Plasmodium berghei ANKA: Selection of resistance to piperaquine and lumefantrine in a mouse model

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A B S T R A C T

We have selected piperaquine (PQ) and lumefantrine (LM) resistant Plasmodium berghei ANKA parasite lines in mice by drug pressure. Effective doses that reduce parasitaemia by 90% (ED90) of PQ and LM against the parent line were 3.52 and 3.93 mg/kg, respectively. After drug pressure (more than 27 passages), the selected parasite lines had PQ and LM resistance indexes (I90) [ED90 of resistant line/ED90 of parent line] of 68.86 and 63.55, respectively. After growing them in the absence of drug for 10 passages and cryo-preserving them at −80 °C for at least 2 months, the resistance phenotypes remained stable. Cross-resistance studies showed that the PQ-resistant line was highly resistant to LM, while the LM-resistant line remained sensitive to PQ. Thus, if the mechanism of resistance is similar in P. berghei and Plasmodium falciparum, the use of LM (as part of Coartem®) should not select for PQ resistance.

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1. Introduction

Malaria is a global public health priority. The control of malaria is hampered by the rapid selection of parasites resistant to antimalarials. Indeed, there is no single antimalarial in clinical use to which the parasite has not yet developed resistance (Nzila, 2006; White, 2004). Current international strategies for treatment depend on the use of combinations of drugs that include artemisinin compounds. Although this strategy is designed to reduce the chance of resistance emerging, there is considerable concern that this will inevitably happen.

Studies devoted to understanding factors that promote the selection of resistance of Plasmodium falciparum to antimalarials have demonstrated that drug elimination profile in the body is one of the key parameters that determine the emergence and selection of resistance (Nzila et al., 2000; Watkins and Mosobo, 1993). When drugs are used in combination, a mismatch between their half-lives can have a substantial impact on the evolution of drug resistance. If one drug is rapidly eliminated, the other drug persists alone and new infections are exposed to sub-therapeutic level of drugs, a fact that promotes the development of resistance (Hastings, 2004).

For instance, the combination of lumefantrine (LM) and artemether (ATM), known as Coartem® has become the first line of treatment of malaria in many African countries, including Kenya (Davis et al., 2005b; Kokwaro et al., 2007; Mutabingwa, 2005; Nosten and White, 2007). Emerging reports indicate that the use of LM in (Coartem®) selects for parasites that show an increased tolerance to Coartem® and these parasites select for wild type genotype in, or show increased copy number of pfmdr1, a gene associated with chloroquine (CQ) and mefloquine (MFQ) resistance (Dokomajilar et al., 2006; Sisowath et al., 2007, 2005). Thus, there is now concern that resistance to LM will be rapidly selected (Hastings and Ward, 2005; Humphreys et al., 2007; Sisowath et al., 2007).

Piperaquine has been combined with dihydroartemisinin (DHA), the drug known as Artekin®. It has undergone successful clinical evaluation in Africa and Asia (Ashley et al., 2004, 2005; Davis et al., 2005a; Denis et al., 2002; Karema et al., 2006;
Karanagewawa et al., 2004). Piperaquine has been used as mono-
therapy for the treatment of malaria infections for several years
(in the 80s and early in the 90s) in China. However, when used
alone, there was rapid selection of resistance in vitro, from 18% in
the 1980s to 98% in 1990s (Fan et al., 1998; Yang et al., 1999,
1992; Zhang et al., 1987). This in vitro-resistance was followed
invariably by the emergence of in vivo resistance (Davis et al.,
2005b; Guan et al., 1983; Pang et al., 1989).

In both combinations, the artemisinin derivatives (ATM and
DHA) and the main components (PQ and LM) have different
pharmacokinetic properties. Indeed, like most artemisinin-based
compounds, ATM and DHA are short acting drugs, with a half-lives
of less than 2 h (Navaratnam et al., 2000; White et al., 1999).

On the other hand, PQ and LM have long half-lives, around 4–6
and 15–20 days, respectively (Ahmed et al., 2008; Hai et al.,
2008; Kokwaro et al., 2007; Tarning et al., 2008). Under these
circumstances, the selective pressure for resistance would be primarily
exerted by the LM and PQ, leading to a rapid selection of PQ and
LM resistance when the drug combinations will come into wide-
spread use. In the case of Coartem® (CQ and AQ), a rapid emergence of parasite
tolerant to LM have been reported following the use of Coartem®
(Dokomajilar et al., 2006; Sisowath et al., 2007, 2005).

Thus, if strategies are to be devised to extend the useful therapeu-
tic lifetime of Coartem® and Artekin®, there is a need to understand
the mechanisms of PQ and LM resistance. However, to date, there are
no well established and characterized PQ- and LM-resistant
P. falciparum strains, which could be used to study the mechanism
of drug resistance.

Here we report the selection of stable LM- and PQ-resistant Plas-
modium berghei ANKA strains by continuous PQ and LM pressure
in vivo. We also report the activity of the antimalarial drugs chlo-
roquine (CQ), amodiaquine (AQ), LM and DHA against the backdrop
of these LM and PQ resistance. These strains represent valuable tools
to study the mechanisms of LM and PQ resistance.

2. Materials and methods

2.1. Parasites, hosts and test compounds

To select PQ resistance, we used a transgenic ANKA strain of
P. berghei expressing Green Fluorescent Protein (GFP), resistant to
pyrimethamine obtained from the MR4 repository (MRA-865, MR4, ATCC®
Manassas, Virginia), while a P. berghei ANKA strain expressing GFP-Luciferase fusion,
(MRA-868, MR4, ATCC® Manassas, Virginia) obtained from Dr. C.J. Janse of Center of Infectious
Diseases Leiden University Medical Center, Netherlands was em-
ployed to induce LM resistance. Male, random-bred Swiss albino
mice (20 ± 2 g), were each infected intraperitoneally with donor
blood containing approximately 2 × 107 parasite red blood cells (PRBC)
in 0.2 ml inoculum. However, during the first 4 passages of selection of PQ resistance, female NMRI mice were used and
were infected intravenously. Infection was assessed by micro-
scopic examination of Giemsa-stained thin smears prepared from
tail snips on day 3, 72 h post-infection in the 1-DT or on day 4,
96 h post-infection in the 4-DT. Percentage chemosuppression of
each dose was then calculated as (A–B)/A × 100, where A is
the mean parasitaemia in the test group (Tona et al., 2001). ED50 and ED90
were estimated using a linear regression line.

2.2. Determination of 50% and 90% effective-dose level (ED50 and ED90)

Fifty percent and 90% effective doses (ED50 and ED90, respecti-
vely) were measured in a quantitative standard method ‘4-day
test’ (4-DT), in which the parasites are exposed to four, daily, drug
doses (Peters, 1975), except for the ED50 and ED90 of the parent strain
and that of the line selected at the 4th passage of PQ pressure
which were measured using the ‘1-day test’ (1-DT), in which
the parasites are exposed to a single drug dose (Vennerstrom et al.,
2004). The first 4 passages of PQ pressure were carried out at Swiss
Tropical Institute (STI), Basel, Switzerland, using the 1-DT. How-
ever, experiments from the 4th passage of PQ pressure and the
all LM pressure were carried out at the Kenya Medical Research
Institute (KEMRI), Nairobi, Kenya, using the 4-DT. Drugs were
administered by oral (p) route on day 1, (24 h post-infection) in
the 1-DT or starting on the day 0, (4 h post-infection) and contin-
uing for a total of four daily doses, days 0–3 (24, 48 and 72 h
post-infection) in the 4-DT. Parasite count was estimated by micro-
scopic examination of Giemsa-stained thin smears prepared from
tail snips on day 3, 72 h post-infection in the 1-DT or on day 4,
96 h post-infection in the 4-DT. Percentage chemosuppression of
each dose was then calculated as (A–B)/A × 100, where A is
the mean parasitaemia in the negative control group and B is the
parasitaemia in the test group (Tona et al., 2001). ED50 and ED90
were estimated using a linear regression line.

2.3. Procedures for exerting drug-selection pressure and assessing the
level of resistance

After inoculation (2 × 107 parasitized red blood cells contained
in 0.2 ml inoculums) in 5 mice, on day zero (D0), mice were then
orally treated once with the drug at concentration equivalent to
ED90, 72 h post-infection (D3). The second and third treatment
followed on D6 (or 7) and 10. Drugs were administered
orally in a volume of 0.01 ml per gram mouse. After the third treat-
ment (D10), parasitaemias were monitored until they reached
≥2% when a mouse was selected for donation of PRBC to the next
naive group of five mice. The parasites were exposed to increasing concentrations of PQ and LM by an
ED90 factor of one in subsequent passages.

During the first 4 passages of PQ drug pressure, after parasite
inoculation (D0), mice (a group of 5) were treated three times with
the drug at concentration equivalent to ED90. The first treatment
was carried out 72 h post-infection (D3). The second and third
treatment followed on D6 (or 7) and 10. Drugs were administered
orally in a volume of 0.01 ml per gram mouse. After the third treat-
ment (D10), parasitaemias were monitored until they reached
≥2% when a mouse was selected for donation of PRBC to the next
naive group of five mice and subsequent steps were carried out as
mentioned in the previous paragraph.

The level of resistance was evaluated at different intervals by
measurement of ED50 and ED90 in the standard 4-DT or 1-DT which
permits the calculation of an ‘index of resistance’, I50 and I90 (de-
efined as the ratio of the ED50 or ED90 of the resistant line to that of
the sensitive, parent line).

The I90 values were grouped into four categories, based on pre-
vious work (Merkti and Richle, 1980): (1) I90 = 1.0, sensitive, (2)
I90 = 1.01–10.0, slight resistance, (3) I90 = 10.01–100.0, moderate
resistance and (4) I90 > 100.0, high resistance.

2.4. Stability study

The stability of PQ and LM resistant line was evaluated by mea-
suring drug responses after (i) making 10 drug free passages fol-
lowed by measurement of ED90, (ii) freeze-thawing of parasites
from −80 °C followed by measurement of ED90. Stable resistance
was defined as the maintenance of the resistance phenotype when
drug-selection pressure was removed for at least 10 passages in
mice (Gervais et al., 1999).
2.5. Cross-resistance studies

The activity of CQ, AQ, LM and DHA against both drug sensitive and resistant lines (after 10 drug free passages) was assessed in the 4-DT. $I_{50}$ was computed as the ratio of the ED$_{50}$ of the resistant line to that of the sensitive, parent line. Cross-resistance was classified into three categories as previously described (Li, 1985; Li et al., 1985): $I_{50} \leq 1.0$ sensitive, $I_{50}$ of 1.01–5.00 as slight cross-resistance, $I_{50}$ of above 5.01 as high cross-resistance. Statistical analyses were carried out using the Student t-test (Minitab Inc. software, State College, PA, USA).

3. Results

The ED$_{50}$ and ED$_{90}$ of PQ against the parent line were 1.30 and 3.52 mg/kg, respectively. After 4 passages under PQ selective pressure (a total of 15 treatments), the PQ ED$_{50}$ and ED$_{90}$ increased to 160.28 and 262.59 mg/kg, respectively, yielding $I_{50}$ of 123.29 and $I_{90}$ of 74.70 (Table 1). However, after cryopreservation and revival of the parasite, this resistance decreased, with ED$_{50}$ and ED$_{90}$ of 7.50 and 21.90 mg/kg, respectively (Table 1).

When exposed to further 23 passages (27th passage) of selection pressure, parasites regained the resistant phenotype and reached a high level of resistance with $I_{50}$ and $I_{90}$ of 129.29 and 63.55, respectively (Table 1). Fig. 1A shows the changing response of the P. berghei ANKA to PQ in the course of PQ drug pressure. After the 5th passage under PQ pressure, a dose of 30 mg/kg (>8 times higher the ED$_{50}$ of the parent strain) suppressed the bulk of parasitaemia, indeed treated mice had parasitaemia of 0.08% only (Fig. 1B) However, at the 20th passage, a dose of 10 mg/kg did not prevent parasitaemia to reach 4.1%, and at 36th, a higher parasitaemia of 6.5% was reached at a dose of 40 mg/kg. The higher level of resistance was observed at

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<td>4th</td>
<td>160.28</td>
<td>123.29</td>
<td>262.59</td>
<td>74.70</td>
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<tr>
<td>(6 months cryopreservation)</td>
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<td>21.90</td>
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<td>16.46</td>
<td>64.50</td>
<td>18.32</td>
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<tr>
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<td>194.00</td>
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<tr>
<td>17th</td>
<td>168.08</td>
<td>129.29</td>
<td>242.38</td>
<td>68.86</td>
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<tr>
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<td>283.71</td>
<td>80.60</td>
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<tr>
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<td>84.64</td>
<td>223.15</td>
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<tr>
<td>27th passage line after</td>
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<td>84.64</td>
<td>223.15</td>
<td>63.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 months cryopreservation</td>
<td>133.14</td>
<td>97.46</td>
<td>230.71</td>
<td>64.50</td>
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</tr>
</tbody>
</table>

Like with PQ, the first 5 passages were not associated with increase in LM resistance. At the 5th passage, a dose 6 mg/kg allowed a parasitaemia of 0.08% only (Fig. 1B) However, at the 20th passage, at the same dose (6 mg/kg), parasite grew and reached 2% parasitaemia, a clear indication of emergence of resistance. This resistance increased further with the number of passages. At 28th, a dose of 10 mg/kg did not prevent parasitaemia to reach 4.1%, and at 36th, a higher parasitaemia of 6.5% was reached at a dose of 40 mg/kg. The higher level of resistance was observed at

**Table 1** Selection of piperazine resistance in Plasmodium berghei GFP ANKA strain using serial technique. Data are presented as effective doses that reduce parasitaemia by 50% and 90% (ED$_{50}$, ED$_{90}$) and as 50% and 90% indexes of resistance ($I_{50}$ and $I_{90}$, defined as the ratio of the ED$_{50}$ or ED$_{90}$ of the resistant line to that of the parent strain).

<table>
<thead>
<tr>
<th>Passage no.</th>
<th>ED$_{50}$ (mg/kg)</th>
<th>$I_{50}$</th>
<th>ED$_{90}$ (mg/kg)</th>
<th>$I_{90}$</th>
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<td>1.0</td>
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<tr>
<td>4th</td>
<td>160.28</td>
<td>123.29</td>
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<tr>
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<td>122.00</td>
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<tr>
<td>27th passage line after</td>
<td>116.34</td>
<td>84.64</td>
<td>223.15</td>
<td>63.39</td>
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<tr>
<td>4 months cryopreservation</td>
<td>133.14</td>
<td>97.46</td>
<td>230.71</td>
<td>64.50</td>
</tr>
</tbody>
</table>

**Table 2** Selection of lumefantrine resistance in Plasmodium berghei GFP-Luciferase ANKA strain using serial technique. Data are presented as effective doses that reduce parasitaemia by 50% and 90% (ED$_{50}$, ED$_{90}$) and as 50% and 90% indexes of resistance ($I_{50}$ and $I_{90}$, defined as the ratio of the ED$_{50}$ or ED$_{90}$ of the resistant line to that of the parent strain).

<table>
<thead>
<tr>
<th>Passage no.</th>
<th>ED$_{50}$ (mg/kg)</th>
<th>$I_{50}$</th>
<th>ED$_{90}$ (mg/kg)</th>
<th>$I_{90}$</th>
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<tbody>
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<td>3.93</td>
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</tr>
<tr>
<td>4th</td>
<td>1.34</td>
<td>0.80</td>
<td>3.49</td>
<td>0.89</td>
</tr>
<tr>
<td>12th</td>
<td>1.58</td>
<td>0.95</td>
<td>3.25</td>
<td>0.83</td>
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<tr>
<td>20th</td>
<td>2.96</td>
<td>1.77</td>
<td>5.25</td>
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<td>28th</td>
<td>9.76</td>
<td>5.84</td>
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<td>36th</td>
<td>42.50</td>
<td>25.33</td>
<td>69.70</td>
<td>17.74</td>
</tr>
<tr>
<td>48th</td>
<td>140.15</td>
<td>83.92</td>
<td>249.75</td>
<td>63.55</td>
</tr>
<tr>
<td>Drug free passages</td>
<td>133.17</td>
<td>79.74</td>
<td>256.21</td>
<td>65.19</td>
</tr>
<tr>
<td>48th line after 2 months cryopreservation</td>
<td>116.34</td>
<td>69.66</td>
<td>204.58</td>
<td>52.06</td>
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</table>
48th passage, with mice harboring parasitaemia of 8% when treated with 80 mg/kg. During all 48 passages, parasitaemias in the untreated controls remained steady, ranging between 9% and 12%, a confirmation of dramatic rise in LM resistance (Fig. 1B). At this point (48th passage), a stable LM-resistant strain was selected (Fig. 1B, Table 2).

These high values of PQ and LM resistance indices led us to test the stability of the resistant phenotypes. We maintained these two resistant strains in absence of the drug pressure for 10 passages (at least 2 months) and then assessed in vivo activity of the drugs. The resulting PQ I50 and I90 remained high, with values of 147.28 and 83.80, respectively, and those of the LM were 79.74 for I50 and 65.19 for I90, a clear indication of the stability of the resistant phenotype. To further check this stability, we cryo-preserved these parasite lines for 2 and 4 months for LM and PQ, respectively. Upon revival, the analysis of the drug activity showed PQ I50 and I90 indexes of 84.64 and 63.39, respectively, and those for LM as 69.66 and 52.06, respectively. These values are slightly lower than those obtained before cryopreservation. However, they remained high, 50–80 times higher than those of the parent lines, a further indication of the stability of the phenotype (Tables 1 and 2).

We also tested the extent to which resistance to PQ and LM affect the activity of other antimalarial drugs, a phenomenon known as cross-resistance, and the results are summarized in Tables 3 and 4. Against the PQ-resistant line, the activity of AQ, CQ and DHA decreased significantly by a factor of 3–7 (*p < 0.01 at least) (Table 3), an indication of the existence of slight cross-resistance of PQ with AQ, CQ and DHA. Surprisingly, the highest level of cross-resistance was recorded with LM, with its activity decreasing 97-fold against this PQ-resistant parasite line (*p < 0.0001).

Table 3

<table>
<thead>
<tr>
<th>Antimalarial drug</th>
<th>ED90 (mg/kg)</th>
<th>Index of resistance (I90)</th>
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<tr>
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<td>Parent strain</td>
<td>Resistant line</td>
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<tr>
<td>AQ</td>
<td>3.72</td>
<td>13.48*</td>
</tr>
<tr>
<td>LM</td>
<td>2.52</td>
<td>246.06*</td>
</tr>
<tr>
<td>CQ</td>
<td>3.57</td>
<td>26.24*</td>
</tr>
<tr>
<td>DHA</td>
<td>4.80</td>
<td>12.06*</td>
</tr>
</tbody>
</table>

| Differences between parent and resistant lines were significant according to Student's t-test. |

Overall, the LM-resistant parasite line retained relative susceptibility to the 4-aminoquinolines, AQ and PQ (Table 4). Indeed, AQ activity did not change (I90 of 1.06), and more interestingly, this parasite line remained susceptible to the bisquinoline PQ (I90 of 0.91). However, a significant decrease in activity was observed with the aminoquinoline CQ, with an I90 of 1.62 (*p < 0.0001), and the endoperoxide DHA, with an I90 of 5.36 (*p < 0.05). Thus the selection of LM resistance is associated with a decrease in CQ and DHA activity and the retention of AQ and PQ susceptibility.

4. Discussion

Our study shows that PQ and LM resistance in *P. berghei* ANKA can be selected within 18 months of continuous drug pressure. To the best of our knowledge, this is the first report of the selection of stable PQ- and LM-resistant strains in murine malaria following drug pressure. A PQ-resistant *P. berghei* strain had been selected in 5 months of selection pressure, but when the drug was removed, the strain reversed to sensitive phenotype (Li, 1985; Li et al., 1985), and a stable phenotype was observed only after mouse–mosquito–mouse passages (Li et al., 1985).

Two approaches by other laboratories have been used to select resistant murine malaria parasites: the 2% relapse technique (2% RT) in which a single and high drug dose is administered at the time of each passage (Peters and Robinson, 1999) and the serial technique (ST), in which drug dose is gradually increased after each passage (Peters, 1999; Peters and Robinson, 1999).

Using 2% RT, a number of phenotypes stably resistant to pyronaridine, amodiaquine, atovaquone and tafenoquine have been selected in *P. berghei* (Peters and Robinson, 1992, 1999, 2000; Peters et al., 2003) and tafenoquine in *Plasmodium yoelii* and *Plasmodium chabaudi* (Peters et al., 2003). However, using this method, stable resistance to sulfadoxine/pyrimethamine in *P. berghei* and artesininisin in *P. yoelii* could not be selected (Peters, 1999; Peters and Robinson, 1999). On the other hand, the ST approach has allowed the establishment of strains stably resistant to various antimalarials, including atovaquone in *P. berghei* (Syafruddin et al., 1999) and mefloquine in *P. chabaudi* (Cravo et al., 2003), artesininisin in *P. chabaudi* (Afonso et al., 2006), halofantrine in *P. yoelii* (Singh and Puri, 2000) and arteether in *P. vinckeii* (Puri and Chandra, 2006). Though failure to select stable resistance to piperazine, chloroquine and primaquine in *P. berghei* has been reported (Li, 1985; Peters, 1999; Peters et al., 2003), overall, the ST approach has proven to be more efficient to select for stably resistant strains than 2% RT (Afonso et al., 2006; Cravo et al., 2003; Puri and Chandra, 2006). Using this approach, we have, for the first time, successfully established stable PQ- and LM-resistant *P. berghei* strains within 12–18 months of drug pressure.

Interestingly, after cryopreservation of both PQ and LM-resistant strains, a decrease in ED50/90 was recorded upon revival of the strains. This is common, it indicates that some of the mechanisms of resistance are the result of epigenetic changes such as gene amplification, protein over expression and protein modifications. However, if resistance is well established, the degree of ED50/90 decrease is small, the strains remained resistant to the drugs, as our data show.

Evaluation of cross-resistance patterns revealed that PQ and AQ retain potency against the LM resistant parasite line. LM is an arylaminoalcohol closely related to mefloquine (MQ), halofantrine and pyronaridine (Schlitzer, 2008). PQ, AQ and CQ are 4-aminoquinoline derivatives, and are likely to share a similar mechanism of action (Raynes, 1999). Resistance to CQ and AQ in *P. falciparum* is reported to be inversely correlated with resistance to arylaminoalcohols (Duraisingh and Cowman, 2005), and the selection of the resistance to arylamino-alcohol MQ results in an increase in CQ sensitivity (Cowman et al., 1994; Peel et al., 1993). In our experi-
ments, LM resistance was not associated with a decrease in PQ efficacy. Similarly, the efficacy of the 4-aminoquinolines AQ and CQ did not change or only slightly decreased against the LM-resistant strain. Assuming that the mechanism of LM resistance is similar in *P. falciparum* and *P. berghei*, these results would suggest a high efficacy of PQ against LM-resistant strains in *P. falciparum*.

It is very interesting to note the activity of LM against PQ-resistant line decreased by 97-fold, a rate which is even higher than its activity against the LM-resistant parasite line selected after 2 years of LM pressure (log of 64). Thus, the selection of PQ resistance is associated with a higher level of LM resistance, while, as discussed earlier, the selection of LM resistance is associated with PQ susceptibility. This demonstrates that two different LM-resistance phenotypes exist. The first phenotype is associated with PQ resistance, while the second is associated with PQ susceptibility. Assuming that the same pattern prevails in *P. falciparum*, the use of either drug could be associated with resistance or susceptibility to the other. For instance, currently, Coartem® is being used to treat malaria, thus the selection of resistance to LM could be associated with susceptibility to PQ (component of Artekin®). While if Artekin® is first used, resistance to this drug may render Coartem® ineffective.

Our data show significant 3- and 5-fold decreases in dihydroartemisinin activity against PQ- and LM-resistant strains, respectively, indicating the existence of a slight cross-resistance between LM and artemisinin, and PQ and artemisinin, in *P. berghei*. In *P. falciparum*, resistance to the arylamino-alcohol mefloquine, as the result of the increase copy number of pfmdr1, is associated with a decrease in activity of artemisinin derivatives (Nelson et al., 2005; Pickard et al., 2003; Price et al., 1999), thus a similar phenomenon may prevail in *P. berghei* with the arylamino-alcohol LM. We report cross-resistance between PQ and artemisinin in *P. berghei*, in agreement with previous work (Li, 1985; Li et al., 1985).

Thus, if the mechanism of LM and PQ resistance is similar in *P. berghei* and *P. falciparum*, the selection of LM and PQ resistance would be associated with a reduced artemisinin derivative efficacy, compromising the potential of artemisinin-based combinations. Consequently, there is an urgent need to clarify the mechanism of LM and PQ resistance and establish the extent of cross-resistance between these important antimalarials. The existence of cross-resistances to chemically and mechanistically unrelated drugs suggests the likely involvement of changes in drug accumulation, i.e. a ‘multi-drug resistance’ phenotype.

The LM and PQ resistant parasite lines have been selected so as to study the mechanism of drug resistance in *P. berghei* and use this information as a platform to explore the resistance mechanism in *P. falciparum*. In the latter species, reports indicate that the use of LM + ATM (Coartem®) selects for 2 haplotypes at 86Y-184Y-1246Y and 86Y-184F-1246D of pfmdr1, a gene associated with changes in susceptibility to chloroquine (Dokomajilar et al., 2006; Humphreys et al., 2007; Sisowath et al., 2007, 2005). The copy number of pfmdr1 has also been reported to increase with the use of LM in field isolates in Thailand (Price et al., 2006), and a decrease in copy number was found to heighten in vitro lumefantrine susceptibility in laboratory selected parasites (Duraisingh and Cowman, 2005; Sidhu et al., 2006). These observations indicate that pfmdr1 will likely contribute to LM resistance, but the full definition of the mechanism of resistance remains to be elucidated since overall, Coartem® retains sensitivity against CQ-resistant isolates. In *P. berghei* and *P. chabaudi*, amplification of the pfmdr1 orthologue is associated with mefloquine resistance (Carlton et al., 2001) as in *P. falciparum* (Cowman et al., 1994; Peel, 2001; Sidhu et al., 2005). Thus, pfmdr1 could also be involved in LM resistance in *P. berghei*.

PQ is a bis-chloroquine derivative (Davis et al., 2005a; Raynes, 1999). Thus one could expect that PQ and CQ would share the same mode of action and perhaps a similar mechanism of resistance. However, PQ remains active against CQ-resistant isolates (Basco and Ringwald, 2003), clearly indicating that though PQ is closely related to CQ, these two drugs have different mechanisms of resistance. To date, no gene or candidate gene has been associated with PQ resistance in *P. falciparum*. Thus, further analysis of this PQ resistant *P. berghei* line could provide insight into the mechanism of PQ resistance.

However, we are aware that mechanism of resistance in *P. falciparum* and murine *Plasmodium* species may be different. For instance, the mechanisms of resistance to CQ are different in *P. falciparum* and in murine malaria and there is still a debate whether those of artemisinin derivatives will be similar (Afonso et al., 2006; Carlton et al., 2001; Hunt et al., 2007, 2004a,b). However, for drugs such as mefloquine, antifolates, and atovaquone, similar mechanisms of resistance have been reported (Carlton et al., 2001). Thus, the use of murine malaria could provide critical information on the mechanisms of resistance to PQ and LM.

In summary, we have selected LM and PQ resistant lines of *P. berghei*. The stability of this phenotype indicates that mechanisms that underlie it are coded into the cell genome. Amplification of pfmdr1 has been associated with resistance to mefloquine, an amino-alcohol (Carlton et al., 2001). We hypothesise that the same could prevail in LM, which is also an amino-alcohol. Studies are underway to explore the mechanisms of resistance to LM and PQ.

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References


P. berghei

P. falciparum

Plasmodium falciparum

Plasmodium

Plasmodium berghei


