Clinical and Laboratory Assessment of Antimalarial Drug Efficacy in the Lao P.D.R

Presentation Plan
1. Introduction
2. Studies conducted before 2000 (in-vivo)
3. Studies after 2000:
   - In-vitro study
   - Molecular study
   - Clinical trials

Abbreviations
CQ = Chloroquine
SP = Fansidar
QN = Quinine
A = Artesunate
M = Mefloquine
AL = Artemether-lumefantrine (Coartem)
DP = Dihydroartemisinin-piperaquine (Artekin)

Before 2005…
• Malaria: important cause of morbidity & mortality in Laos (Prevalence ~ 14%)
• Chloroquine (CQ) & sulfadoxine-pyrimethamine (SP): did not work anymore in neighboring countries
• But CQ & SP: Lao nationally recommended antimalarials for treatment of uncomplicated malaria!

Efficacy of Antimalarial Drugs in Laos before 2000
   - Antimalarial drugs: CQ, SP, QN + SP
   - Nam-Ngum dam, Vientiane (1968); N = 108

<table>
<thead>
<tr>
<th></th>
<th>CQ (n=64)</th>
<th>SP (n=26)</th>
<th>QN+SP (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>59 (92%)</td>
<td>25 (96%)</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>RI</td>
<td>5 (8%)</td>
<td>1 (4%)</td>
<td>0</td>
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</tbody>
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A Lao technician said in 2000:
“…..in Laos, chloroquine still works OK and there is no resistence of malaria parasites. If you don’t believe me, let’s see in your study….”
- Antimalarial drug: CQ
- Nam-Ngum dam, Vientiane (1975-76)
- N = 48 (follow up: 7 days)

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<thead>
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<tbody>
<tr>
<td>Sensible</td>
<td>28 (58%)</td>
</tr>
<tr>
<td>RI</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>RII</td>
<td>10 (21%)</td>
</tr>
<tr>
<td>RIII</td>
<td>2 (4%)</td>
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Results: 42%

- Antimalarial drug: CQ
- Nam-Ngum dam, Vientiane (1989)
- N = 15 (10 days of follow up ?)

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sensitive</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>RI</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>RII</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>RIII</td>
<td>2 (13%)</td>
</tr>
</tbody>
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Results: 40%

4. Pillai et al., 2001 (JID 183: 789 - 95)
- Antimalarial drug: CQ (28 days – DOT)
- Vangvieng District, Vientiane (1998-99)
- N = 39

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<table>
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<tbody>
<tr>
<td>Sensitive</td>
<td>21 (54%)</td>
</tr>
<tr>
<td>RI</td>
<td>5 (13%)</td>
</tr>
<tr>
<td>RII - RIII</td>
<td>13 (33%)</td>
</tr>
</tbody>
</table>

Results: 46%

5. Guthmann et al., 2002 (Ann Trop Med Parasitol 96: 553 -7)
- Antimalarial drug: CQ (28 days – DOT)
- Sekong Province (1999 - 2000)
- N = 88

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<tbody>
<tr>
<td>ARC</td>
<td>53 (60%)</td>
</tr>
<tr>
<td>ETF</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>LTF</td>
<td>29 (33%)</td>
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</table>

Results: 40%
In vitro drug sensitivity assays

- Parasites taken from malaria patients
- Adjust parasites to culture condition
- Expose parasites to different antimalarials compared to controls (no drug exposure)
- Measure EC 50

In vitro antimalarial susceptibility patterns of *P. falciparum* malaria in Laos

(Phanlanxay District, Savannakhet 2003 - 2004)

*P. falciparum* isolates defined as resistant (N = 108):

- Chloroquine 65 %
- Quinine 40 %
- Mefloquine 08 %

(Mayxay et al., 2007: *Am J Trop Med Hyg* 76:245-50)

**HOW TO ASSESS ANTIMALARIAL EFFICACY**

3 methods:
1. *In vitro* drug sensitivity assays
2. Molecular method
3. *In vivo* assessment of drug efficacy

**Concentration - effect curve**

- Blood spots collected on filter papers
- DNA extraction
- PCR to study gene mutation

**Molecular methods**

**In vitro drug sensitivity assays**

* Emax
EC 50

**Concentration - effect curve**

Log drug concentrations
Distribution of \textit{P. falciparum} molecular markers associated with SP & CQ in Laos

Collaboration with Dr. Tim Anderson, Texas, USA

* Countrywide survey
* \(\sim 884\) blood spot samples on filter paper from 17 provinces
* Molecular markers: \textit{PfDHFR & DHPS}, \textit{PfCRT}

\textbf{Dhfr} gene mutation in parasites (resistant to \textit{P}) from 17 provinces of Laos

(Mayxay \textit{et al.}, 2007: \textit{Am J Trop Med Hyg} 77: 36 - 43)

\textbf{Dhps} gene mutation in parasites (resistant to \textit{S}) from 17 provinces of Laos

(Mayxay \textit{et al.}, 2007: \textit{Am J Trop Med Hyg} 77: 36 - 43)

\textbf{pfCRT} 76-allele frequency in parasites (resistant to CQ) from 17 provinces of Laos

(Mayxay \textit{et al.}, 2007: \textit{Am J Trop Med Hyg} 77: 36 - 43)

\textbf{In vivo assessment of drug efficacy}

- Patients take antimalarial drugs
- Follow up (7, 14, 28, 42, 63… days)
- Assessment:
  * Cure rate
  * Fever clearance time
  * Parasite clearance time

\textbf{Distribution of \textit{P. falciparum} molecular markers associated with SP & CQ resistance in Laos}

- Countrywide (884 blood spot samples on from 17 provinces)
- Molecular markers: \textit{pfdhfr} & \textit{pfdhps}, \textit{pfcsr}
- Proportion of mutation:
  * \textit{Pf-dhfr} (resistant to pyrimethamine): 77% with \(\geq 1\) mutations
  * \textit{Pf-dhps} (resistant to sulphadoxine): 21% with \(\geq 1\) mutations
  * \textit{Pf-crt} (resistant to chloroquine): 85% with 76T mutations

Therapeutic Efficacy of Chloroquine plus Sulphadoxine/Pyrimethamine Compared with Monotherapy with Either Chloroquine or Sulphadoxine/Pyrimethamine in Uncomplicated *Falciparum* Malaria in Laos


**Attapeu Province (14 day-follow up) N = 107**

<table>
<thead>
<tr>
<th>Results</th>
<th>CQ (n=29)</th>
<th>SP (n=28)</th>
<th>CQ+SP (n=24)</th>
<th>MQ (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>16 (55%)</td>
<td>23 (82%)</td>
<td>20 (83%)</td>
<td>26 (100%)</td>
</tr>
<tr>
<td>ETF</td>
<td>3 (10%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>LTF</td>
<td>10 (35%)</td>
<td>4 (14%)</td>
<td>3 (13%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Oral CQ & SP are no longer effective

* What is the optimum combination treatment in Lao PDR ?

* What are the cost implications ?

Follow up of patient at home

Randomized Comparison of Chloroquine + Sulphadoxine-Pyrimethamine vs Artemunate + Mefloquine (AM) vs Artemether + Lumefantrine (AL) for Treatment of Uncomplicated *falciparum* Malaria

(Maysay et al., 2004: *Clinical Infectious Diseases* 39: 1138-47)

**Phalanxay District (42 day-follow up) N = 330**

<table>
<thead>
<tr>
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<th>CQ+SP (n = 110)</th>
<th>AM (n = 110)</th>
<th>AL (n = 110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment success</td>
<td>102 (95%)</td>
<td>110 (100%)</td>
<td>107 (97%)</td>
</tr>
<tr>
<td>Treatment failure</td>
<td>8 (7%)</td>
<td>0</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>ETF</td>
<td>6 (5%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
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* COMPARISON OF PARASITE CLEARANCE TIMES

**COMPARISON OF PARASITE CLEARANCE TIMES**

- CQ + SP
- AM
- AL

* Significant difference compared to other two groups, P < 0.001
**COMPARISON OF FEVER CLEARANCE TIMES**

- **CQ + SP**: Hours = 30
- **AM**: Hours = 20
- **AL**: Hours = 15

* Significant difference compared to other two groups

\[ P < 0.001 \]

**GAMETOCYTAEMIA AFTER TREATMENT**

- **CQ + SP**: 28/110 (25.5%)
- **AM**: 4/110 (3.6%)
- **AL**: 5/110 (4.5%)

* Significant difference compared to the other two groups \( P < 0.001 \)

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**PROBABLE SIDE EFFECTS AFTER TREATMENT**

- **Neuro-psychiatric**: AM
- **Nightmares**: AM
- **Sedative**: AM
- **Diarrhoea**: AM
- **Vomiting**: AM
- **Diabetes**: AM
- **Pain**: AM

* Significant difference from other groups

**COSTS PER 3 DAYS-TREATMENT COURSE IN ADULTS (in $US)**

- **Artesunate + mefloquine**: $3.5
- **Artemether - lumefantrine (Coartem®)**: $2.4
- **Dihydroartemisinin - piperaquine (Artekin®)**: $1.2

**Therapeutic efficacy of artemether-lumefantrine and artemunate-mefloquine for treatment of uncomplicated *Plasmodium falciparum* malaria in Luang Namtha Province, Lao People’s Democratic Republic**

(Stolten et al., 2004: *Trop Med Int Health* 9: 1175 - 83)

Luang Namtha Province (42 day-follow up) \( N = 100 \)

<table>
<thead>
<tr>
<th></th>
<th>AM ( n = 53 )</th>
<th>AL ( n = 47 )</th>
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<tbody>
<tr>
<td>Treatment success</td>
<td>53 (100%)</td>
<td>44 (94%)</td>
</tr>
<tr>
<td>Treatment failure</td>
<td>0 (0%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>LTF</td>
<td>0 (0%)</td>
<td>3 (6%)</td>
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**An open, randomized comparison of artemunate + mefloquine (AM) vs dihydroartemisinin - piperaquine (Artekin®) for the treatment of uncomplicated *falciparum* malaria in Laos**

(Mayxay et al., 2006: *Trop Med Int Health* 11: 1157-65)

Phalanxay District (42 day-follow up) \( N = 220 \)

<table>
<thead>
<tr>
<th></th>
<th>AM = 110</th>
<th>Artekin = 110</th>
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<tbody>
<tr>
<td>Treatment success</td>
<td>106/107 (99%)</td>
<td>105/106 (99%)</td>
</tr>
<tr>
<td>Treatment failure</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>2</td>
<td>3</td>
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</table>
A Phase III, randomized, non-inferiority trial, to assess the efficacy and safety of Dihydroartemisinin+Piperaquine (DHA+PPQ, Artekin) in comparison with Artesunate+Mefloquine (AS+MQ) in patients affected by acute, uncomplicated *Plasmodium falciparum* malaria in Asia.
Conclusions

1. Chloroquine & sulphadoxine-pyrimethamine are not longer efficacious for the treatment of uncomplicated falciparum malaria in Laos

2. Artemisinin combination therapy (artesunate + mefloquine, artemether –lumefantrine or coartem, dihydroartemisinin-piperaquine or artekin): work very well in Laos

Thank you for your attention