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CLINICAL AND NEUROPHYSIOLOGICAL STUDY OF THE EFFECTS OF MULTIPLE DOSES OF ARTEMISININ ON BRAIN-STEM FUNCTION IN VIETNAMESE PATIENTS

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Abstract. The qinghaosu (artemisinin) group of drugs is the most important new class of antimalarials developed in the last fifty years. Although there has been no clinical evidence of neurotoxicity, an unusual pattern of damage to specific brain-stem nuclei has been reported in experimental animals receiving high doses of arteether or artemether. Detailed clinical examinations, audiometry, and brain stem auditory evoked potentials (BSAEPs) were assessed in 242 Vietnamese subjects who had previously received up to 21 antimalarial treatment courses of artemisinin or artesunate alone and 108 controls from the same location who had not received these drugs. There was no evidence of a drug effect on the clinical or neurophysiological parameters assessed. In this population there was no clinical or neurophysiological evidence of brain-stem toxicity that could be attributed to exposure to artemisinin or artesunate.

INTRODUCTION

The qinghaosu (artemisinin) group of drugs, first isolated by Chinese scientists from the plant *Artemesia annua* in 1972, is now in widespread use throughout Southeast Asia and is used increasingly elsewhere in the tropical world for the treatment of both uncomplicated and severe malaria.[1–3] Several different compounds are available, including the parent compound artemisinin, and the derivatives artesunate, artemether, and arteether. Several million patients have now been treated with this group of drugs and, to date, no unequivocal drug-attributable toxicity has been reported. Recent large comparative studies of patients with severe falciparum malaria have confirmed that artemether is an acceptable, well-tolerated alternative to quinine. No significant adverse effects were found. In a double-blind trial of 560 Vietnamese adults treated with artemether, which included thorough neurological evaluation and double blind audiometry, no evidence of iatrogenic neurotoxicity was found. In large studies of uncomplicated malaria in Thailand, again no evidence of significant toxicity was found.[3–5] Two recently completed meta-analyses of the use of artemisinin derivatives in uncomplicated and severe malaria conducted under the auspices of the Cochrane Group and the World Health Organization (WHO) have provided no evidence of neurotoxicity.[6,7] The excellent safety profile of the qinghaosu compounds contrasts with the relatively frequent minor toxicity reported with the quinoline antimalarials, quinine, chloroquine, and mefloquine.[8] However, concerns have been raised by *in vitro* and animal studies. In laboratory experiments using the oil-based parenteral artemisinin derivatives arteether and artemether, selective neurotoxicity was noted in the brain-stem nuclei with a dose-related site-specific necrosis primarily in the pons and medulla of rats, dogs, and monkeys.[9–12] Use of the water soluble qinghaosu compound, artesunate, and oral administration of all the compounds produced considerably less neurotoxicity in animal experiments.

Despite the lack of clinical reports of neurotoxicity, these data from animals are a cause for concern. In order to evaluate the possibility of brain-stem neuropathology in patients who have received multiple treatment courses of the qinghaosu compounds, we assessed the auditory pathway of the brain stem with studies of brain-stem auditory evoked potentials (BSAEPs) together with thorough audiometric and neurological examinations.

The BSAEPs occur in the brain stem during the first 10 msec after a transient auditory stimulus. The potentials are recorded as a characteristic series of waveforms. These are detected by scalp electrodes and correspond to anatomic structures in the auditory pathway of the brain stem. Specifically, the auditory nerve and the cochlear nucleus are the generators of peaks I and II, the superior olivary complex generates peak III, the lateral lemniscus generates peak IV, and the inferior colliculus generates peak V (Figure 1).[13] Together, the series of waveforms encompass these nuclei and the relays between them. The BSAEPs are used to demonstrate the integrity of the neuronal pathway from the cochlea, via the auditory nerve to the brain stem, allowing localization of dysfunction within this pathway. The neuropathological damage reported in experimental animals treated with artemisinin is highly localized to the auditory and vestibular nuclei, and the trapezoid body. If there was significant iatrogenic damage to these centers then measurement of BSAEPs in patients treated with repeated doses of artemisinin, or its derivatives, would be expected to be abnormal.

The purpose of this study was to determine whether there was clinical or neurophysiological evidence of neurotoxicity in humans exposed to multiple doses of the qinghaosu compounds.

MATERIALS AND METHODS

Study site. The study was carried out at the Khanh Phu Medical Station between May and November 1997. Khanh Phu is in the Central Highlands of Vietnam, 450 km north of Ho Chi Minh City. Malaria transmission is high and Khanh Phu Village has the highest incidence of malaria reported in Vietnam. The entomological inoculation rate (EIR)

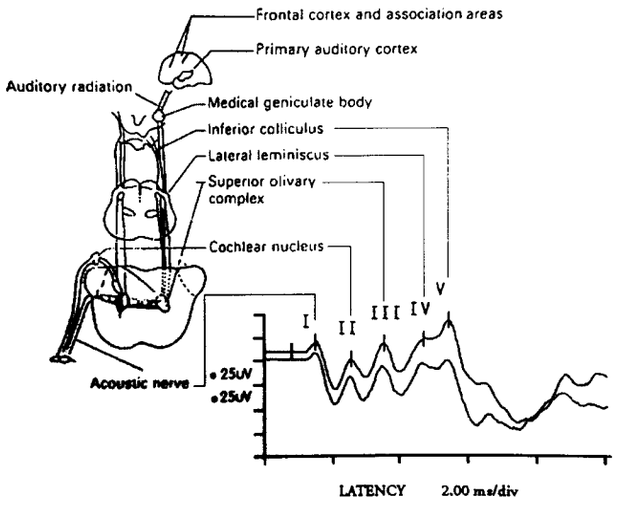

FIGURE 1. A representative example of the brain-stem auditory evoked potentials generated from a single subject in the study. The anatomical structures from auditory nerve (wave I) to the inferior colliculus (wave V) corresponding to this characteristic wave form are shown.

is approximately 100 per year; 70% of infections are *Plasmodium falciparum,* 25% *Plasmodium vivax,* and 5% *Plasmodium malariae.* Sporozoite rates are 3–4%. The artemisinin drugs became available in the village from 1995 onwards, and are supplied at no cost by the medical station. Every patient attending the clinic with fever has a blood smear examined, and is treated with antimalarial drugs onsite if the blood smear is positive and if there are also clinical features of malaria. Asymptomatic parasite-positive patients are not treated. If the patient lives far from the clinic they are given a course of treatment to take at home; if they live nearby they return daily for supervised administration of drugs. Every clinic attendance is recorded along with basic clinical information, results of smears, and drugs dispensed. All the treatment episodes from 1995 until May 1997 were recorded. There is little incentive in this extremely poor village to seek external medical help or to purchase drugs elsewhere. Thus, the details of all malaria episodes from the introduction of artemisinin in 1995 are likely to include all antimalarial treatments.

Drug usage. Both artesunate (Guilin Factory, Guangxi, China) and artemisinin (Factory 19, Ho Chi Minh City) were used. The artesunate treatment regimen that has been used by the Medical Station since 1995 is as follows: if less than 1 year of age, give 150 mg total dose; 1–5 years, 300 mg; 5–13 years, 550 mg; and older than 13 years, 600 mg. For artemisinin the total doses have been: less than 1 year of age, 750 mg total dose; 1–5 years, 1,500 mg; 5–13 years, 2,750 mg; and older than 13 years, 3,000 mg. Both drugs were given as five-day regimens. Five hundred mg/kg artemisinin as the total cumulative dose of drug received was selected *a priori* to look at the heavy exposure in an analysis suited to a binary outcome.

Mefloquine (15 mg/kg monotherapy) was given as the antimalarial treatment between July and December, 1995 at a time when artemisinin derivatives were not available. Mefloquine was never used in combination therapy with an artemisinin derivative. Using the dates of drug administration and the present age of the subject, the age of each patient at the time of receiving the drug was back-calculated. Using these ages and a standardized height and weight growth chart for Vietnamese children for ages ranging from birth to 18 years, the total weight-adjusted dosage was calculated for all subjects who had previously received antimalarial treatment. Demographic details of the subjects and controls are shown in Table 1A.

For subjects aged eighteen years or younger who received antimalarial therapy the mean difference in the estimated weight (from height and weight growth charts) and the weight recorded was 1.88 kgs (95% normal range −5.69 up to 9.45 kgs). The magnitude of the difference between the measured and estimated weight was significantly positively correlated with age (Spearman's rank correlation coefficient $= 0.524$, $P < 0.001$). Table 1B shows the mean difference in estimated weight (from height and weight growth charts) and the weight measured during the study.

Study design. Subjects were randomly selected from the village population of 1,764 people; the controls were the total number of people in the village who had received no artemisisin derivative. All subjects were assessed clinically using a standard neurological examination. Particular emphasis was placed on identifying abnormalities in hearing, vestibular and cerebellar function, and the control of voluntary movement. The presence of nystagmus or tremor was noted, and abnormalities in auditory localization, finger to nose movement, Romberg's test, gait, repetitive alternating movements, and fine motor control were assessed. Otoscopic examination of the external auditory canal was performed with particular reference to the presence of scarring, perforation, or pus. Audiological examinations were performed on both ears on those over 4 years old (Kamplex Screening Audiometer, AS7, Japan). The initial screening was at 40 dB with a frequency of 250 Hz, subsequently increasing to 500 Hz, 750 Hz, 1,000 Hz, 1,500 Hz, 2,000 Hz, 3,000 Hz, 6,000 Hz, and 8,000 Hz. All the data were recorded together with the patients' clinical history and exposure to all drugs. The neurological, otoscopic, and audiological assessments were performed blinded to the treatment histories of the subjects. Ethical approval and scientific review for this study was obtained from the Scientific and Ethical Committee of the Centre for Tropical Diseases, Ho Chi Minh City, and by the Scientific and Ethical Committee of the Khanh Hoa Provincial Health Services. Informed consent was obtained from the patients or their parents or guardians.

The BSAEP measurements were performed throughout by a single investigator using a Bio-Logic Traveller® Express E Auditory Evoked Potential Machine (SLE instruments, Croyden, Surrey, United Kingdom) according to the manufacturer's instructions. Briefly, surface electrodes were applied to the vertex and to both mastoids, and the subject was asked to lie down. Electrical impedance was checked and kept below 10 kOhm for all recordings. The 100 μs click stimulus was delivered to each ear in turn, via the headphones at a rate of 11.1 per second with an intensity of 80 dB. Hearing in the contralateral ear was masked with 40 dB noise. The bilateral BSAEPs of 1,024 clicks were amplified, averaged, and recorded. At least two recordings were made for each ear to ensure reproducibility. The wave forms were

TABLE 1A
Demographic details

	Controls (n = 108)	Qinghaosu compound alone (n = 242)	Qinghaosu compound + mefloquine (n = 98)	Mefloquine alone (n = 10)
Median age	13.5	10.0	8.0	10
(range)	4–65	1–57	4–48	7–17
n (%)				
2–5 yr	2 (1.9%)	67 (27.7%)	31 (31.6%)	0
6–15 yr	70 (64.8%)	94 (38.8%)	58 (59.2%)	9 (90%)
16+	36 (33.3%)	81 (33.5%)	9 (9.2%)	1 (10%)
Male sex n (%)	50 (46.3%)	108 (44.6%)	45 (45.9%)	5 (50%)
Mean (SD)	36.9 (0.4)	37.0 (0.5)	37.0 (0.4)	36.9 (0.2)
Temperature	n = 105	n = 222	n = 79	n = 9
Range	36.1–38.0	36.0–46.0	36.1–37.8	36.5–37.1
Body mass index				
Median	16.0	15.63	14.72	15.12
Range	11.75–34.0	6.85–25.95	11.34–21.37	13.42–17.85
Otoscope exam				
Dry perforation	10.4% (11/106)	19.7% (45/229)	19.4% (18/93)	12.5% (1/8)
Otitis media	13.4% (14/106)	11.4% (26/229)	8.6% (8/93)	12.5% (1/8)
Otitis and/or perforation	23.6% (25/106)	31.0% (71/229)	28.0% (26/93)	25.0% (2/8)
Audiometry	n = 102	n = 153	n = 60	n = 9
Right ear 4000				
≤30	89 (87%)	123 (80%)	47 (78%)	7 (78%)
35–45	11	28	12	2
50–55	1	0	1	0
>60	1	2	0	0
Left ear 4000				
≤30	81 (79%)	124 (81%)	53 (88%)	8 (89%)
35–45	18	20	5	1
50–55	3	6	2	0
≥60	0	3	0	0

identified subsequently and marked by a single investigator, who was blind to information regarding drug exposure. All the wave forms and markings were subsequently checked, again blind to all subject details, by a single experienced neurophysiologist and these values accepted. Interpeak wave latencies were calculated and the wave forms assessed for other abnormalities. Subsequently, all BSAEP measurements were checked (again blind to all subject details) by the neurophysiologist. The accuracy of the Bio-Logic Traveller® Express E Auditory Evoked Potential Machine is 0.1 msec; variation below this level was considered not significant. The interpeak latencies between waves III and V were considered prospectively to most likely be affected if the same pattern of neurological toxicity observed in experimental animals was found in the subjects. The analysis concentrated on the I to V and III to V latencies.

Statistical analyses. Data were analyzed using SPSS for Windows (SPSS Inc., Chicago, IL). Categorical data were compared using the χ^2 test. Normally distributed continuous data were compared by the Student's *t*-test. Data not conforming to a normal distribution were compared using the Mann-Whitney U test. A *P*-value of < 0.05 was considered statistically significant. In comparison with published normative data, a value of the mean plus 2.5 SD was regarded as within the normal range.

TABLE 1B
Mean difference for estimated weight (from height and growth charts) minus recorded weight (95% normal range)

Age group	Mean difference kilograms (95% normal range)
1–4 yr	0.08 (−2.43, 2.59)
5–9	1.61 (−2.33, 5.55)
10–14	2.36 (−5.97, 10.69)
15–18	4.28 (−8.73, 17.29)

RESULTS

Neurological and otoscopic examinations, and assessments of auditory localization, audiometry, and BSAEPs were made in 242 subjects who had received previous antimalarial treatment with either artemisinin or artesunate alone (QHS), in 98 who had received both artemisinin and mefloquine (QM), in 10 who had been treated with mefloquine alone (M), and in 108 controls from the same location who had never received these drugs. The controls were older with a median of 13.5 years (range 4 to 65) compared with the other groups: QHS 10 years (1 to 57), QM 8 years (4 to 48), and M 10 years (7 to 17), $P < 0.001$. The controls also had a larger body mass index (BMI) (median [range] 16.0 [11.75 to 34]) compared with the other groups: QHS 15.63 [6.85 to 25.95], QM 14.72 [11.34 to 21.37], and M 15.12 [13.42 to 17.85]. The controls also had a lower body temperature (mean [SD] °C: 36.9[0.4]°C) compared with the other groups: QHS 37.0 [0.5], QM 37.0 [0.4], and M 36.9 [0.2]. The groups were otherwise comparable (Table 1A).

TABLE 2
Frequency of qinghaosu total doses (weight adjusted) given to subjects

	All qinghaosu compounds n = 337*	Qinghaosu compound alone n = 240	Qinghaosu compound + MFQ n = 97
Weight-adjusted total dose†			
Median (range)	168 (11, 2705)	130 (11, 2705)	237 (19, 2646)
0–200 mg/kg	188 (55.8%)	148 (61.7%)	40 (41.2%)
201–500	82 (24.3%)	47 (19.6%)	35 (36.1%)
501–1000	44 (13.1%)	29 (12.1%)	15 (15.5%)
>1000	23 (6.8%)	16 (6.7%)	7 (7.2%)

MFQ = mefloquine.
* 3 subjects without weight measured.
† The artesunate dose multiplied by 5.

Intensity of drug exposure. The qinghaosu compounds dispensed by the Medical Station were either artemisinin or artesunate, depending on availability. The median (range) number of treated episodes in individual patients over the previous two years was 2 (1–21) separate courses of artemisinin. Artemisinin is approximately five times less potent in terms of antimalarial activity compared with artesunate. The weight-adjusted (milligram per kilogram) dosing regime for each treatment course of artemisinin was therefore five times that for artesunate; artesunate dosage was multiplied by five in order to express all treatment in terms of artemisinin. The median (range) of treatment dose was 168 mg/kg (11–2,705). Overall, 20% of subjects received total doses of artemisinin (or the adjusted equivalent of artesunate) exceeding 500 mg/kg (Table 2 and Figure 2).

Neurological exam results. A detailed neurological examination was conducted in 375 (81.5%) of the study subjects. This total was limited by the ability to follow commands. There was one subject with nystagmus in the control group. Otherwise no abnormalities were found on neurological examination in any of the treatment groups or controls.

Of the 436 otoscopic exams 124 (28%) were found to have pus and/or perforation. Consistent with the natural history of otitis media, the rates of otitis as determined by pus in the external canal and at the tympanic membrane were much higher in younger children. Sixteen percent (15 of 92) in the 2 to 5 year age group, 14% (31 of 221) in the 6 to 15 year-old age group, and 2.4% in those over 16 years. As there were no controls in the youngest age group, comparisons cannot be made between rates of infection in control versus treatment groups in the very young. For children over 5 years of age, the rates of dry perforation were as follows: 10.6% (11/104) in controls, 17.9% (30/168) in the QHS group, 14.1% (9/64) of the QM group, and 12.5% (1/8) of the M group (P value = 0.43). The rates of otitis were 11.5% (12/104) of controls, 9.5% (16/168) of the QHS group, 7.8% (5/64) of the QM group, and 12.5% (1/8) of the M group (P = 0.87).

Audiometry results. There were no differences between the groups (Table 1A).

Auditory evoked potentials results. Analysis of the interpeak latencies (IPLs) was performed separately for each ear (Table 3). No differences in the IPLs of the left ear were observed between the qinghaosu compounds and the left ear of the controls. On univariate analysis, the IPLs I–V and I–III of the right ear were significantly longer for those patients in the QM group compared to the controls. Patients in the QHS group had a significantly longer I–V IPL of the right ear compared to right ear of the controls. There were no differences for the left ear.

As age is related to IPLs, and there were only two subjects less than 5 years of age in the control group (Table 1A), a

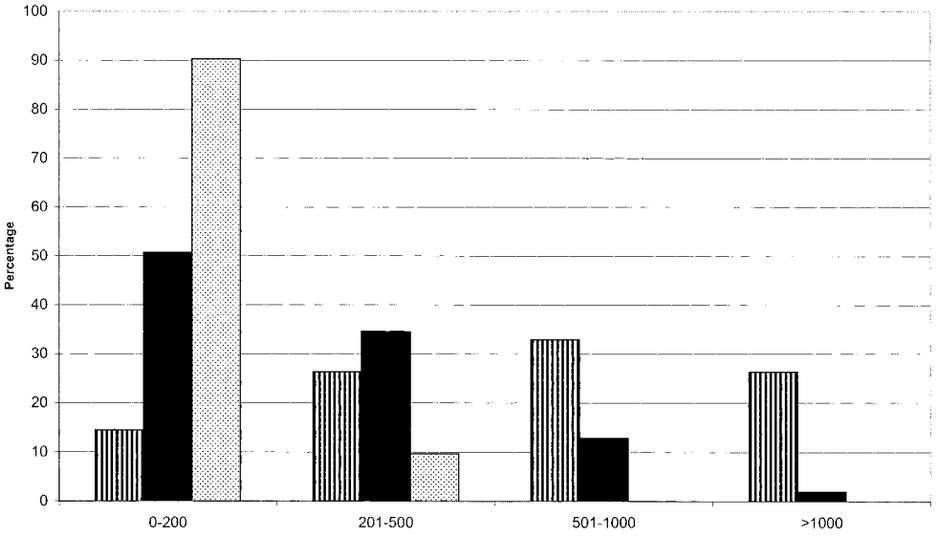

FIGURE 2. Percentage of subjects receiving a total dose of QHS of either < 200, 201–500, 501–1,000, or >1,000 mg/kg.

TABLE 3
Descriptives of inter-peak latencies (msec)

Inter-peak latencies*	Controls	All qinghaosu compounds	Qinghaosu compound alone	Qinghaosu compound + mefloquine
Right I–III	1.98 (0.15)	2.02 (0.17)	2.01 (0.17)	2.03 (0.17)
	1.66–2.44	1.52–2.60	1.56–2.52	1.52–2.60
		$P = 0.035$	$P = 0.075$	$P = 0.029$
Right I–V	3.74 (0.18)	3.81 (0.22)	3.81 (0.23)	3.81 (0.19)
	3.20–4.12	3.20–4.74	3.20–4.74	3.43–4.40
		$P = 0.006$	$P = 0.011$	$P = 0.011$
Right III–V	1.78 (0.17)	1.79 (0.17)	1.80 (0.18)	1.78 (0.14)
	1.28–2.28	1.40–2.78	1.40–2.78	1.44–2.32
		$P = 0.324$	$P = 0.254$	$P = 0.758$
Left I–III	2.00 (0.16)	2.02 (0.17)	2.02 (0.18)	2.04 (0.16)
	1.64–2.64	1.52–2.76	1.52–2.76	1.54–2.48
		$P = 0.289$	$P = 0.507$	$P = 0.108$
Left I–V	3.78 (0.20)	3.81 (0.21)	3.80 (0.21)	3.83 (0.22)
	3.40–4.24	3.16–4.72	3.16–4.72	3.32–4.56
		$P = 0.219$	$P = 0.364$	$P = 0.122$
Left III–V	1.78 (0.16)	1.79 (0.18)	1.80 (0.18)	1.78 (0.18)
	1.44–2.30	1.32–2.54	1.36–2.54	1.32–2.24
		$P = 0.690$	$P = 0.552$	$P = 0.900$

* mean (SD), range.
P-value for treatment group versus controls, unpaired *t*-test.

sub-analysis was performed in which IPLs of the treatment groups were compared with those of the control group in those study subjects greater than five years old (Table 4). This revealed very small but statistically significant prolongation for the right IPLs I–III (2.03 versus 1.98 ($P = 0.027$)) and I–V (3.82 versus 3.74 ($P = 0.007$)) in the QM group compared to the control group. In each case, the difference from controls was less than the accepted 0.1 msec measurement accuracy range of the equipment. Again, there were no differences for the left ear.

In those individuals less than 4 years of age (for whom there were no controls in the population studied), the IPLs were also compared with published normative age matched data (normal values for IPL I–V 1 years old < 5.01; 2 years old < 4.76; 3 years old < 4.75; for IPL III–V 1 years old < 2.9; and 2 years old < 2.688; 3 years old < 2.664). One patient had an IPL longer than 2.5 SD from the mean IPL for their age (2 years old IPL III–V 2.78). This child also had pus in the external auditory canal obstructing the tympanic membrane.

Throughout the analyses there were no differences between the right and left ear within the treated groups, the apparent prolongation of the right IPL I–III and I–V is due to the shorter IPL in the right ear of the controls (Table 3).

Effect of total drug exposure on IPL. A comparison was made of the effects on cumulative quantity of drug received (less than or equal to 500 mg/kg or $>$ 500 mg/kg of artemisinin or artesunate dose times five). There was no significant difference in the IPLs between these two groups except for IPLs I–III (2.07 ms in the greater than 500 mg/kg group versus 2.00 ms in the less than or equal to 500 mg/kg group [$P = 0.014$]) and I–V (3.89 msec versus 3.79 msec [$P = 0.014$]) of the right ear. However, because of the effect of age on IPL, a sub-analysis was undertaken (Table 5A and

TABLE 4
Descriptives of inter-peak latencies for subjects older than 5 years of age

Inter-peak latencies*	Controls n = 106	All qinghaosu compounds n = 241	Qinghaosu compound alone n = 174	Qinghaosu compound + mefloquine n = 67
Right I–III	1.98 (0.15)	2.00 (0.16)	1.99 (0.16)	2.03 (0.15)
	1.66–2.44	1.56–2.48	1.56–2.48	1.72–2.40
		$P = 0.180$	$P = 0.511$	$P = 0.027$
Right I–V	3.74 (0.18)	3.80 (0.21)	3.79 (0.22)	3.82 (0.20)
	3.20–4.12	3.20–4.64	3.20–4.64	3.43–4.40
		$P = 0.018$	$P = 0.069$	$P = 0.007$
Right III–V	1.78 (0.17)	1.80 (0.17)	1.81 (0.17)	1.80 (0.15)
	1.28–2.28	1.40–2.40	1.40–2.40	1.44–2.32
		$P = 0.178$	$P = 0.178$	$P = 0.420$
Left I–III	2.00 (0.17)	2.02 (0.17)	2.01 (0.17)	2.03 (0.16)
	1.64–2.64	1.52–2.44	1.52–2.44	1.54–2.44
		$P = 0.409$	$P = 0.551$	$P = 0.321$
Left I–V	3.78 (0.20)	3.81 (0.20)	3.80 (0.20)	3.84 (0.22)
	3.40–4.24	3.16–4.56	3.16–4.52	3.48–4.56
		$P = 0.225$	$P = 0.515$	$P = 0.063$
Left III–V	1.78 (0.16)	1.79 (0.17)	1.79 (0.17)	1.80 (0.18)
	1.44–2.30	1.36–2.44	1.36–2.44	1.48–2.24
		$P = 0.624$	$P = 0.699$	$P = 0.595$

* mean (SD), range.
P-value for treatment group versus controls, unpaired *t*-test.

TABLE 5A
Mean (SD) inter-peak latency (msec) for subjects in the artemisinin group ≤ 5 years of age

	Drug dosage		
	≤ 500 mg/kg n = 30	> 500 mg/kg n = 36	*P*-value
Right I–III	2.03 (0.18)	2.08 (0.18)	0.258
I–V	3.79 (0.19)	3.89 (0.29)	0.125
III–V	1.76 (0.15)	1.80 (0.24)	0.436
Left I–III	1.99 (0.17)	2.07 (0.20)	0.105
I–V	3.80 (0.20)	3.85 (0.27)	0.456
III–V	1.81 (0.22)	1.79 (0.18)	0.764

TABLE 5B
Mean (SD) inter-peak latency (msec) for subjects in the artemisinin group older than 5 years of age

	Drug dosage			
	Controls n = 106	≤ 500 mg/kg n = 165	> 500 mg/kg n = 8	*P*-value
Right I–III	1.98 (0.15)	1.99 (0.16)	2.01 (0.21)	0.782
I–V	3.74 (0.18)	3.79 (0.22)	3.88 (0.15)	0.113
III–V	1.78 (0.17)	1.80 (0.18)	1.85 (0.17)	0.308
Left I–III	2.00 (0.17)	2.01 (0.17)	2.07 (0.15)	0.627
I–V	3.78 (0.20)	3.80 (0.20)	3.79 (0.22)	0.789
III–V	1.78 (0.16)	1.80 (0.17)	1.73 (0.15)	0.476

5B). We compared the IPLs between those who received less than or equal to 500 mg/kg with those who were older than five years of age and received more than 500 mg/kg. The same comparison was made separately for those younger than or equal to five years old. In each case, when correcting for age, there were no significant differences in IPLs between the group receiving higher cumulative dosages and the group receiving lower cumulative dosages.

DISCUSSION

The artemisinin (qinghaosu) group of compounds are now the first-line antimalarial drugs in Southeast Asia, and they are being used increasingly in Africa and South America. Their use has increased because of their ease of drug delivery, lack of adverse effects, and rapid therapeutic responses, even against multidrug-resistant falciparum malaria.[14] The demonstration in experimental animals of a reproducible pattern of selective damage to the brain stem following large doses of artemisinin derivatives has been a major concern.[15] Neurotoxicity in mice, rats, dogs, and rhesus monkeys, has resulted from the administration of large intramuscular doses of arteether (AE), or artemether (AM)—two lipophilic derivatives of artemisinin. Following administration of 10–50 mg/kg/day AE for eight to twenty-eight days, a dose-dependent, region-specific pattern of damage was noted in the pons and medulla of beagle dogs and Sprague-Dawley rats.[9–11] In rhesus monkeys no pathological effects could be demonstrated after 7 days of treatment with arteether (8–24 mg/kg/day), but dose-dependent neuropathological lesions were noted in the reticular, vestibular, and auditory nuclei after 14 days of treatment. Foci of neurotoxicity were also found in the brainstem nuclei of the reticular formation, the vestibular system, and the auditory system (including the superior olivary nuclear complex and most consistently, the trapezoid nuclear complex).[11] A marked species variation was noted with a ranking of susceptibility of the nervous system in the examined species: dog > rat > rhesus monkey. In cell culture, toxicity has been demonstrated against neuronal cell types.[16,17] It has also been noted that considerably higher doses of oral artemether or arteether, or the water-soluble artemisinin derivatives given by any route, are required to produce neuropathological lesions. This suggests that the development of neurotoxicity with parenteral AE and AM results from the slow release of drug following intramuscular injection and thus sustained exposure of the central nervous system.

Khanh Phu village, the site for this study, is a remote community in a region of high malaria transmission with an entomological inoculation rate of 100 per year and sporozoite rates of 3–4%. The population may have had a higher cumulative dose of artemisinin or artesunate than any other group of people in the world. The subjects had received up to 21 separate treatment courses. This provided an unusual opportunity to assess possible neurotoxicity. The treated group was younger than the control group, but there were no other significant differences in demographic details, or in the results of otoscopic examination and audiometry. The clinical examination concentrated on the assessment of hearing, vestibular function, cerebellar, and control of voluntary movement, as these were considered *a priori* to be most likely affected by neurotoxicity. No significant differences were found between cases and controls.

Brain-stem auditory evoked potentials (BSAEPs) are a sensitive, non-invasive technique to assess the integrity of the auditory pathways from the cochlea to the mid-brain. The interpeak latencies (IPLS) are the least variable and most independent of subject, stimulus, and recording parameters compared with other measures derived from the BSAEP, and were therefore used throughout this study.[18,19] The accuracy of the recording system in this study was 0.1 ms; variation below this level cannot be assessed accurately. In this study, a very small but statistically significant differences in IPLS were found in recordings from the right side of subjects over four years old who had received artemisinin or artesunate compared to those who had not. No differences were found on the left side. The differences were below the accepted sensitivity of the recording device. As there is no reason to believe neurotoxicity should occur preferentially on one side of the brain, these findings are not considered pathologically significant.

In this study there were no untreated controls under 5 years of age and therefore, for those subjects less than 5 years old, IPL values were compared with published normative data.[18] Using a conservative definition of the upper limit of normal as 2.5 SD greater than the mean, only one subject in the treatment group had an IPL outside the accepted normal range for their age. This was a two-year old child with pus in the external auditory canal (EAC) such that the ear drums could not be visualized, and in whom the rest of the clinical neurological assessment was normal. While we cannot rule out absolutely a neuropathological process in this individual, severe inflammation in the unilateral EAC in a two year old would be an acceptable clinical AR cause of a prolonged IPL 1–V. The contralateral ear was

normal and had normal IPLs. All other subjects had IPL within the normal range for their age.

If artemisinin and its derivatives are neurotoxic with cumulative exposure, there should be some relationship between total dose and effect. There was no evidence for dose-dependency in IPLS. This provides further support for lack of neurotoxicity.

The artemisinin derivatives have been deployed mainly in China and Southeast Asia, where the main at-risk populations live in areas of low malaria transmission and infrequent infection. The situation in Khanh Phu is unusual in its transmission intensity, and thus the inhabitants of the village receive extensive courses of treatment with the artemisinin derivatives. This study aimed at careful neurological evaluation of the population exposed to multiple treatments with the artemisinin derivatives. As a field study in a small rural community it could not meet the rigorous criteria of a drug-safety study. However, the lack of any convincing clinical or neurophysiological evidence of brain-stem neurotoxicity, despite thorough examination in subjects receiving up to twenty-one treatment episodes in a two-year period, is reassuring.

It is possible that the combination of detailed clinical assessment plus brain-stem neurophysiology used in this study is not sensitive enough to detect covert neuropathology. The BSAEP measures neuronal activity from the acoustic nerve through the cochlear nucleus, the superior olivary complex, the lateral lemniscus, the inferior colliculus, and finally via the medial geniculate body to the primary auditory cortex. This pathway is directly adjacent to the structures damaged by arteether in male rhesus monkeys; the reticular formation (pontine nuclei, medullary gigantocellular nuclei), the vestibular system, and the auditory system (superior olivary and trapezoid nuclear complex).[11] These nuclei and their projections are intimately connected to the neuronal pathway assessed by the BSAEP. If the combined clinical and neurophysiological assessments conducted in this study are not sensitive enough to detect a potential lesion, then these defects would have to be very subtle indeed. This study provides further reassurance, in addition to the recent Cochrane Group meta-analysis,[6,7] that there is no evidence of neurotoxicity from studies in man that would lead one to restrict the use of appropriate antimalarial treatment regimens with these drugs. The safety of these drugs in continuous use (e.g., prophylaxis) has not been established and cannot be inferred from these data.

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