Endectocides for controlling transmission of mosquito-borne diseases

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1 Introduction

Controlling malaria parasite transmission among endemic communities by targeting vectors is a cornerstone of successful malaria control, and critical to future malaria elimination and eradication efforts. This concept is underscored by examining the simple models of *Anopheles* spp. vectorial capacity (VC) for malaria transmission and the basic reproductive number (R_0) of malaria; proportionally, the two most influential variables affecting VC and R_0 are the daily mosquito survival rate (*p*) and the human biting rate (*a*). Targeting these variables usually consists of attacking *Anopheles* vectors with insecticides, for example, with long-lasting insecticidal nets (LLIN) or indoor-residual spraying (IRS). The problem is that mosquito populations can rapidly adapt to these control methods to maintain transmission. In the face of extensive vector control, certain *Anopheles* species or sub-species can rise in prominence and/or proportion to the pre-control vector population and maintain *Plasmodium* transmission, particularly those with exophagic and exophilic habits [1-4]. Other sub-populations adapt through behavioural changes, such as more crepuscular biting when people are not under LLIN [2,3]. Lastly, genetic changes driving metabolic resistance mechanisms and/or target site sensitivity for insecticides can occur [5]. New mosquitocidal agents need to be developed and they need to be delivered in novel ways [6].

2 The idea

Our idea (as part of our Grand Challenge Exploration grant; Round 1, 2008) was to develop a control method that targets the most important variables of VC and R_0 so malaria parasite transmission would be maximally impacted, but that did so by targeting mosquitoes through human blood meals. This would a) target *all* malaria vectors around a community, regardless of when and where they bite, and b) ensure that the effector molecules directly entered the midgut of mosquitoes rather than needing to be efficiently applied in the environment and then efficiently penetrate the vectors' cuticle. This concept had been developed by one of the authors (BDF) since his time as a graduate student in efforts to discover *Anopheles gambiae* antigen targets that could be developed as components of a mosquitocidal vaccine [7-9]. Such a vaccine would produce mosquito-targeting immunological components in human blood that would be directly ingested by the mosquito when it bites, and reduce mosquito survival and/or delay its re-feeding ability. In a parallel idea, a drug ingested by humans which circulates in the blood, that has mosquitocidal properties, that has a reasonably long half-life in the blood stream, and that is administered to much of the malaria-endemic community, might work in a similar fashion.

We also wanted to specifically target the most efficient malaria vectors (e.g. *Anopheles gambiae*) through blood meals in order to take advantage of two very important concepts that define malaria transmission: 1) the extrinsic incubation period (EIP), defined as the time it takes for *Plasmodium* parasites to develop from gametocytes in the blood meal into infectious sporozoites in the salivary glands, which is ≥9 days and, 2) the most efficient malaria vectors, like *Anopheles gambiae,* are highly anthropophagic and take blood meals frequently, often every two days, likely because they utilise much of the blood meal as an energy source in addition to a protein source for egg production. When these two concepts are assessed together, the unique potential of targeting malaria vectors via the blood meal becomes apparent. A mosquito that has acquired *Plasmodium* gametocytes after biting an infected person will be able to spread these parasites to another person only after at least 9 days of parasite development, but in that same length of time, the mosquito will take bloodmeals from up to 5 more people without transmitting parasites. If only one of these 5 people has a mosquito-lethal concentration of endectocide circulating in their blood at the time of the bite, their blood will kill the mosquito and the mosquito will never transmit parasites.

Literature searches quickly identified ivermectin as a drug that likely fit the profile we envisioned above. Soon after the avermectins (precursors to ivermectin [IVM]) were discovered in bioassays showing mice were cured of

https://doi.org/10.5281/zenodo.10894657

a parasitic nematode [10], researchers at Merck Sharpe and Dohme discovered IVM also had potent insecticidal and acaracidal properties [11,12]. The first tests against mosquitoes that blood fed on treated mice were published in 1985, where the authors discovered that *An. stephensi* were more susceptible than either *Aedes aegypti* or *Culex quinquefasciatus* [13]. In 1987, French authorities approved IVM's use in humans to combat onchocerciasis (or river blindness). Our proposal to test IVM for malaria parasite transmission control also seemed to fit the expressed desires of the BMGF and GCE programmes; it was a novel idea, but at the same time, if successful, it could be repurposed relatively quickly given that the ivermectin is very safe and already given in mass drug administrations (MDA) to humans around the world. Of the publications studied as we were forming and testing our ideas, three most influenced our ideas going forward: Wilson [14] reviewed much of the initial literature published on the insecticidal properties of the avermectins and concluded that what was lacking was understanding the potential of the avermectins to influence the variables of VC and thus influence transmission of disease by vectors. Using a natural experiment, Bockarie *et al*. [15] cleverly looked at ivermectin's effects against the Papua New Guinean vectors of malaria and lymphatic filariasis (LF) as the drug was implemented in villages for LF control. The effects were striking; 100% of the wild indoor-resting blood-fed *An. punctulatus* and *An. koliensis* collected 1-3 days after a village treatment were killed within 9 days of being collected compared to 33% and 17% of those caught pre-treatment and 28 days post-treatment, respectively. The proportion of vectors that were infected with *Wuchereria bancrofti* dropped from ~10% to 0% after the MDA, however, because the drug treatments (diethylcarbamazine was also used in one village) mainly kill the microfilaria (transmission) stages of *W. bancrofti* in the villagers, the authors would have been unable to distinguish any separate contribution that the mosquitocidal effect may have had on decreased *W. bancrofti* infections in the collected vectors. The authors also measured human biting rates to assess whether the mosquitocidal effect suppressed the total LF vector population. Here, they found that the biting rate significantly *increased* in the months after treatment, likely reflecting the transient nature of the mosquitocidal effect and suggesting that the biting rate over the long term is much more influenced by environmental factors and the standing larval reservoir at the time of MDA. Lastly, Foley *et al.* [16] demonstrated a strong and lasting mosquitocidal effect of ivermectin by directly blood feeding colonised *An. farauti* on a single treated human volunteer. Groups of mosquitoes had significantly increased mortality if they blood fed on the volunteer up to 14 days post treatment. The authors also used deterministic models to predict the effects that increasing proportions of treated hosts relative to untreated ones (either human or animal) would have on variables of malaria parasite transmission (the human inoculation rate, sporozoite rate, VC) when the vectors were either zoophilic or anthropophilic. The model predicted that treating humans could dramatically reduce variables of malaria parasite transmission regardless of the host preference of the vectors. From our own ideas and these three key publications, we devised experiments to test the effects of ivermectin and other endectocides against colonised *An. gambiae*, and to perform similar field tests first conducted by Bockarie *et al.*, but in a highly malaria endemic area of Africa that is also endemic for onchocerciasis, and thus receiving annual ivermectin MDA for onchocerciasis control. BDF was lucky enough to be introduced to MS in 2005 when MS was visiting Colorado State University for training. MS had been working in malaria- and onchoceriasis-endemic south-eastern Senegal for many years and was very familiar with the local people, primary health care personnel, and with villages that received ivermectin MDA, all of which was critical for this work to be performed. Collaboration was planned and both authors conducted preliminary mosquito sampling in a Senegalese treated village in August of 2006 to see if they could replicate the results of Bockarie *et al.* The preliminary field data suggested that the ivermectin MDA indeed caused a mosquitocidal effect against wild African *Anopheles* malaria vectors and these data corroborated our preliminary laboratory data with colonised *Anopheles gambiae*. KCK entered into our collaboration in 2007, first as a research associate and later as a graduate student, and was instrumental in gathering data in both the field and laboratory, as well as helping shape subsequent ideas and experiments. We initially submitted a proposal on these ideas to the U.S. National Institutes of Health (NIH) in October of 2006, but it was not funded. We subsequently submitted a smaller, more focused proposal on the same ideas to the NIH in October 2007, and then submitted a proposal in May of 2008 to the GCE program Round 1, to extend these ideas and experiments. Both of these proposals were funded.

3 Results

Experiments were conducted in the laboratory testing ivermectin and other systemic drugs against colonised *An. gambiae*, *Ae. aegypti*, and *Culex* mosquitoes using *in vitro* membrane-feeding assays. Our first experiments measured the LC_{50} and other effects against our various colonised mosquitoes and compared it to the known pharmacokinetics of ivermectin in human blood after a standard treatment [17]. The LC_{50} of ivermectin for *An. gambiae s.s.* G3 strain was 22.4 ng/ml [18.0, 26.9], but was more than 20 times higher for *Ae. aegypti* Rexville D strain. This showed that the activity against *An. gambiae* was well within the range of IVM blood concentrations after standard MDA, but human blood concentrations from approved

doses were too low to effectively target Culicine mosquitoes. Other anthelmintic drugs that are also given via MDA, diethylcarbamazine, pyrantel and albendazole (sulfoxide), exhibited no effect against mosquitoes. Ivermectin blood concentrations predicted from the first two nights after a standard MDA also delayed mosquito re-blood feeding frequency and faeces defecation rates. Finally, consecutive blood meals containing ivermectin concentrations that reflect human blood concentrations on days following MDA enhanced the mosquitocidal effect. We published a subsequent study examining various *Ae. aegypti* strains and showed that approximately 3 fold differences in ivermectin susceptibility can occur between different strains of the same mosquito species, and that metabolic cross-resistance to permethrin may influence ivermectin susceptibility [18]. Finally, we tested six other veterinary-approved systemic insecticides against *An. gambiae* to compare them with ivermectin. Of the six, only eprinomectin, a similar drug also derived from avermectin, demonstrated comparable effects to ivermectin. We also showed that a blood meal containing sub-lethal mosquito concentrations of either of these drugs significantly inhibited the ability of *An. gambiae* to 'recover' (fly through a cone to access sugar and water) after the blood meal [19].

Our field studies were conducted during the rainy malaria transmission seasons in southeastern Senegal, where onchocerciasis is also endemic and ivermectin MDAs are conducted yearly to control this disease. We aspirated wild indoor-resting blood fed *Anopheles* from houses of pair-matched control and treated villages, over three separate time periods in 2008 and 2009. Backpack aspiration collections were performed from 13 days prior to MDAs in the treated villages, to up to 12 days after treatment. The captured mosquitoes were held in an insectary for 5 days post-capture to measure their survival. We demonstrated that blood fed *Anopheles gambiae s.s.* captured from the treated villages between 1-6 days after MDA suffered significant mortality relative to mosquitoes captured prior to the MDA, to those captured >7 days after the MDA, and to all those captured from the control villages at all time intervals [20]. A similar trend was shown for captured *An. arabiensis*, but the data were less robust as we only caught this species during the third trial. We then modelled the impact of our field-measured reduction in the daily probability of mosquito survival relative to controls (-10.4%) on malaria R_0. The model showed that significant reductions in R_0 could occur depending on the ivermectin MDA treatment interval. Lastly, we processed the captured *An. gambiae s.s.* that survived for 5 days in the field insectary for *Plasmodium* infection. These data showed that the mean sporozoite rate in mosquitoes from the treated villages decreased by 79% from those caught before to after MDA, while the sporozoite rate in mosquitoes from the control villages increased by 246% over the same timeframe [21].

4 Discussion

Our findings strongly validated our hypotheses with respect to malaria transmission, which was the primary focus of our GCE project. We already had data on the high susceptibility of colonised *An. gambiae* to low concentrations of ivermectin at the time of our GCE submission, but we were unsure whether similar effects could be observed from the natural field experiments we were proposing, and we were appropriately sceptical that any effect on sporozoite rates would be observed at all. To our surprise, the effects of ivermectin in the field were far stronger than what we expected from our laboratory data. This is highlighted by our observations from controlled *in vitro* membrane feeding experiments in the lab that colonised *An. gambiae* were only susceptible to ivermectin concentrations that would be expected in human blood for about 2 nights following an MDA [17]. However, field data showed significantly enhanced mortality of wild *An. gambiae* that blood fed on treated people up to 6 days after MDA [20], and the treatment resulted in unexpectedly large reductions in sporozoite rates [21]. It is even more surprising that these data were collected despite all the uncontrolled variables that are inherent to such natural experiments, including the fact that ~17% of villagers didn't participate in the MDA, and we couldn't decipher on which persons mosquitoes had fed.

Our project provided definitive answers on the direct effects of ivermectin on *An. gambiae s.s.* mortality in both the laboratory and the field. We currently infer that the stronger-than-expected field data we observed are the result of a range of effects ivermectin has on *Anopheles* beyond direct mortality. We have collected unpublished laboratory data suggesting that ivermectin is more active against older mosquitoes. Our published laboratory data [17,19] also suggests that sub-lethal ivermectin concentrations, imbibed by wild mosquitoes that bite for many days after the MDA, inhibits mosquito flight and other physiological systems so that the mosquitoes are less likely to survive in nature. Finally, we hypothesised that sub-lethal ivermectin concentrations may also inhibit sporogony of *Plasmodium* in *Anopheles*, and thus affect another variable of VC (*b*). Our recently published laboratory data, collected at the Walter Reed Army Institute of Research, confirms this hypothesis [22]. We currently hypothesise that these effects combine to significantly curtail the proportion of infectious (sporozoite-containing) mosquitoes for a period of at least several weeks after the MDA.

5 Future perspectives

We believe that our GCE project, combined with our NIH funding, has helped to stimulate a new research agenda centred on developing ivermectin and other endectocides for malaria transmission control. While we made signifi-

cant progress with the GCE award, especially in conducting key proof-of-principal experiments, many questions remain. In particular, how exactly do the anti-mosquito and anti-sporogony effects of ivermectin combine to reduce sporozoite rates, and for how long? There is no evidence in our and other's observations that a single MDA significantly reduces biting mosquito populations around treated villages over an extended period. Indeed, the modelling we have done suggests adult mosquitoes are only transiently reduced by around 26% in the week following the MDA. Rather, the transient mosquitocidal effects of ivermectin likely shift the population structure of the surviving adult mosquito population to younger age classes for weeks following the MDA [23], but work remains to demonstrate this with empirical data. We also do not know whether anti-sporogony effects occur in the field, and if so, what contribution they make towards reducing sporozoite transmission. Data remains to be collected on how susceptible exophagic and exophilic vectors are to ivermectin MDA. Maybe most importantly, we need to precisely measure the length of time that a single MDA inhibits *Plasmodium* transmission, and define the critical operational, biological and environmental parameters that influence this. As we answer these questions, the future clearly points to controlled clinical trials where we determine if ivermectin MDAs can work to reduce malaria infections and clinical episodes among people. It could be expected that such treatments would integrate with other malaria control measures (antimalarial treatments, ITNs and IRS) and would simultaneously work to control helminth infections and other neglected tropical diseases in the community.

6 Acknowledgements

We like to acknowledge all people who contributed to making this project a success, including Dr. Moussa Dieng Sarr and the Senegalese Ministry of Health, Dr. Doudo Sene, Dr. Saul Lozano-Fuentes, Dr. Anna Winters, Dr. Phillip Chapman, Dr. Kelsey Deus, Dr. Karla Saavedra-Rodriguez, Dr. William Black IV, Dr. Jason Rasgon, Tan Hongyu, Meg Gray, Ines Marques da Silva, Matthew Butters, Bren Wheeler, Mactar Mansaly, and the people of Ndebou, Ibel, Damboucouye, Boundoucondi, Nathia and Bandafassi. We would also like to acknowledge funding from the U.S. NIH for grants R21 AI079528-01, N01 AI25489, R01 AI060960-01A1, from the U.S. Military Infectious Disease Research Program, and especially from the Bill and Melinda Gates foundation for GCE grant 51995.

The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the U.S. Department of the Army or the U.S. Department of Defense.

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Grant recipient publications directly related to this GCE project

Kobylinski KC, Deus KM, Butters MP, Hongyu T *et al.* The effect of oral anthelmintics on the survivorship and re-feeding frequency of anthropophilic mosquito disease vectors. *Acta Trop*. 2010, **116**:119-126.

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Competing interests: No competing interests declared.