not only within the mosquito but also following transmission to subsequent vertebrate populations, demonstrating their cial outputs not only within the mosquito but also following trans-

ACKNOWLEDGMENTS
A.M.B. thanks MMV (Geneva), the Fraunhofer Institute (Delaware, USA), and MV1-PATH (USA) for funding. A.M.B. is funded by MVI-PATH (http://www.mariavaccine.org/). I.M.U. is funded by MV1 and MMV. K.A.S. and P.M.B. are funded by MVI. T.S.C. was funded by a Junior Research Fellowship from Imperial College London. P.W.G. is a Medical Research Council (United Kingdom) Career Development Fel-
nov. K00669X) and acknowledges support from the Bill and Melinda Gates Foundation (no. OPP1068440) and the Medicines for Malaria Venture. M.J.D. is funded by the Bill and Melinda Gates Foundation (no. OPP1068048). A.C.G. acknowledges funding from the Bill and Melinda Gates Foundation (no. K00669X) and acknowledges support from the Bill and Melinda

REFERENCES


