Intra-host model of malaria infection: characterizing artesunate resistance

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Artemisinin Resistance in \textit{Plasmodium falciparum} Malaria

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Problem:
Increasing parasite clearance time in patients receiving artesunate monotherapy have been observed in Western Cambodia.

Hypothesis:
This phenomenon results from reduced drug efficacy on the ring stage of the parasite life cycle.
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Hypothesis:
This phenomenon results from reduced drug efficacy on the ring stage of the parasite life cycle.
Possible causes of long clearance time

- Poor absorption of drug
- High initial load of parasites
- Reduced activity of drug
 Parasite Staging & Artesunate

**Stage-specific**

Very young rings (< 6 hrs) and very mature schizonts (> 44 hrs) are insensitive to the drug.
Parasite Staging & Artesunate

*Stage-specific*

Very young rings (< 6 hrs) and very mature schizonts (> 44 hrs) are insensitive to the drug.

*Plasmodium falciparum* staging (in vitro culture)
Data for the model

In each study sites, the patients were divided into two groups for different dose regimens, AS7 and MAS3.

- **AS7** - 2mg/kg/day of artemisate for 7 days.
- **MAS3** - artemisate 4mg/kg/day for 3 days followed by mefloquine 15/mg/kg on day 3 and 10 mg/kg on day 4.

**Parasite count**

Parasitemia was assessed on admission, 4, 8 and 12 hours after start of antimalarial treatment and then every 6 hours until 2 consecutive slides were negative.

**Dihydroartemisinin(DHA) concentration**

Plasma concentrations of DHA were taken at 15 and 30 minutes, 1, 1.5, 2, 3, 4, 5, 6, 8 and 12 hours after the first artesunate dose.
Our models can be divided into three main parts:

1. initial age distribution
2. changing in time
3. killing effect
The number of parasites distribute over their lifetime follow the normal distribution. The life-cycle is fixed at 48 hours.

Every one hour the distribution graph is moved to the right. Number of parasites at the age 48 hours will be multiplied with a multiplication rate for being the number of new parasites at the age 1 hour.
Parasite age distribution & Changing in time

Initial age distribution
The number of parasites distribute over their lifetime follow the normal distribution. The life-cycle is fixed at 48 hours.

Changing in time
Every one hour the distribution graph is moved to the right. Number of parasites at the age 48 hours will be multiplied with a multiplication rate for being the number of new parasites at the age 1 hour.

without drug
The parasites will be killed or removed from the system if they have the ages in the kill zones. The drug concentrations are used in the calculation of the killing percentage in each kill zone.

The parasites ages from young rings to schizonts are divided into threes parts and in each part has its own concentration-effect relationship.
Killing effect

Killing Effect

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Parasite Kill Zones

The parasites ages from young rings to schizonts are divided into threes parts and in each part has its own concentration-effect relationship.
### Model parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbols</th>
<th>Sampling ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial load of parasites</td>
<td>$N_0$</td>
<td>$10^{10} - 10^{12}$</td>
</tr>
<tr>
<td>Mean age of parasites (hrs)</td>
<td>$\mu$</td>
<td>[4, 16]</td>
</tr>
<tr>
<td>Standard deviation (hrs)</td>
<td>$\sigma$</td>
<td>[2, 8]</td>
</tr>
<tr>
<td>Parasite multiplication rate</td>
<td>$PMR$</td>
<td>[6, 12]</td>
</tr>
<tr>
<td>Parasite lifespan (hrs)</td>
<td>$LC$</td>
<td>48</td>
</tr>
<tr>
<td>Slope of the sigmoid curve</td>
<td>$\gamma$</td>
<td>[1.5, 9.5]</td>
</tr>
<tr>
<td>50% effect concentration (ng/ml)</td>
<td>$EC_{50}$</td>
<td>[0, 100]</td>
</tr>
<tr>
<td>Maximum killing effect (%)</td>
<td>$E_{max}$</td>
<td>[0, 99.9999]</td>
</tr>
<tr>
<td>The speed of killing (1/hr)</td>
<td>$\alpha$</td>
<td>[3, 12]</td>
</tr>
<tr>
<td>begin/end kill zone (Rings)</td>
<td>$KZR_b$, $KZR_e$</td>
<td>$KZR_b = 6$, $KZR_e \in [20, 30]$</td>
</tr>
<tr>
<td>begin/end kill zone (Trophozoites)</td>
<td>$KZT_b$, $KZT_e$</td>
<td>$KZT_b = KZR_e + 1$, $KZT_e = 38$</td>
</tr>
<tr>
<td>begin/end kill zone (Schizonts)</td>
<td>$KZS_b$, $KZS_e$</td>
<td>$KZS_b = 39$, $KZS_e = 44$</td>
</tr>
</tbody>
</table>
Fitting Model

The main output of the model is the parasitemia over time.

100,000 sets of parameters for one patient data

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Root Mean Square Deviation

$$RMSD = \sqrt{\frac{\sum (X_{obs,i} - X_{mod,i})^2}{m}}$$

where $m$ is the number of points in each patient data.
The main output of the model is the parasitemia over time.

Root Mean Square Deviation

\[
\text{RMSD} = \sqrt{\frac{\sum (X_{\text{obs}} - X_{\text{mod}})^2}{m}}
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Fitting Model

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Fitting Model

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Root Mean Square Deviation

\[ RMSD = \sqrt{\frac{\sum (X_{obs} - X_{mod})^2}{m}} \]

where \( m \) is the number of points in each patient data.
Fitting model with the individual data

<table>
<thead>
<tr>
<th>Study Site/Regimen</th>
<th>RMSD (in log10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang Pha/ AS7</td>
<td>0.34(n=20)</td>
</tr>
<tr>
<td>Pailin/ AS7</td>
<td>0.45(n=19)</td>
</tr>
<tr>
<td>Wang Pha/ MAS3</td>
<td>0.26(n=20)</td>
</tr>
<tr>
<td>Pailin/ MAS3</td>
<td>0.39(n=20)</td>
</tr>
</tbody>
</table>

** n = number of patients

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Intra-host model of malaria infection
Concentration-Effect curves of each stage

Pailin (MAS3)-Western Cambodia

Wang Pha (MAS3)-Western Thailand
Concentration-Effect curves of each stage

Pailin (AS7)-Western Cambodia

Wang Pha (AS7)-Western Thailand
Resistance Index

Resistance Index (RI) = \( \frac{EC_{50}}{E_{max} \times \gamma \times \alpha} \)

The higher the resistance index the more resistant that stage of that parasite is to the drug.
Comparing RI between stages and sites

The first 10 best fit parameters of each patient were used in the calculation of RI for every stages. These RIs were compared using the mixed-effect model.

**Multilevel mixed-effect model**

\[ y_{ij} = X_{ij} \beta + u_i^{(1)} + u_{ij}^{(2)} + \epsilon_{ij} \]

- \( y_{ij} \) - the logarithms of RI for \( n_{ij} = 10 \)
- \( X_{ij} \) - a set of regressors (stage, site)
- \( u_i^{(1)} \) - a random intercept at the patient level
- \( u_{ij}^{(2)} \) - a random intercept at the model level in each patient

(i=1 Wang Pha, 2 Pailin, j=1 Rings, 2=Trophozoites, 3=Schizonts)
Comparison RI between stages and sites

Using a two-level model mixed-effects linear regression, it shows that

- RI of rings was significantly higher than trophozoites and schizonts regardless of location.
- RI for trophozoite stage parasites was not significantly different to RI of schizonts regardless of location.
- RI for rings in western Cambodia was significantly higher than in western Thailand.
Conclusion from the model

- The model can reproduce the observed data.
- The results from the model support the hypothesis that increasing of parasite clearance time results from reduced efficacy of the drug at the ring stage of parasite life-cycle.
- The model can be used for designing alternative dosing regimens.
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ขอบคุณค่ะ
köszönöm! ซ่าท dekuji
mahalo  고맙습니다
thank you
merci  谢谢 danke
شكرا  どうもありがとうございます  gracias
Age distribution

If the initial number of parasites is $N_0$ then the initial number of parasites at age $a$ is

$$N(a, 0) = \frac{N_0}{\sum_{a=1}^{L} p(a)} p(a)$$  \hspace{1cm} (1)

where $a = 1, 2, 3 \ldots L$ and $p(a)$ - is the probability density function at time interval $a$,

$$p(a) = \frac{\exp \left( \frac{(a-\mu)^2}{2\sigma^2} \right)}{\sqrt{2\pi\sigma}}$$  \hspace{1cm} (2)
Parasite Killing

For each stage, the concentration-effect relationship is

$$ E(c(t)) = E_{\text{max}} \frac{c(t)^\gamma}{c(t)^\gamma + EC_{50}^\gamma} $$  \hspace{1cm} (3)

In the kill zone, the decrease in parasite number per time step can be described as:

$$ N(a + 1, t + 1) = N(a, t) \exp(-k_j(t)) $$  \hspace{1cm} (4)

where $N(a, t)$ is the number of parasites with the age $a$ at time $t$ and $k_j(t)$ is the decay constant at time $t$ of the zone $j$ ($j=1$ Rings, $2=\text{Trophozoites}$, $3=\text{Schizonts}$).

$$ k_j(t) = \alpha \ln\left(\frac{100}{100 - E_j(c(t))}\right) $$  \hspace{1cm} (5)
Peripheral blood parasite counts (as number of parasites/µL) were converted into total body parasite biomass by assuming a total blood volume of 80 ml/kg.

\[
\text{total biomass} = \text{peripheral blood parasitemia} \times \Psi
\]  \hspace{1cm} (6)

with \( \Psi = 80,000 \times \text{body weight} \).
The relationship between the observed peripheral blood parasitemia (circulating parasites) and the corresponding total body parasite biomass at time $t$ was described as:

$$N_{\text{obs}}(t) = \sum_{a=1}^{L} N(a, t)g(a)$$  \hspace{1cm} (7)

where $N_{\text{obs}}(t)$-the circulating parasitemia, $g(a)$-the probability function of seeing the parasite age $a$ hours from the blood sample

$$g(a) = \begin{cases} 
1 & \text{for } a < 11 \\
\exp \ln(0.5)(a - 11)/3 & \text{for } a \geq 11 
\end{cases}$$  \hspace{1cm} (8)
The concentration data of each patient were divided into an absorption phase (from the first point to maximum concentration point) and an elimination phase (from the maximum concentration point to the last point).

\[ c(t) = \begin{cases} qt \\ r \exp(-st) \end{cases} \]

absorption elimination

(9)
Indivaria

Indivaria = Individual + Malaria

Indivaria is a Mathematica package developed by MORU Mathematical modelling team. It is the package for doing within host models of malaria infections.

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TROPICAL MEDICINE RESEARCH PROGRAMME
Splitting Dose

Giving artesunate twice a day for 7 days.
Give artemisinin 4 mg/kg for 7 days (Using DHA concentration of MAS3 in AS7 patients).
Give artesunate 4 mg/kg for 7 days.
Lowest $E_{max}$ at Ring stage

The $E_{max}$ of each stage from the sets of parameters that have rmsd < 1.0 in each patient were compared for the minimum.