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A CASE-CONTROL AUDITORY EVALUATION OF PATIENTS TREATED WITH ARTEMISININ DERIVATIVES FOR MULTIDRUG-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA

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Abstract. The artemisinin derivatives are now used widely in areas with multidrug-resistant *Plasmodium falciparum* malaria such as Southeast 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). To investigate whether a similar toxic effect occurs in patients treated with these compounds, clinical neurologic evaluation, audiometry and early latency auditory evoked responses were measured in a single-blind comparison of 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 northwestern border of Thailand. There were no consistent differences in any of these test results between the cases and controls. This study failed to detect any evidence of significant neurotoxicity in patients treated previously with oral artemether or artesunate for acute malaria.

Artemisinin derivatives are now used widely for the treatment of *Plasmodium falciparum* malaria in Southeast Asia. On the western border of Thailand where *P. falciparum* has developed resistance to nearly all antimalarials, the combination of mefloquine and artesunate was introduced in 1994 as a first-line treatment.[1] More than 3,000 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 neurologic examinations, has failed to identify any serious adverse effects associated with the 2 most widely used compounds, oral artesunate or artemether.[2] However, 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 2 oil-soluble derivatives) develop dose-dependent damage to certain brain stem nuclei. Neurologic findings included gait disturbance, loss of spinal, brain stem, and pain responses, and, eventually, death. Pathologic changes in these experimental animals were found even in the absence of any detectable neurobehavioural symptoms.[4,5] The neuropathologic 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.[6,7]

Despite the lack of reported toxicity in extensive clinical studies to date,[2,9] it remains possible that the artemisinin compounds produce a similar pattern of neurotoxicity in humans. To assess possible toxic effects on the brain stem in patients treated with repeated courses of artemether or artesunate, brain stem auditory evoked responses (BAERs) were measured in a case-control study. These have been used[10] to detect neurotoxicity in situations as diverse as subclinical mercury poisoning[11] and interferon toxicity.[12]. They should be particularly useful when the auditory pathways or cochlea are involved specifically, as in the case of experimental artemisinin animal neurotoxicity.[3–8]

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 2 occasions during the previous 3 years were eligible for the study provided that they gave fully informed consent. This investigation was part of a series of studies of antimalarial treatment regimens approved by the Ethics Committee of the Faculty of Tropical Medicine of Mahidol University and the Karen Refugee Committee. The subjects were selected randomly from those still resident in the camps. Patients with previous severe or cerebral malaria,[13] a history of chronic ear pathology, or head trauma were excluded. A complete medical history was obtained and all previous antimalarial treatments were checked against the medical records. Control subjects were selected from the community provided that they had never received an artemisinin derivative, and had no ear pathology or previous head trauma, and also gave informed consent. Since all antimalarial treatments were documented in this community, and the artemisinin derivatives were not available outside the health structures, the history of artemisinin exposure was reliable. These subjects were matched for age and sex with the cases.[14] All subjects were assessed initially for concurrent illness on the day of testing, and if healthy, underwent a full otologic and neurologic examination. Audiometry was then performed and auditory evoked responses were recorded by examiners unaware of whether the subject was a case or a control.

Neurologic test. A clinical assessment for neurotoxicity was performed that included the following: Romberg's test, assessment of gait and balance (tandem gait), fine finger dex-

TABLE 1
Different drug regimens given to the patients[15–18]

MAS3	Mefloquine, 25 mg/kg + artesunate, 12 mg/kg total for 3 days
MAS7	Mefloquine, 25 mg/kg + artesunate, 12 mg/kg total for 7 days
MA	Mefloquine, 15 mg/kg + artesunate, 10 mg/kg (3 doses in 1 day)
MAM3	Mefloquine, 25 mg/kg + artemether, 12 mg/kg total for 3 days
AS5	Artesunate, 12 mg/kg total for 5 days
AS7	Artesunate, 12 mg/kg total for 7 days
AM7	Artemether, 12 mg/kg total for 7 days
CO-AM	Lumefantrine (benflumetol), 6–12 mg/kg + artemether, 1–2 mg/kg total for 3 days
AS7TET7	Artesunate, 12 mg/kg total + tetracycline, 250 mg 3 times a day for 7 days
AS7DOX7	Artesunate, 12 mg/kg total + doxycycline, 100 mg once a day for 7 days

terity (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 behavior abnormality. This has been a standard procedure in all drug trials conducted at this site since 1994.[15]

Audiometry test. Both right and left ears were tested using a portable Kampex™ AS7 screening audiometer (P. G. Werth, London, United Kingdom). The starting point was 40 decibels (dBnHL) with a frequency of 250 Hz. This was increased gradually to 500 Hz, 750 Hz, 1,000 Hz, 1,500 Hz, 2,000 Hz, 3,000 Hz, 4,000 Hz, 6,000 Hz until 8,000 Hz.

Auditory evoked response test. The BAER test was performed using a portable computerized system (Bio-logic Traveler Express E Auditory Evoked Potential Analyzer; SLE Instruments, Croydon, Surrey, United Kingdom). Ag/AgCl surface electrodes were applied to the vertex (cz-position) 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 <10 kOhm for all electrodes. A rarefaction click-stimulus delivered by headphones was used to elicit the auditory evoked potentials. The duration of 1 click was 100 μsec and the clicks were presented monoaurally 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 1,024 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, and V for the ipsilateral recording (i.e., the test ear) and III' and 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 auditory evoked response (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 were 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 5 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).

TABLE 2.
Baseline characteristics of the 79 tested pairs

	Cases (n = 79)	Controls (n = 79)	*P*
Height, cm			
Mean (SD)	140 (22)	141 (20)	0.26
Range	91–176	95–174	
Weight, kg			
Mean (SD)	39.8 (15.4)	39.6 (14.67)	0.96
Range	12.5–68.5	13–69.5	
Audiometry (decibel threshold)			
Right 4,000 Hz			
≤30	41 (52%)	49 (62%)	
35–45	31 (39%)	25 (32%)	
50–55	5	3	
≥60	1	1	
Left 4,000 Hz			
≤30	47 (60%)	42 (66%)	
35–45	26 (33%)	22 (28%)	
50–55	2	3	
≥60	4	1	

Drug regimens. In this study the cases were mainly patients who had been recruited to antimalarial drug studies between 1991 and 1997.[1,15–18] They were treated under supervision with oral artesunate (Guilin Pharmaceutical Factory No.1, Guilin, People's Republic of 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), lumefantrine (Novartis, Basel, Switzerland), or tetracyclines (Table 1), and followed up prospectively for 42–63 days to establish the efficacy of the various regimens. Patients who were not part of these studies were treated with the standard 3-day artesunate plus mefloquine regimen and followed in the same way. Since all antimalarial treatments are recorded at 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 were described 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 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 analyzed using SPSS for Windows (SPSS, Inc., Chicago, IL).

RESULTS

In May 1997, 79 patients and 79 age- and sex-matched controls were tested. Overall 56 pairs (71%) were males. The median age was 15 years (range = 3–53 years). Two cases (2.5%) were <5 years old, 37 cases (47%) were 5–14 years old, and 40 cases (51%) were >14 years old. Sixteen pairs (20%) differed by ≥ 1 year of age. There were no significant

TABLE 3
Mean (SD) interpeak latencies for the cases and controls (msec)

Interpeak latencies		Cases	Controls	*P**
Right I–III	n = 69	2.14 (0.19)	2.08 (0.19)	0.049
Right I–V	n = 69	3.97 (0.21)	3.91 (0.21)	0.079
Right III–V	n = 71	1.83 (0.15)	1.84 (0.18)	0.720
Left I–III	n = 70	2.14 (0.25)	2.10 (0.17)	0.275
Left I–V	n = 70	3.95 (0.23)	3.93 (0.23)	0.603
Left III–V	n = 71	1.81 (0.20)	1.83 (0.18)	0.519

* By Student's paired *t*-test.

differences in height and weight between cases and the controls (Table 2).

The cases had received a mean (SD) dose of 38.9 mg/kg (16.3) with a range of 24–108 mg/kg of an oral artemisinin derivative (either artesunate and/or artemether). The number of exposures per case ranged from 2 to 9. The median (range) time from most recent exposure was 385 (31–1,963) days. In 51 cases (65%), the first treatment received was a combination of an oral artesunate plus mefloquine, and in 16 cases (20%) the treatment consisted of a combination of oral artemether plus lumefantrine (co-artemether) for 3 days. All other cases received a different regimen of an artemisinin derivative alone, or in combination, with doxycycline or tetracycline (Table 1). Two-thirds (67%) of the cases studied received 3 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 3 times had a median (range) time of 77 (21–516) days between their second and their third exposures. All results of neurologic examinations were normal except for the hearing test results in 1 case and 2 controls. The case could not hear with the left ear while tested for hearing acuity, and had hearing loss on the left side at 8,000 Hz. There were no abnormalities found on 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 because of either profound hearing loss or technical problems that could not be resolved. The two controls could not hear with their left ears while testing hearing acuity, but the results of both audiometry tests were in the normal range. The corresponding AER for 1 control did not give reproducible waveforms for both ears, and the other control showed no waveforms in the left ear. The AER test results with no reproducible waveforms were regarded as missing in the final analysis. No abnormalities were seen in the results of the Romberg's test, and in assessments of tandem gait, fine finger dexterity, visual acuity, eye movements, and behavior. Audiometry at 4,000 Hz for both the right and left ears was similar for the cases and controls (Table 2).

The AERs are summarized in Table 3. There was no significant difference between cases and controls for the interpeak latencies (IPLs) I–V and III–V on either the left or right sides of the brain stem. There was a very small but significant difference between cases and controls for the IPL 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 >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 *P. 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 that have included more than 5,000 patients.[2] 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 neuropathologic changes seen in rats, dogs, and monkeys are usually limited to certain brain stem nuclei, including those involved in hearing and sound localization. The toxicity described in animals is dependent upon dose, route of administration, and time. No neurologic abnormalities were observed following administration of 25 or 30 mg/kg/day of parenteral arteether for 6 or 8 days to rats, but neurologic abnormalities were observed following administration of 50 mg/kg/day for 5–6 days.[3–6] High doses of arteether or artemether (20 mg/kg/day given intramuscularly for 8 days) are lethal in dogs, causing a progressive syndrome of clinical neurologic defects culminating in cardiorespiratory collapse.[6] Route of administration influences the toxicity: oral intermittent dosing of the same drugs is considerably less toxic.[19,20] Administration of lipid-soluble intramuscular artemether and arteether is more toxic than intramuscular or intravenous water-soluble artesunate[21] (Brewer TG, unpublished data). 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 whereas efficacy does not require sustained exposure of the parasite population to parasiticidal blood concentrations.[22] Wesche and others have compared the neurotoxicity of the artemisinin analogs *in vitro*.[8] Dihydroartemisinin, the common metabolite of the artemisinin derivatives, has the most potent antimalarial activity, and it is also the most toxic of the analogs tested. The precise cause of neuronal damage remains unknown although it is known to be potentiated by heme. Indeed, neurotoxicity cannot be dissociated from antimalarial activity, suggesting a common mechanism of action.[23,24]

The artemisinin derivatives have been used since 1994 along the western border Thailand in combination with mefloquine as the standard treatment for multidrug-resistant *P. falciparum* malaria. This study was a 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 3 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 because interpretation would be difficult since fever[24] and illness cause acute changes in the BAER.[25] The

results of neurologic tests and audiometry in these healthy subjects showed no significant difference between the cases and the controls. The auditory evoked response tests the auditory pathway through its central connections and allows identification of the level at which abnormalities occur. Based on animal studies,[3–8] 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 IPLs I-V and III-V for both the 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 ($P = 0.049$). There was also no evidence of dose-related toxicity. In the 9 cases who received the highest doses of artesunate (>60 mg/kg), no difference between cases and controls was seen for the 3 different tests. Mefloquine, which is known to produce adverse neurologic effects,[27] was also used in many patients but no persistent abnormalities related to this drug were found. These results are encouraging but not definitive. The measurement of BAERs, although the most sensitive, noninvasive approach generally available to assess brain stem function, has not been validated in the animal models as a good correlate of the neuropathologic abnormalities described. 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.[28]

We conclude from this study that there is no evidence of residual clinical brain stem pathology detectable with the AER 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, i.e., higher thalamic and auditory cortical functions, could be more sensitive for the detection of neuropathology caused by the artemisinin derivatives (Brewer TG, unpublished data). The development of sensitive physiologic tests for which the results in experimental animals correlate with neuropathologic changes are needed. Neither auditory evoked potentials nor any other neurophysiologic assessment have been validated as sensitive predictors of neuronal damage by these drugs. Indeed, it may never be possible to be absolutely sure that currently used treatment regimens with artemisinin derivatives are completely safe. Loss of a few neurones would be impossible to detect. Nevertheless, with increasing negative evidence from detailed electrophysiologic 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 neurologic toxicity should not limit appropriate use of these valuable antimalarials.

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