Artemether-lumefantrine: a new treatment combination for multi-drug resistant falciparum malaria

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A CASE CONTROL AUDITORY EVALUATION OF PATIENTS TREATED WITH ARTEMISININ DERIVATIVES FOR MULTI-DRUG RESISTANT FALCIPARUM MALARIA

Running title: Auditory evaluation of artemisinin derivatives

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Chapter 9: A Case Control Study: Evaluation of Efficacy of New Antimalarials for Multiresistant Plasmodium falciparum Malaria
Summary

The artemisinin derivatives are now used widely in areas with multidrug resistant falciparum malaria such as South East Asia, but concerns remain over their potential for neurotoxicity. Mice, rats, dogs, and monkeys treated with high doses of intramuscular artemether or arteether, develop an unusual pattern of focal damage to brain stem nuclei (particularly those involved in auditory processing). In order to investigate whether a similar toxic effect occurs in patients treated with these compounds clinical neurological evaluation, audiometry and early latency auditory evoked responses were measured in 79 patients who had been treated with ≥ 2 courses of oral artemether or artesunate within the previous 3 years, and 79 age and sex matched controls living in a malaria endemic area on the North Western border of Thailand. There were no significant differences in any of these tests between the cases and controls. This study failed to detect any evidence of significant neurotoxicity in patients treated with oral artemether or artesunate.

Introduction

Artemisinin derivatives are now used widely for the treatment of falciparum malaria in South East Asia. On the western border of Thailand where P. falciparum has developed resistance to nearly all antimalarials, the combination of mefloquine and artemesunate was introduced in 1994 as first line treatment.1 Over 3000 patients with uncomplicated falciparum malaria have been recruited subsequently in prospective studies to optimize antimalarial treatment. Detailed prospective follow up of these patients, including simple neurological examinations, have failed to identify any serious adverse effects associated with the two most widely used compounds oral artesunate or artemether.2 But animal studies have raised concerns about the potential neurotoxicity of some of the artemisinin derivatives.3–8 These studies showed that rodents, dogs and monkeys treated with intramuscular
arteether or artemether (the two oil soluble derivatives) develop dose dependent
damage to certain brain-stem nuclei. Neurological findings included gait
disturbance, loss of spinal, brain stem and pain responses and, eventually, death.
Pathological changes in these experimental animals can be found even in the
absence of any detectable neurobehavioural symptoms.\textsuperscript{4,5} The neuropathological
lesions were unusual in that they were confined to the neuronal cells of certain
brain stem nuclei, whereas adjacent nuclei were often unaffected. The nuclei
affected included those in the auditory relay.\textsuperscript{6,7} Despite the lack of reported toxicity
in extensive clinical studies to date,\textsuperscript{2,9} it remains possible that the artemisinin
compounds produce a similar pattern of neurotoxicity in humans. In order to
assess possible toxic effects on the brainstem in patients treated with repeated
courses of artemether/артесуnate, brainstem auditory evoked responses (BAER)
were measured in a case control study. BAER have been used to detect
neurotoxicity in situations\textsuperscript{10} as diverse as subclinical mercury poisoning\textsuperscript{11} and
interferon toxicity.\textsuperscript{12} BAER should be particularly useful when the auditory
pathways or cochlea are involved specifically as in the case for artemisinin animal
neurotoxicity.

\textbf{Patients and methods}

This study was conducted in 1997 at the Shoklo Malaria Research Unit located on
the western border of Thailand. Karen subjects who had received oral antimalarial
treatment with either artemether or artesunate on at least two occasions during the
previous three years were eligible for the study provided that they gave fully
informed consent. Patients with previous severe or cerebral malaria\textsuperscript{13} or a history
of chronic ear-pathology or head-trauma were excluded. A complete medical
history was taken and all previous antimalarial treatments were checked against the
medical records. Control subjects were selected from the community providing
that they had never received an artemisinin derivative, and had no ear-pathology or previous head-trauma, and also gave informed consent. These subjects were matched for age and sex with the cases. All subjects were assessed initially for concurrent illness on the day of testing, and if healthy, underwent a full otological and neurological examination. Audiology was then performed and auditory evoked responses recorded.

**Neurological test.** A clinical assessment for neurotoxicity was performed which included the following: Romberg's test, assessment of gait and balance (tandem gait), fine finger dexterity (ability to pick up a small tablet), tests of clinical assessment for hearing acuity (using a 256 Hz tuning fork) and assessment of eye movements, nystagmus, and behaviour abnormality. This has been a standard procedure in all drug trials conducted at this site since 1994.

**Audiometry test.** Both right and left ears were tested using a portable Kampex™ AS7 screening audiometer. The starting point was 40 dBnHL with a frequency of 250 Hz. This was increased gradually to 500 Hz, 750 Hz, 1000 Hz, 1500 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz until 8000 Hz.

**Auditory Evoked Response test.** The BAER test was performed using a portable computerized system (Bio-logic Traveller Express E Auditory Evoked Potential Analyser). Ag/AgCl surface electrodes were applied to the vertex (exposition) and to both mastoids and the ground cable was applied to the forehead. Patients lay on a flat couch and were allowed to relax before testing. Electrode impedance was checked for each individual and was maintained <10kOhm for all electrodes. A rarefaction click-stimulus delivered by headphones was used to elicit the auditory evoked potentials. The duration of one click was 100 μs and the clicks were presented monaurally in a rate of 11.1/sec with an intensity of 80 dBnHL. The contra-lateral ear was masked using white noise at 40 dBnHL. A total of 1024
sweeps were recorded by the computer and averaged. Two replications were made to determine reliability. This procedure was performed for both ears separately. The wave-forms were labeled I, II, III, IV, V for the ipsilateral recording (i.e. the test-ear) and III', V' for the contralateral recording (masked-ear) by an investigator unaware of the volunteer status (case or control). The wave form analysis was performed by the AER-technician and then reviewed by a clinical neurophysiologist unaware of the patient allocation. The latencies for each of these waves were established and the inter-peak times determined (I to III, III to V and I to V). The auditory evoked response test represents the auditory pathway up to the midbrain. The five vertex-positive potentials (I-V) relate to different levels in the auditory system: cochlea and acoustic nerve (I), medulla (II), caudal pons (III), rostral pons (IV) and midbrain (V).

**Drug regimen.** In this study the cases were patients who had been recruited to antimalarial drug studies between 1991 and 1997. They were treated under supervision with oral artesunate (Guilin Pharmaceutical Factory No.1, Guilin, China) or artemether (Kunming Pharmaceutical Factory, Kunming, People’s Republic of China) either alone or in combination with mefloquine (Lariam®, Hoffman-La Roche, Basel, Switzerland), or lumefantrine (Novartis, Basel, Switzerland) or tetracyclines (Table 2), and followed up prospectively for 42-63 days to establish the efficacy of the various regimens.

As all antimalarial treatments are recorded in this site. Unrecorded administration of an artemisinin derivative outside the health structure would not have been possible in this setting.

**Statistical analysis.** Continuous normally distributed data were described by the mean (standard deviation, range) and non-normally distributed data by the median (range). Percentages were given for categorical data. The Student’s paired $t$ test was performed to determine whether there were significant differences between cases and controls for
Auditory evaluation of artemisinin derivatives

the I to III, I to V and III to V inter-peak latencies identified prospectively as being the most likely to show abnormalities if any were present. The Spearman's rank correlation coefficient was used to assess the association between the inter-peak latencies and total amount of drug the patient received. Data were analysed using SPSS for Windows (SPSS Inc, Chicago, Illinois, USA).

Results

In May 1997, 79 patients and 79 age and sex matched controls were tested. Overall 56 pairs (70.9%) were males. The median age was 15 years (range 3 to 53 years). Two cases (2.5%) were under five, 37 cases (47%) were 5-14 years old, and 40 cases (51%) were over 14 years of age. Sixteen pairs (20.2%) differed by one year of age. There were no significant differences in height and weight between cases and the controls (Table 1).

Table 1: Baseline characteristics of the 79 tested pairs

<table>
<thead>
<tr>
<th>Feature</th>
<th>Cases</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>79</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>140 (22)</td>
<td>141 (20)</td>
<td>P = 0.263</td>
</tr>
<tr>
<td>Range</td>
<td>91-176</td>
<td>95-174</td>
<td></td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>39.8 (15.4)</td>
<td>39.6 (14.67)</td>
<td>P = 0.962</td>
</tr>
<tr>
<td>Range</td>
<td>12.5-68.5</td>
<td>13-69.5</td>
<td></td>
</tr>
<tr>
<td><strong>Audiometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R 4000-&lt;30</td>
<td>41 (52%)</td>
<td>49 (62%)</td>
<td></td>
</tr>
<tr>
<td>35-45</td>
<td>31 (39%)</td>
<td>25 (32%)</td>
<td></td>
</tr>
<tr>
<td>50-55</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>L 4000-&lt;30</td>
<td>47 (60%)</td>
<td>42 (66%)</td>
<td></td>
</tr>
<tr>
<td>35-45</td>
<td>26 (33%)</td>
<td>22 (28%)</td>
<td></td>
</tr>
<tr>
<td>50-55</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
The cases had received a mean (SD) dose of 38.9 mg/kg (16.3) with a range of 24 to 108 mg/kg of an oral artemisinin derivative (either artesunate and/or artemether). The number of exposures per case ranged from two to nine. In 51 cases (64.6%) the first treatment received was a combination of an oral artesunate plus mefloquine, and in 16 cases (20.3%) the treatment consisted of a combination of an oral artemether plus lumefantrine (co-artemether) for three days.

Table 2: Different drug regimens given to the patients

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAS3</td>
<td>Mefloquine 25 mg/kg + Artesunate 12 mg/kg total for three days</td>
</tr>
<tr>
<td>MAS7</td>
<td>Mefloquine 25 mg/kg + Artesunate 12 mg/kg total for seven days</td>
</tr>
<tr>
<td>MA</td>
<td>Mefloquine 15 mg/kg + Artesunate 10 mg/kg (3 doses in 1 day)</td>
</tr>
<tr>
<td>MAM3</td>
<td>Mefloquine 25 mg/kg + Artemether 12 mg/kg total for three days</td>
</tr>
<tr>
<td>AS5</td>
<td>Artesunate 12 mg/kg total for five days</td>
</tr>
<tr>
<td>AS7</td>
<td>Artesunate 12 mg/kg total for seven days</td>
</tr>
<tr>
<td>AM7</td>
<td>Artemether 12 mg/kg total for seven days</td>
</tr>
<tr>
<td>CO-AM</td>
<td>Artemether 1-2 mg/kg + Benflumetol 6-12 mg/kg total/dose four times</td>
</tr>
<tr>
<td>AS7TET7</td>
<td>Artesunate 12 mg/kg total + Tetracycline 250 mg t.i.d. for seven days</td>
</tr>
<tr>
<td>AS7DOX7</td>
<td>Artesunate 12 mg/kg total + Doxycycline 100 mg o.d. for seven days</td>
</tr>
</tbody>
</table>

All the other cases received a different regimen of an artemisinin derivative alone, or in combination, with doxycycline or tetracycline (Table 2). Two thirds (67.1%) of the cases studied received three different artemisinin regimens. Patients who were treated twice had a median (range) time of 69 (6-969) days between their first and second exposures to an artemisinin derivative. Patients who were treated three times had a median (range) time of 77 (21-516) days between their second and their third exposures.

All the neurological examinations were normal except for the hearing tests in one case and in two controls. The case could not hear with the left ear while testing the
hearing acuity, and had hearing loss on the left side at 8000 Hz. There were no abnormalities found while testing the hearing acuity for the right ear and there was no hearing loss on the right side. The corresponding AER did not give reproducible waveforms for both right and left side due either to profound hearing loss or technical problems. The two controls could not hear with their left ear while testing hearing acuity, but both audiometry-tests were in the normal range. The corresponding AER for one control did not give reproducible waveforms for right and left ears, and for the other control there were no waveforms on the left, possibly due to profound hearing loss. AER tests with no reproducible waveforms were regarded as missing in the final analysis. No abnormalities were seen in the Romberg’s test, and in assessments of tandem gait, fine finger dexterity, visual acuity, eye movements and behaviour. Audiometry at 4000 Hz for both the right and left ears was similar for the cases and controls (Table 1).

The auditory evoked responses (AER’s) are summarised in Table 3. There was no significant difference between cases and controls for the interpeak latencies (IPL) I-V and III-V in either left or right sides of the brainstem.

<table>
<thead>
<tr>
<th>Inter-peak latencies</th>
<th>Cases</th>
<th>Controls</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right I-III n= 69</td>
<td>2.14 (0.19)</td>
<td>2.08 (0.19)</td>
<td>0.049</td>
</tr>
<tr>
<td>Right I-V n= 69</td>
<td>3.97 (0.21)</td>
<td>3.91 (0.21)</td>
<td>0.079</td>
</tr>
<tr>
<td>Right III-V n= 71</td>
<td>1.83 (0.15)</td>
<td>1.84 (0.18)</td>
<td>0.720</td>
</tr>
<tr>
<td>Left I-III n= 70</td>
<td>2.14 (0.25)</td>
<td>2.10 (0.17)</td>
<td>0.275</td>
</tr>
<tr>
<td>Left I-V n= 70</td>
<td>3.95 (0.23)</td>
<td>3.93 (0.23)</td>
<td>0.603</td>
</tr>
<tr>
<td>Left III-V n= 71</td>
<td>1.81 (0.20)</td>
<td>1.83 (0.18)</td>
<td>0.519</td>
</tr>
</tbody>
</table>

* - Student’s paired t test
There was a very small but significant difference between cases and controls for the interpeak latency I-III, but only on the right side. No correlation was observed between the total dose (mg/kg) of artemisinin administered and the left or right IPLs. The patients given the highest cumulative doses of artemisinin derivatives, [90th percentile; total dose of more than 60 mg/kg (n=9)], showed no significant differences in any of the IPLs compared with their controls.

Discussion

The artemisinin derivatives are an essential component of antimalarial treatment in areas where multidrug resistant Plasmodium falciparum is prevalent, and their use is likely to increase with the more widespread introduction of combinations to combat resistance. No toxicity has been seen in clinical trials which have included more than 5000 patients. However concerns have been raised by the consistent findings of neurotoxicity in animals following parenteral administration of the oil based derivatives arteether and artemether. Central nervous system neuropathological changes, seen in rats, dogs and monkeys are usually limited to certain brain stem nuclei, notably those involved in hearing and sound localisation. The toxicity described in animals is dependent upon dose, route of administration, and time. No neurological abnormalities were observed following administration of 25 or 30 mg/kg/day of parenteral arteether for six or eight days to rats, but neurological abnormalities were observed following administration of 50 mg/kg/day for five to six days. High doses of arteether or artemether, [20 mg/kg/day IM for eight days] are lethal in dogs, causing a progressive syndrome of clinical neurologic defects culminating in cardiorespiratory collapse. Route of administration influences the toxicity: oral intermittent dosing of the same drugs is considerably less toxic. Administration of the lipid soluble intramuscular artemether and arteether is more toxic than intramuscular or intravenous water-soluble artesunate (TG Brewer: personal communication). Pharmacokinetic differences between the different formulations and routes of administration
provide a plausible explanation for this observation. Artesunate is absorbed and eliminated very rapidly whether given orally or parenterally. Oral artemether is also absorbed rapidly. In contrast artemether and arteether are both absorbed slowly and erratically from an intramuscular depot giving sustained blood concentrations. Neurotoxicity seems to result from these sustained blood concentrations. Wesche et al. have compared the neurotoxicity of the artemisinin analogs in vitro. Dihydroartemisinin, the common metabolite of the artemisinin derivatives, has the most potent antimalarial activity, and it is also the most toxic of analogues tested. The precise cause of neuronal damage remains unknown although it is known to be potentiated by haem. Indeed neurotoxicity cannot be dissociated from antimalarial activity suggesting a common mechanism of action. Along the Thai-Burmese border the artemisinin derivatives have been used since 1994 in combination with mefloquine as the standard treatment for multidrug resistant Plasmodium falciparum malaria. This study is the first detailed attempt to assess neurotoxicity beyond standard clinical examination in this population. Patients who received multiple doses of an artemisinin derivative (during a period of three years) were included and matched for age and sex with controls. Data were not recorded in the acute phase after intake of an artemisinin derivative as interpretation would be difficult since fever and illness cause changes acutely in the BAER. The neurological tests and audiometry in these healthy subjects showed no significant difference between the cases and the controls. The auditory evoked response test represents the auditory pathway up to the midbrain allowing identification of the level at which abnormalities occur. Based on the animal studies, it was considered prospectively that the III to V latency would be the most likely to be affected by toxicity. There was no significant difference between cases and controls in the interpeak-latencies I-V and III-V, for both right and left ears. There was a small but significant prolongation of the interpeak-latency I-III in the cases, but this was only on the right side (no difference was seen on the left side).
Any toxicity related to a cumulative drug effect would have been expected to produce bilateral prolongation of the interpeak-latency, (especially of waves III to V). Furthermore the difference was of borderline significance \((p=0.049)\). Furthermore there was no evidence of dose related toxicity. In the nine cases who received the highest doses of artesunate (more than 60 mg/kg), no difference between cases and controls was seen for the three different tests. Mefloquine, which is known to produce adverse neurological effects, was also used in many patients but no persistent abnormalities related to this drug were found either. These results are encouraging but not definitive. In this study the artemisinin doses were given over a prolonged time in divided doses. The total dose administered in our patient-population might be below that associated with toxicity. Alternatively reversible toxicity might have occurred, although there was no evidence of this in the acute clinical evaluations.

We conclude from this study that there is no evidence of residual clinical brainstem-pathology detectable with the auditory evoked response test, after administration of multiple doses of oral artemisinin derivatives. Temporo-spatial sound discrimination, which is thought to be related to the middle and late latency evoked potentials ie higher thalamic and auditory cortical functions, could be more sensitive for the detection of neuropathology caused by the artemisinin derivatives. The development of sensitive physiological tests in experimental animals which correlate with neuropathological changes is needed. It may never be possible to be absolutely sure that currently used treatment regimens with artemisinin derivatives cause no neuronal damage. Loss of a few neurones would be impossible to detect. Nevertheless with increasing negative evidence from detailed electrophysiological studies such as this, and reassuring information on the safety of the oral compounds in animal models, it seems likely that there is a significant margin of safety. More studies are needed, but concerns of neurological toxicity should not limit appropriate use of these valuable antimalarials.
Acknowledgements

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References


