The Effect of Quinine on the Electroretinograms of Children with Pediatric Cerebral Malaria

Jonathan Lochhead,† Armand Movaffagh,* Benedetto Falsini,* Peter A. Winstanley,* Edward K. Mberu,* Charles E. Riva,* Malcolm E. Molyneux,* Terrie E. Taylor,† and Simon P. Harding†

1St. Paul’s Eye Unit, Royal Liverpool University Hospital, and 2Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, United Kingdom; 3Institut de Recherche en Ophtalmologie, Sion, Switzerland; 4Istituto di Oftalmologia, Università Cattolica del Sacro Cuore, Roma, Italy; 5Kenya Medical Research Institute Collaborative Project/Wellcome Research Laboratories, Nairobi; 6The Malaria Project, Queen Elizabeth Hospital, Blantyre, Malawi

To investigate the effects of quinine on the electroretinograms (ERGs) of children with cerebral malaria (CM), we recruited subjects during a single malaria season in Blantyre, Malawi. Seventy ERG investigations were performed, on 34 children with CM. Time recorded from completion of the most recent quinine infusion was termed “quinine elapsed time” (QET). In a subgroup of 16 children, whole-blood quinine concentrations were estimated in a sample of capillary blood, for validation. A significant positive association was found between QET and both maximal-response A-wave amplitude (MRAWA; \(P = .03\)) and cone A-wave amplitude (\(P = .04\)). Longitudinal analysis demonstrated a significant trend of increasing MRAWA with increasing QET (\(P = .03\)). Parenteral quinine administered in therapeutic doses to a pediatric population appears to cause a transient depression in photoreceptor function. No evidence of ocular quinine toxicity was found at the therapeutic doses used.

Overdose of quinine is known to be toxic to the eye. A number of case reports have described irreversible retinal and neurological abnormalities associated with very high levels of quinine in blood [1, 2]. Retinal electrophysiological changes include altered electroretinogram (ERG) and depressed electrooculogram (EOG), in addition to reduced or absent visual-evoked potential (VEP) [3]. However, there are no published reports of the effects of quinine, at therapeutic doses, on the eye.

Quinine is the current treatment of choice for Plasmodium falciparum malaria, which includes cerebral malaria (CM) [4]. A typical regimen of quinine treatment comprises an initial intravenous loading dose of 20 mg/kg, followed by infusions of 10 mg/kg every 12 h until oral treatment is possible [5]. As part of an ongoing program to study the eyes in African children with CM [6, 7], we recorded the ERGs of children who had been clinically diagnosed with CM, and we investigated the ERGs’ relationship to quinine-exposure levels.

Subjects and methods. Between January 1998 and April 1998, all children who had been admitted to the Malaria Project pediatric unit at the Queen Elizabeth Central Hospital in Blantyre, Malawi, and had been diagnosed with CM were assessed for inclusion in this study. CM was defined as the inability to localize pain (sternal pressure), in the presence of asexual P. falciparum parasitemia, in a child whose coma could not be explained by hypoglycemia, convulsions, sedatives, or other encephalopathies [5]. All children were examined for the presence of CM-associated retinopathy, which consists of retinal hemorrhages (frequently with white centers), vessel delineation, and retinal whitening (elsewhere described as “cotton-wool spots”) (figure 1) [6, 7]. When present, the different components of retinopathy were recorded and quantified using a specifically developed classification system [8, 9]. All urgent medical interventions (correction of hypoglycemia, severe anemia and hypovolemia, and commencement of quinine infusion) were performed before any electrophysiological investigation.

ERG recordings were performed by use of a miniganzfeld stimulator (LACE Eletronica), which can provide, at a predetermined luminance and flicker frequency, a uniform light stimulus to the entire retina, thus stimulating only selected retinal-cell populations. This stimulator was connected to a computer fitted with an amplification and signal-acquisition system. Software was designed for the study (LACE Eletronica), and calibration followed International Society for Clinical Electrophysiology of Vision standards. A standard flash of 2 candelas/m²/s was used.

The left eye of each subject was studied. Subjects underwent an initial period of 15 min of darkness adaptation, by use of an eye patch, which was followed by a period of 5 min within
Figure 1. Cerebral malaria retinopathy that consists of retinal hemorrhages (frequently with white centers), retinal whitening, and vascular delineation.

a darkened room. The eye being studied was anesthetized with 0.5% oxybuprocaine hydrochloride, and a corneal electrode was inserted (JET). Recordings were referenced to a skin electrode that was positioned at the temple and grounded at the glabella. Darkness-adaptation recordings were made by use of a scotopic dim-flash rod response, calibrated at 2.5 log units of attenuated standard flash, followed by a maximal response elicited by use of the standard flash. After 5 min of light adaptation at 20 candelas/m²/s, a photopic cone response was recorded by use of the standard flash followed by a 30-Hz flicker response. Bright-flash VEP was recorded from a skin electrode that was positioned 3 cm above the inion, and recordings were referenced to a second skin electrode that was positioned at the mastoid. Oscillatory potentials were derived from the maximal-response B-wave (MRBW) by use of LACE software.

The waveform analysis was performed by 2 observers (J.L. and A.M.) using a consensus protocol, with independent sample validation performed by a third observer (B.F.). Missing or poor data points (defined as having excessive artifact or noise, which renders the waveforms unrecognizable) were excluded from further analysis.

Quinine-therapy data were measured as the time elapsed since cessation of the most recent infusion, which was termed “quinine elapsed time” (QET). QET was measured from the end of an infusion, a point at which it could be assumed that whole-blood concentration had reached a peak and that subsequent decay of blood levels might follow a predictable course.

To minimize unnecessary sampling of blood from these critically ill children, we studied the QET. However, because of the indirect nature of this measure, we investigated the correlation between QET and whole-blood quinine concentration (QN), in a small subgroup of patients who had been selected according to clinical status. One hundred microliters of capillary blood was collected via finger prick, after completion of infusion, and was air-dried on filter paper. QN was measured by use of high-performance liquid chromatography [10].

Cross-sectional analysis of data was performed by use of a correlation matrix and multiple regression analysis. ERG variables consisted of amplitudes and implicit times of the maximal-response cone A waves and maximal-response cone B waves, 30-Hz flicker, and VEP P100. Other variables that were tested included age, QN, QET, and presence of retinopathy.

Results. Of the 34 children diagnosed with CM who were recruited into the study, 22 had malaria retinopathy. Seventy ERG investigations were performed for cross-sectional and longitudinal analysis. For 10 patients, 2 complete data sets recorded at different QET intervals were completed. Twenty-one blood samples were collected for QN measurement, from 16 children.

QNIs were in keeping with published data [5]. Linear regression analysis of QNIs against QETs revealed a significant correlation ($R^2 = 0.714; P < .001$). With a higher QET value, this relationship was skewed by 3 points; with removal of these points, the relationship lost significance ($P = .4$). The most complete data set (after exclusion of poor data points) was selected for each of the 34 cases. Analysis by correlation matrix revealed the following statistically significant correlations: increasing QET with increasing maximal-response A-wave amplitude (MRAWA) ($P = .03$) and increasing QET with increasing cone A-wave amplitude, at 10 ms ($P = .04$). QET and
maximal cone A-wave amplitude showed a similar nonsignificant positive trend association ($P = .07$).

Stepwise multiple regression analysis demonstrated a significant positive correlation between QET (predictor variable) and MRAWA (dependant variable), independent of either age or presence of retinopathy ($n = 34; R^2 = 0.19; P = .03$). To provide further confirmation of the findings in the larger QET data set, linear regression analysis of QN data was also performed. A similar correlation between MRWA and QET was found ($figure 2; P = .002$). This relationship was skewed, however, and it became less significant with removal of 2 points with a higher QET ($P = .02$).

A total of 10 children had multiple QET measurements, and 5 had >1 QN measurement. The first and last of these examinations were used for longitudinal analysis. The mean QET was 6.5 h for the initial ERG and 25.6 h for the final ERG. The initial ERG examinations provided a mean (95% confidence interval [CI]) MRAWA of 146.8 (±1.6) μv, and the final ERG provided a mean (95% CI) MRAWA of 190.2 (±1.0) μv. This trend of increasing MRAWA with increasing QET was found to be statistically significant ($P = .03$). A similar trend was seen when QN was plotted against MRAWA ($n = 5$). At the earliest of the investigations, the mean (95% CI) MRAWA was 179.4 (±2.4) μv (mean QET, 4.2 h; mean QN, 7.5 mg/L). In contrast, at the latest of the investigations, the mean (95% CI) MRAWA was 212.6 (±1.9) μv (mean QET, 8.7 h; mean QN, 5.7 mg/L). This trend of increasing MRAWA with decreasing QN was found to be approaching significance, despite the very small numbers ($P = .06$). No significant correlations were found between either QET or QN and any other ERG parameters.

**Discussion.** Our study shows for the first time a transient effect of quinine, at therapeutic doses, on retinal electrophysiology. The most significant effect appears to be a depression of the MRAWA, with a similar trend indicated for cone A-wave function. The A-wave of the ERG is attributed to retinal photoreceptors; our findings therefore suggest a preferential effect at that level. In contrast, our study did not detect an effect of quinine on other ERG parameters, a finding that suggests little or no effect on retinal bipolar cells.

The bulk of previous literature concerned with the effect of quinine on the eye relates to toxicity, which typically occurs after deliberate overdose, with total quinine concentrations in plasma that are >10 mg/L$^{-1}$ [11]. Fundoscopic changes that have been reported to occur early after overdose include retinal edema, macular cherry-red spot, and dilatation of retinal vessels, which are followed by vessel attenuation, disk pallor, and atrophy [1, 2]. Reduced or absent responses to all electrophysiological stimuli have been reported, with the least effect on responses from photoreceptors and the greatest effect on postsynaptic cells and pathways [1, 2, 12, 13]. One study, which used a cat model of quinine overdose, reported that doses of 104 mg/kg resulted in dose-dependent toxicity with recovery after 4 days but that higher doses resulted in permanent blindness [1]. Also, electrophysiological and histopathologic evidence indicated a predominant effect on ganglion and bipolar cells, with photoreceptors being affected to a lesser extent. The absence of retinal ischemia indicated a direct cellular toxicity. This evidence of post-photoreceptor involvement in large overdose is in contrast with our chief finding, which is the identification of a photoreceptor effect at therapeutic doses. In 2 of the overdose studies [12, 13], an abnormal EOG occurred in the acute phase, which indicates loss of function at the photoreceptor/RPE level (a loss that, in those studies, was reversible). Because of the need for voluntary eye movements, it was not possible for us to perform EOG examinations of our study population, but the results reported by these other studies are at least indirectly compatible with our observations that support a greater but more transient susceptibility of deeper retinal layers to quinine.

Retinal edema is a typical feature of overdose and has been attributed to cloudy swelling of the inner retina [2]. An anticholinergic action of quinine has been proposed as a possible mechanism for disruption of neurotransmission in the inner retinal layer [12]. This theory has led to concern that doses of quinine used in the treatment of children with CM could be responsible for the retinal whitening seen in severe cases. Detailed fundoscopy was performed as part of our investigation, and the clinical retinal features of retinopathy, including retinal whitening, were often present before administration of intravenous quinine and were also seen in children treated with alternative therapies.

Our patients’ QN levels were within the currently accepted therapeutic range [5]. Our findings suggest that quinine ad-
ministered at the doses that we prescribed is unlikely to produce retinopathy or neurological deficit. The majority of visual defects that follow CM are accompanied by other neurological sequelae, and these defects appear to indicate a form of cortical damage, rather than retinal toxicity [14].

CM is a multifactorial disease with several abnormalities of physiology, such as hypoglycemia and convulsions, that can influence electrophysiological findings. Known potential confounding factors were corrected on all cases before any ERG examinations were performed. In our ongoing studies of the eyes in children with CM, we have found an association between the presence of retinopathy and the dysfunction of the retinal bipolar cells that are located in the inner retinal layers (authors’ unpublished data). No such associations were found between the presence of retinopathy and any ERG A-wave functions; therefore, the changes related to CM retinopathy and quinine appear to be distinct.

It was not possible to perform control examinations in this population. ERG recordings were possible only after initial clinical resuscitation was complete and quinine treatment had commenced and, in those children who recovered, only for as long as coma persisted.

For reasons already mentioned, we decided prospectively to use QET as a surrogate marker for quinine activity. We have demonstrated a correlation between QET and transient retinal photoreceptor dysfunction.QN correlations indicated a trend that, in most cases, was not found to be significant. It is possible that whole-blood QN may not adequately represent tissue bioavailability in the retina. In addition, malaria induces, especially in children, a rise in α-1 acid glycoprotein, to which quinine binds avidly [15]. As a result, unbound QNs are likely to be considerably lower in patients with more severe cases of malaria. Further studies are required to clarify which pharmacokinetic measure of quinine activity is most closely correlated with ERG variables.

This study comprises the first set of ERG observations recorded in a human population that has received quinine at therapeutic doses. Our findings appear to indicate that therapeutic doses of intravenous quinine in children are associated with a significant depression of photoreceptor function. The transient response of this effect suggests that toxicity at these doses is unlikely.

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References