Repurposing isoxazoline veterinary drugs for control of vector-borne human diseases

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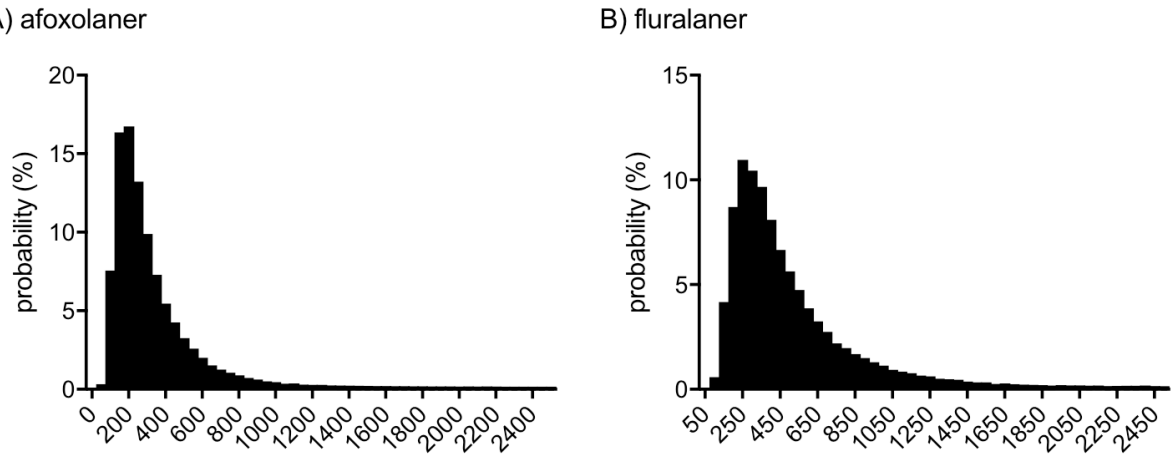
SI Appendix

Figures

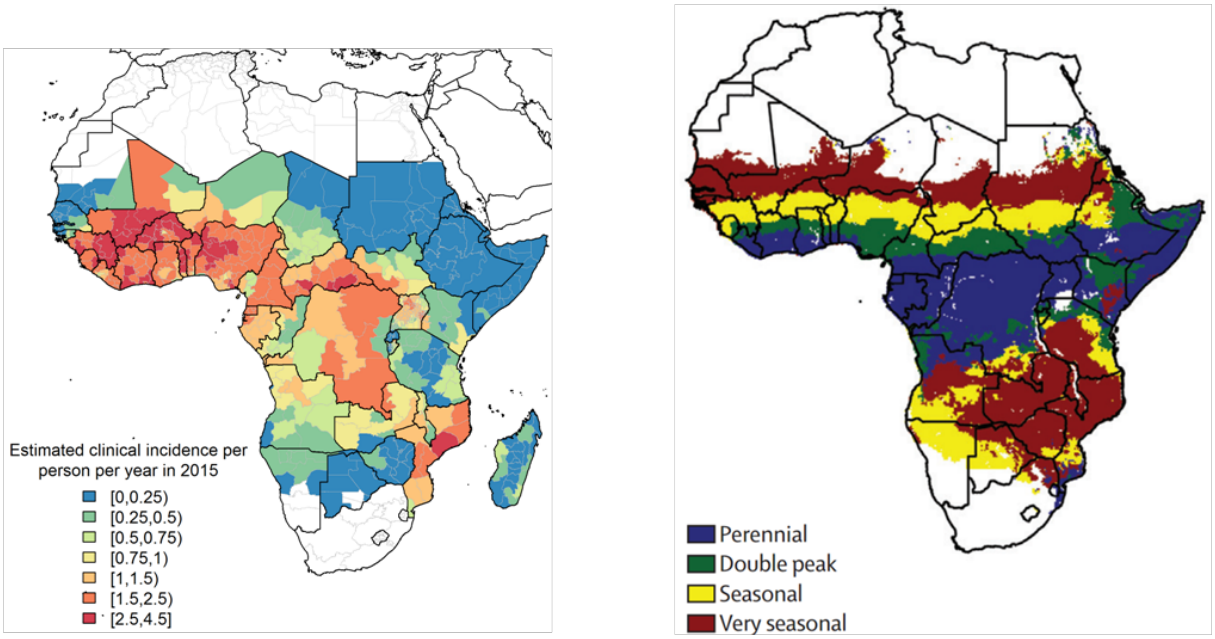


	value
Minimum	40.77205
25% Percentile	177.01635
Median	257.64385
75% Percentile	406.69165
Maximum	6266.119
Mean	360.439004427828
Std. Deviation	369.012165373013

	value
Minimum	65.08561
25% Percentile	277.788125
Median	407.8749
75% Percentile	647.822675
Maximum	9840.97
Mean	578.470227481782
Std. Deviation	607.818054911775

SI Appendix Figure S1:

Predicted human dose levels. Stochastic modeling was used to estimate the doses providing circulating drug levels above the *Anopheles/Aedes* IC_{99} up till day 90 post dosing. The figure shows the distribution of the dose predictions for afoxolaner (A) and fluralaner (B) and the cognate summary statistics. For an average 70 kg subject the predicted median doses were 258 mg for afoxolaner and 408 mg for fluralaner which were rounded up to the nearest 10 mg in main body of the text to give values of 260 and 410 mg respectively.



SI Appendix Figure S2:

Estimated clinical incidence based on prevalence-incidence relationship in the Imperial College malaria model and the Malaria Atlas Project estimates for 2015 prevalence and seasonality profile based on rainfall data (1-3).

Tables

	Target	Sodium channel			Acetylcholine esterase		GABACl
	Mutation type	kdr			Ace		rdl
	Family	pyrethroid			carbamate	organo-phosphate	
	Molecule	0.75% Permethrin	0.05% Delta-methrin	4% DDT	0.1% Propoxur	1.0% Feni-trothion	4% Dieldrin
Anopheles gambiae	Kisumu	100	100	100	100	100	96
	Tiassale 13	22	10	1	67	100	22
Aedes aegypti	New Orleans	100	100	91.7	100	100	100
	Cayman	13	42	13	7.75	100	91

SI Appendix Table S1:

Characterization of the insecticide resistance in different strains of *Anopheles* and *Aedes* mosquitoes housed at the Liverpool Insect Testing Establishment using the standard WHO paper contact assay following exposure to the drugs indicated in the table. The results are shown as percentage mortality (on over 100 mosquitoes in each case), the red numbers indicate values that fall within the criteria for resistance (4).

		Dog pharmacokinetics	Predicted human pharmacokinetics
Fluralaner	Oral bioavailability	11-34%	25%
	CL (mL/min/kg)	0.0972	0.0612
	V (L/kg)	3.1	3.1
	Half-life (days)	15.0	24.4
Afoxolaner	Oral bioavailability	74%	74%
	CL (mL/min/kg)	0.0825	0.0519
	V (L/kg)	2.68	2.68
	Half-life (days)	15.5	24.4

SI Appendix Table S2:

Estimation of pharmacokinetics parameters in human based on the published values found in dogs (5, 6). CL stands for plasma clearance and V for volume of distribution.

	Rodent Study - No Adverse Effect Dose	**Rodent Study - Human Equivalent Dose**	**Dog Study - No Adverse Effect Dose**	**Dog Study - Human Equivalent Dose**
Fluralaner	Acute Rat/ Single Dose: 2,000 mg/kg	19,350 mg	24- Week; 3 Doses at 56 Day Intervals: 280 mg/kg	9,333 mg
	4-Week Rat; Daily Repeat Dose: 60 mg/kg	580 mg		
	90-Day Rat; Daily Repeat Dose: 40 mg/kg*	387 mg		
	90-Day Rat; Daily Repeat Dose: 400 mg/kg*	3,870 mg		
Afoxalaner	Acute Rat/ Single Dose: 300 mg/kg	2,900 mg	18-Week; 3 Doses at 30 Day Intervals followed by 3 doses every 2-weeks: 31.5 mg/kg	1,050 mg
	7 or 12 Day Mouse; Daily Repeat Dose: 550 mg/kg	2,682 mg	70-Day; Doses every other Week: 25 mg/kg	833 mg
	14-Day Rat; Daily Repeat Dose: 10 mg/kg	96 mg		

SI Appendix Table S3:

Summary of published toxicological studies (7-12) carried out in rats, mice and dogs for approval of afoxolaner and fluralaner as a veterinary drug. Estimation are based on published guidelines (12), assuming a 60 kg human subject. (*) NOAEL varies in interpretation based on EMA (10) (40mg/kg) and Australian MSDS (11) (400mg/kg).

Supplemental Materials & Methods

Allometric scaling of pharmacokinetics parameters

The dog PK parameters for both compounds have been reported previously (5, 6). Clearance (Cl) and volume of distribution (Vd) were scaled using fixed exponents of 0.75 and 1.0 respectively (typical values for both parameters (13, 14)) using the equations below.

$$Cl_{human} = Cl_{dog} * (BWT_{human}/BWT_{dog})^{0.75}$$

$$Vd_{human} = Vd_{dog} *(BWT_{human}/BWT_{dog})^{1.0}$$

The predicted human PK parameters for afoxolaner using this approach are Cl_{human} = 5.23 L/d and Vd_{human} = 188 L and for fluralaner Cl_{human} = 6.17 L/d and Vd_{human} = 217 L assuming a bodyweight (BWT) of 11 kg for dog at 70 kg in man.

Human PK model and dose prediction

A single compartment model with a first order absorption at rate Ka, of drug with bioavailability F and first order rate of elimination Ke (=Cl/Vd) was used to describe the concentration-time profile. The dose to achieve a target plasma concentration can be calculated from rearranging the equation that describes the plasma-concentration C(t) at time t using this model.

$$C(t) = F*Dose*Ka/(Vd*(Ka-Ke))*(e^{-Ke*t}-e^{-Ka*t})$$

A nominal absorption rate (Ka) of 1 h^{-1} was used with nominal F values of 0.74 for afoxolaner (matched to the reported value in dog (5)) and 0.25 for fluralaner (range in dog is 0.20 to 0.34 (6)). The target plasma concentrations at day 90 were set at 65 nM (36 ng/mL) for fluralaner and 129 nM (81 ng/mL) for afoxolaner which correspond to the highest IC_{99} values of each compound among all tested *Aedes* and *Anopheles* species, based on data displayed in Figure 1C. To estimate variability, stochastic simulation (total of 100,000 simulations) was adopted using a fixed coefficient of variation of 20% with a log-normal distribution for each parameter used in the single compartment model: Cl (L/d), V (L), Ka (1/d) and F. No covariance has been used. Simulation artefacts with dose values tending to infinity (theoretically possible in the lognormal distribution) were removed by rejecting samples beyond the 99.9% quantile.

For *Culex* mosquitoes and *Phlebotomus* sand flies, the same PK model and doses were used to estimate the time frame with plasma concentration above IC_{99} (for *Culex* mosquitoes, 200 nM=125 ng/mL for afoxolaner and 105 nM=58 ng/mL for fluralaner; for *Phlebotomus*, 407 nM=255 ng/mL for afoxolaner and 1,445 nM=804ng/mL for fluralaner). For *Lutzomyia* sand flies, as well as for *Phlebotomus* sand flies in the case of fluralaner, these doses were not sufficient to obtain a plasma concentration above IC_{99} at any time point.

Modeling of malaria incidence

An existing transmission model describing the impact of another mosquitocidal drug, ivermectin, on malaria was extended to simulate the impact of afoxolaner or fluralaner on malaria incidence. All model parameters were as described previously (15) with the adaptations as described below. For fluralaner and afoxolaner we modeled a 90-day efficacy period, and a 2-year intervention scheme (1 intervention per year at the beginning of the transmission period). The model assumes that mosquitoes taking a bloodmeal containing either drug on any day during the 90 day efficacy window will experience reduced survival – mean lifespan is reduced from 7.6 days pre-intervention (1) to 2 days during the intervention. Mosquitoes were assumed to bite once every three days. This translates to less than 1% of the mosquitoes being able to survive until they complete sporogony (here conservatively estimated at 10 days). As the model is deterministic, we are only concerned with the ratio of mosquitoes to human, which in this simulation is assumed to vary between 13.7 mosquitoes per human at the peak of the rainy season and 0.006 at the lowest point of the dry season, with an average value of 4.9 across the whole year (in the absence of intervention). The vector to human ratio was selected to give a level of prevalence and incidence in the model that is commonly observed in areas of Africa with highly seasonal transmission (16) (i.e. prevalence by microscopy in 2-10 year olds of 27% and 0.9 cases of malaria per 0-5 year old per year). We assume no movement of mosquitoes into and out of the intervention area. Finally, we assume that a proportion (c) of the human population over the age of 5 is treated, and only bloodmeals taken from treated individuals result in reduced survivorship. We simultaneously track the infectious state of the mosquito (susceptible, latently infected or infectious) to link the increase in vector mortality to a reduction in the infectious vector density and thus the rate of infections in humans.

The impact of afoxolaner/fluralaner was estimated in all malaria endemic areas in Africa. Each 1st administrative unit (top-level regional divisions) has a specific transmission intensity based on a prevalence-incidence relationship in the Imperial College malaria model and the Malaria Atlas Project estimates for 2015 prevalence, and seasonality profile based on rainfall data (3) (SI Appendix Figure S1). Mass drug administration (MDA) with a mosquitocidal drug efficacious for 90 days and with a coverage of 30% of the population over the age of five was simulated in each 1st administrative unit. The MDA was conducted at an optimal time based on the specific seasonality profile of each administrative unit. The map presented in Figure 3 is a simplified and illustrative approach to estimating the true impact of this intervention across Africa – in reality a wider range of complexities would need to be

considered, such as the vector species in each location (currently assumed to be all *Anopheles gambiae*-like), movement of individuals between locations, each individual country's national malaria strategy (in terms of planned increases in current interventions such as distribution of long-lasting insecticide-treated bed nets and access to treatment) and true achievable coverage and compliance in each area.

Modeling of Zika incidence

An existing Zika transmission model (17) was adapted to include an increased rate of vector mortality during the 90 day efficacy period of the drug of $0.5cp\kappa$/day where 0.5 translates into a mean lifespan of 2 days for mosquitoes biting a treated subject, c represents drug coverage in individuals over 5 years of age, p is the proportion of the population over 5 years of age (=0.908 for the demography assumed) and κ is the biting rate per adult female mosquito (=0.5/day). We simulate treatment occurring in one of twenty spatially coupled geographic regions (parameterized to represent Latin America) in a population with historical prior exposure to Zika but at a point in time where herd immunity has declined to the point where a new epidemic is able to occur. Treatment started within 2 months of the start of the new epidemic and is repeated exactly one year later. We track the annualized incidence of infection, and the cumulative infection incidence since the start of the epidemic.

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