Pharmacokinetics of retinyl palmitate and retinol after intramuscular retinyl palmitate administration in severe malaria

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ABSTRACT

Retinol (vitamin A alcohol) is an accepted adjunctive treatment in infections such as measles. There is also indirect evidence from in vitro, animal and human studies that retinol supplementation may be beneficial in severe malaria. There have, however, been no studies that have examined the pharmacokinetics of acute retinol supplementation in severe illness. To establish whether mobilization of intramuscular retinyl palmitate (RP) and its availability as retinol are adequate in complicated falciparum malaria, we administered a single dose of 400 000 i.u. of RP to six Vietnamese adults with severe malaria. Another 28 patients were not given RP. All patients had blood samples taken over 96 h for RP and retinol assay using HPLC, and received conventional anti-malarial and supportive therapy. Admission serum retinol concentrations were below the lower limit of the reference range (< 1.0 μmol/l) in 74% of the 34 patients. In supplemented patients, analysis of serum RP between 0 and 96 h using a multi-compartmental model revealed a median (range) delay in mobilization of 6.9 h (0.7–15.1 h), a bioavailability of 55% (19–100%) and an elimination half-life of 13.5 h (4.2–23.7 h). The area under the serum retinol curve expressed as an absolute or percentage change from baseline was greater in supplemented than in unsupplemented patients (P < 0.05). The separation in median serum retinol concentrations in the two groups was maximal at 48 h. The model-derived retinol half-life [1.5 (0.7–15.8) h] suggested rapid uptake, metabolism and/or excretion. In conclusion, there is variable RP bioavailability in severe malaria, but a significant if delayed increase in serum retinol over that associated with recovery from the infection. In severe infections, RP supplementation appears simple, well tolerated and of potential benefit once anti-microbial and supportive therapy have been established.

INTRODUCTION

Most studies of retinol supplementation suggest that it has beneficial effects on morbidity and mortality associated with infections [1–4]. The apparently discrepant results in some other reports [5–8] may relate, in part, to whether adjunctive retinol is given prophylactically or in the acute phase of the illness. Although supplementary retinol may act to inhibit the spread of infection rather than contributing to initial defensive barriers [2,9], there is a decrease in levels of serum retinol and its binding proteins in acutely ill patients even with normal prior vitamin A status [10]. Thus prophylactic retinol may not be as effective as retinol given during acute illness.

Although restoration of tissue retinol stores may assist...
in the acute response to infection, retinol kinetics after supplementation in this situation are unknown. Since absorption of oral formulations and mobilization of oil-based parenteral supplements can be unreliable, especially in vomiting patients and those with reduced splanchnic blood flow, intramuscular injection of 50000–400000 i.u. of water-miscible retinyl palmitate (RP) has been used in a number of studies [3,11]. Esters such as RP are taken up by the liver for storage or dé-esterification and release into the circulation as retinol bound to retinol-binding protein (RBP). Retinol–RBP is, in turn, reversibly complexed to transthyretin and transported to target tissues. In a study in healthy volunteers [12], intramuscular RP had a bioavailability approaching 50%, a half-life ($t_{1/2}$) of 12 h, and reached peak plasma concentrations after 12–24 h. Its effect on plasma retinol per se was not measured [12], perhaps because normal retinol levels are likely to have been maintained homoeostatically despite adjunctive RP.

In severe infections, hepatic dysfunction might impair RP uptake, its conversion into retinol, the synthesis of RBP and transthyretin, and the release of retinol-RBP [9,10,13]. This would limit the efficacy of RP supplementation, at least in the acute phase when it may be most needed. However, in renal impairment, recycling of complexed retinol and reduced transthyretin metabolism may keep serum retinol near physiological levels [14,15], while urinary retinol losses are reduced in oliguria [16]. These considerations suggest that the efficacy of adjunctive retinol depends on hepatic and renal function, a further consideration when differences in outcome between intervention studies are assessed.

Falciparum malaria is an acute illness that can be complicated by hepatic and renal dysfunction [17]. Depressed serum retinol concentrations occur in malaria in an inverse relationship with parasitemia and severity [18–21]. There is also in vitro evidence that retinol inhibits the growth of *Plasmodium falciparum* at actual media concentrations close to physiological [22]. Retinol supplementation may thus have direct and indirect beneficial effects in severe malaria. We investigated the disposition of RP and retinol after a single intramuscular injection of RP in severely ill adults with falciparum malaria, and compared the changes in serum retinol with those in patients with severe malaria who did not receive RP supplementation.

**METHODS**

**Patients**

We studied 34 Vietnamese adults with severe falciparum malaria [17] who were transferred to Cho Ray Hospital, Ho Chi Minh City, or Lamdong Hospital, Dalat City, for management of complications. Of these, 28 (82%) had received prior anti-malarial therapy, and 24 (71%) had cerebral malaria (Glasgow Coma Score (GCS) < 11). Other major complications were renal impairment (serum creatinine > 250 μmol/l after rehydration) in nine cases (27%), jaundice (serum bilirubin > 50 μmol/l plus serum transaminase more than twice normal) in 13 cases (38%), and hyperparasitaemia (parasite density > 250000/μl) in seven cases (21%). Each patient, or a first-degree relative in the case of comatose patients, gave informed consent to study procedures, which were approved by the Ministry of Health, Hanoi.

**Clinical procedures**

After full initial clinical assessment, venous blood was taken for routine laboratory tests and a parasite count. A further blood sample was drawn for assay of baseline serum retinol, RP, vitamin E and total cholesterol. Rehydration, resuscitation and parenteral therapy with quinine or artesunate were started promptly, as was monitoring of vital signs, fluid balance, conscious level and blood glucose. Parasite counts were taken at least twice daily. Complications were managed as described previously [17].

In 28 patients (Group 1), morning blood samples were taken daily until at least day 4 (96 h). As soon as these patients could be fed by mouth, intravenous fluids were stopped and a normal diet started. No vitamin supplementation was given. Group 1 included 24 patients whose serial serum retinol concentrations have been reported previously [20]. In six other patients (Group 2), 400000 i.u. of water-miscible RP (Aravit®; Roche, Basel, Switzerland) was given as a single intramuscular injection into the anterior thigh immediately after the baseline blood sample was taken (0 h). Group 2 patients were unselected, but had no evidence of coagulopathy. For these patients also, intravenous fluids were stopped and a normal diet started as soon as their condition allowed. They received no vitamin supplements other than RP, and were managed in the same way as the Group 1 subjects. Further blood samples were taken from Group 2 patients at 1, 2, 4, 8, 12, 18, 24, 36, 48, 72 and 96 h. Each patient was assessed daily, and gastrointestinal, neurological and dermatological symptoms and signs were recorded on standard forms.

**Sampling and assay procedures**

All blood samples for vitamin assays were protected from light, kept on ice and centrifuged within 1 h. Separated sera were stored in light-proof tubes at $< -20 ^\circ C$ and transported on solid CO$_2$ before assay. RP, retinol and vitamin E were measured in deproteinized, hexane-extracted sera using HPLC with $\gamma$-tocopherol acetate as internal standard [20,23]. Inter-assay precision was $< 9.0\%$ for retinol, $\leq 6.0\%$ for RP and $< 9.2\%$ for vitamin E. Assay detection limits were 0.1 μmol/l for retinol and RP, and 2.0 μmol/l for vitamin E. Other
biochemical tests were performed using Chem 1 (Bayer Diagnostics, Tarrytown, NY, U.S.A.) or Cobas Mira (Roche) automated analysers.

Kinetic analysis
A linear multi-compartment model (Figure 1) was developed to describe serum RP and retinol concentration profiles following intramuscular RP. More complex models have used radioactive tracers [24], but our approach was constrained by the study protocol and context. The SAAM II program (SAAM Institute, Seattle, WA, U.S.A.) was used for model development and fitting. All rate constants were assumed to be first order. Under the model, RP is introduced as a bolus into compartment 1, the injection site. From compartment 1, RP is either transported to serum or lost [k(1,0)]. Two pathways from compartment 1 to the serum RP pool (compartment 2) were required to adequately describe the data. A proportion of RP is transported directly from compartment 1, while the balance is transported more slowly through compartment 3, which comprises a series of five compartments with equal but adjustable residence times. All serum RP was assumed to be taken up into compartment 4. This compartment may represent the liver, where RP is hydrolysed and released into serum (compartment 5) bound to RBP. Serum retinol kinetics were described by the single compartment 2 with first-order kinetics. To account for the initial serum retinol concentration, a constant input of retinol independent of that derived from administered RP was required. The input was a product of the initial retinol concentration and the turnover of the serum retinol pool.

The model is uniquely identifiable with a priori one solution for all transfer constants. Identifiability analysis was performed using the Globi2 program [25]. The model was fitted to individual data sets with weighting based on assay precision for retinol and RP (see above). In some subjects the data were only partially informative, resulting in large errors in model-derived parameters. To overcome this problem, we used population kinetic analysis to determine the model parameters for individual subjects. We used the Iterative Two Stage method [26], based on the population mean at each iteration as a priori for improving individual parameter estimates.

Statistical analysis
Because there were small numbers in Group 2, and because not all variables conformed to a normal distribution by the Kolmogorov–Smirnov test, statistical analysis was by non-parametric methods (SPSS; SPSS Inc., Chicago, IL, U.S.A.). Area under the curve (AUC) was calculated using the trapezoidal rule. A two-tailed level of significance was used. Unless otherwise stated, summarized data are given as median (range).

RESULTS

Clinical course
Baseline patient characteristics are shown in Table 1. Age, gender, body mass index, haematocrit and hepatorenal function were similar in the two groups (P > 0.1 in each
Table 1 Admission details of patients in Group 1 (who received no supplementation) and Group 2 (who were given a single intramuscular dose of RP)

Data are median (range). Significance of differences: *P < 0.05, **P < 0.01 compared with Group 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
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<tr>
<td>Number</td>
<td>28</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 (18–63)</td>
<td>26 (20–37)</td>
</tr>
<tr>
<td>Male/female</td>
<td>23/5</td>
<td>5/1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>18.3 (15.6–21.9)</td>
<td>19.0 (15.4–22.0)</td>
</tr>
<tr>
<td>GCS</td>
<td>9 (4–15)</td>
<td>15 (10–15)</td>
</tr>
<tr>
<td>Venous haematocrit (%)</td>
<td>31.5 (9.0–45.0)</td>
<td>30.7 (18.0–45.0)</td>
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<td>Parasite density (no./l)</td>
<td>26500 (20–97000)</td>
<td>200 (20–11300)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>152 (64–1013)</td>
<td>312 (102–765)</td>
</tr>
<tr>
<td>Serum bilirubin (µmol/l)</td>
<td>46 (4–316)</td>
<td>23 (7–351)</td>
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</table>

Table 2 Baseline and 96-h serum retinol, vitamin E and cholesterol levels and vitamin E/cholesterol ratios in the two groups of subjects

AUC values, expressed as both absolute and percentage changes from baseline, are also shown. Data are median (range). Significance of differences: *P < 0.05, **P < 0.01 compared with Group 2.

<table>
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<td></td>
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<tr>
<td>Number</td>
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<td>6</td>
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<tr>
<td>Serum retinol (µmol/l)</td>
<td>0.69 (0.12–1.84)*</td>
<td>1.50 (0.60–3.00)</td>
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<tr>
<td>Serum vitamin E (µmol/l)</td>
<td>6.5 (&lt; 2.0–22.7)</td>
<td>4.5 (&lt; 2.0–14.0)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>2.4 (1.1–4.0)</td>
<td>3.1 (1.6–4.1)</td>
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<tr>
<td>10⁻³ × Vitamin E/cholesterol ratio</td>
<td>3.3 (0.3–7.7)</td>
<td>1.5 (0.6–4.5)</td>
</tr>
<tr>
<td>96 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Serum retinol (µmol/l)</td>
<td>1.21 (0.35–3.82)**</td>
<td>3.00 (1.80–6.60)</td>
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<tr>
<td>Serum vitamin E (µmol/l)</td>
<td>14.5 (&lt; 2.0–31.8)</td>
<td>8.5 (&lt; 2.0–16.0)</td>
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<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>3.2 (2.1–3.6)</td>
<td>3.2 (2.5–3.7)</td>
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<tr>
<td>10⁻³ × Vitamin E/cholesterol ratio</td>
<td>5.7 (3.8–6.7)**</td>
<td>3.0 (0.5–5.6)</td>
</tr>
<tr>
<td>AUC (change from baseline; µmol/l⁻¹·h)</td>
<td>19.7 (--30.5 to 128.4)*</td>
<td>120.6 (39.6–145.6)</td>
</tr>
<tr>
<td>AUC (change from baseline; %/h)</td>
<td>3132 (--3266 to 17462)*</td>
<td>5760 (2400–19000)</td>
</tr>
</tbody>
</table>

Figure 2 Serum retinol concentrations during the first 96 h of treatment for severe malaria in patients who received intramuscular RP (Group 2; ○) and in those who did not (Group 1; ●)

Data are group medians (circles) and ranges (vertical bars). The shaded area represents the range for healthy Vietnamese adults. Numbers of patients are shown in parentheses for time points at which this was less than the group total. Significance of differences: *P < 0.05, **P < 0.01 compared with Group 1.

Serum retinol

In the total series, 25 out of 34 patients (74%) had an admission serum retinol below the range (mean ±2SD) for healthy Vietnamese adults (1.04–2.68 µmol/l) [20]. The six females had significantly lower serum retinol concentrations than the males (median (range), 0.41 (0.15–0.60) µmol/l and 0.79 (0.12–3.00) µmol/l respectively; P = 0.0008). There was no association between baseline serum retinol and age (r_s = 0.23, P = 0.19), parasitaemia (r_s = −0.21, P = 0.95), serum bilirubin...
Retinyl palmitate kinetics in severe malaria

Figure 3 Changes in serum retinol from baseline in Group 1 (●) and Group 2 (○) patients
The upper panel shows absolute changes, and the lower panel shows percentage changes. Data are medians and ranges. Numbers of patients are shown in parentheses when this was less than the group total. Significance of differences: *$P < 0.05$, **$P < 0.01$ compared with Group 1.

$(r_s = -0.17, P = 0.34)$ or serum creatinine $(r_s = 0.30, P = 0.09)$.

The baseline serum retinol concentrations in Group 1 patients were lower than those in Group 2 patients $(P = 0.03$; Table 2). Group 96-h serum retinol profiles are shown in Figure 2. The 96-h serum retinol concentration in Group 1 patients remained significantly lower than that in Group 2 patients $(P < 0.05$ by Friedman test; Table 2). To allow for the different baseline serum retinol concentrations in the two groups, AUC values for changes in serum retinol from baseline over the period 0–96 h were calculated in absolute ($\mu$mol/l) and percentage terms (Table 2, Figure 3). In both cases, the AUC values were significantly lower in Group 1 compared with Group 2 patients $(P < 0.05)$. In Group 1 patients, a significant inverse association was observed between baseline serum retinol and the percentage increase over 96 h $(r_s = -0.482, P = 0.023)$, but there was no such relationship in Group 2 patients $(P = 0.33)$. Serum retinol concentrations were available from daily sampling in surviving Group 1 patients who were still in hospital on days 5 (120 h; $n = 18$) and 6 (144 h; $n = 13$). The median (range) serum retinol concentrations were 1.30 (0.33–4.18) $\mu$mol/l and 1.69 (0.56–3.97) $\mu$mol/l respectively at these times, with 80% and 85% of values respectively within the reference range (see Figure 2).

Pharmacokinetic analysis
RP could not be detected in any serum sample from four representative Group 1 patients over 96 h. In Group 2, all six patients showed an increase in serum RP after intramuscular injection, with a peak between 24 and 72 h (Figure 4). Pharmacokinetic analysis revealed a median delay of 7 h for the slow component of RP mobilization from compartment 3. There was a median bioavailability of over 50% (Table 3). The estimated median $t_{1/2}$ was just over 13 h for RP and 1.5 h for retinol. A graph showing serum retinol and RP data for a representative patient, together with fitted curves, is shown in Figure 5.

Figure 4 Serum RP concentrations in Group 2 patients
Data are medians and ranges.

Table 3 Pharmacokinetic parameters describing RP and retinol disposition in six patients with severe malaria who received a single intramuscular injection of 400 000 i.u. of RP
For details of the pharmacokinetic model used, see the text. Definitions: $k(i,j)$, fractional transport rate from compartment $i$ to compartment $j$ per unit time; delay, mean transit time for RP passing from compartment 1 to compartment 2 via the delay compartment 3; bioavailability, fraction of RP administered that enters the systemic circulation; $t_{1/2}$, time taken for concentration to fall by one-half.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k(1,0)$ (h$^{-1}$)</td>
<td>0.010</td>
<td>0.001–0.022</td>
</tr>
<tr>
<td>$k(4,0)$ (h$^{-1}$)</td>
<td>0.481</td>
<td>0.044–1.048</td>
</tr>
<tr>
<td>$k(1,2)$ (h$^{-1}$)</td>
<td>0.001</td>
<td>0.001–0.005</td>
</tr>
<tr>
<td>$k(3,2)$ (h$^{-1}$)</td>
<td>0.746</td>
<td>0.331–6.757</td>
</tr>
<tr>
<td>$k(1,3)$ (h$^{-1}$)</td>
<td>0.013</td>
<td>0.002–0.025</td>
</tr>
<tr>
<td>$k(5,4)$ (h$^{-1}$)</td>
<td>0.011</td>
<td>0.004–0.014</td>
</tr>
<tr>
<td>$k(2,4)$ (h$^{-1}$)</td>
<td>0.056</td>
<td>0.029–0.166</td>
</tr>
<tr>
<td>Delay (h)</td>
<td>6.9</td>
<td>0.7–15.1</td>
</tr>
<tr>
<td>RP $t_{1/2}$ (h)</td>
<td>13.5</td>
<td>4.2–22.7</td>
</tr>
<tr>
<td>Bioavailability of RP (%)</td>
<td>55</td>
<td>19–100</td>
</tr>
<tr>
<td>Retinol $t_{1/2}$ (h)</td>
<td>1.5</td>
<td>0.7–15.8</td>
</tr>
</tbody>
</table>
Figure 5 Serum retinol (○) and RP (●) concentrations in a representative Group 1 patient
The model fits for both retinol (broken line) and RP (solid line) are also shown.

Figure 6 Serum vitamin E concentrations in Group 1 (●) and Group 2 (○) patients
Data are group medians and ranges. The shaded area represents the range for healthy Vietnamese adults. Numbers of patients are shown in parentheses when this was less than the group total.

**Effect of RP supplementation on vitamin E metabolism**

Of the 34 patients studied, 22 (65%) had a baseline serum vitamin E level below the reference range (9.9–28.7 μmol/l) [20]. When vitamin E levels were corrected for the serum cholesterol in the form of a ratio [27], 44% had subnormal values [reference range (2.6–5.8) × 10⁻³] [20]. Baseline serum vitamin E concentrations were similar in the two groups, whether expressed in μmol/l or as a ratio (P = 0.44 and 0.11 respectively; Table 2). The serum vitamin E concentration increased more in Group 1 than in Group 2 patients over 96 h (Figure 6). The median 96-h concentration in Group 1 was close to double that in Group 2 (P = 0.06), and there was also a significantly greater 96-h vitamin E/cholesterol ratio (P = 0.009; Table 2).

**DISCUSSION**

The present study is the first to examine the pharmacokinetics of retinol after acute parenteral supplementation in human infection. We aimed to establish whether, in severely ill patients with well characterized falciparum malaria, there would be significant bioavailability of intramuscular RP and whether, through hepatic uptake, hydrolysis and release into the circulation, the absorbed RP would be made available to the tissues as retinol. Our results confirm an adequate, albeit variable, bioavailability of RP and a significant, if delayed, increase in serum retinol over and above that associated with recovery from the infection. The half-life of retinol was short, suggesting that it is quickly taken up by the tissues, metabolized and/or excreted.

Although a limited number of our patients received RP, they were previously healthy young adults who were demographically and anthropometrically similar to the unsupplemented patients. All Group 2 patients exhibited a rise and fall in serum RP after intramuscular injection, despite the severity of their illness, while serum RP remained undetectable in Group 1 subjects. The use of a large control group was important for the modelling and interpretation of changes in serum retinol per se. We have shown previously that the mean serum retinol concentration rises in unsupplemented severely ill patients to reach the bottom of the reference range after 96 h of treatment [20]. As this rise occurs as serum transaminase concentrations are falling and in the presence of persistently depressed serum carotenes [20], it relates primarily to improving hepatic function rather than to dietary factors. Nevertheless, patients in the two groups were managed clinically and nutritionally in the same way, to minimize potential bias due to the unblinded nature of the study.

In the only other published study of kinetics following intramuscular RP, Hartmann et al. [12] gave a single dose of 100000 i.u. to seven volunteers. Bioavailability ranged from 32 to 52% (mean 42%), and the elimination tₜ was between 6 and 16 h (mean 12 h). These data are comparable with those in the present study, even if there was a relatively wide scatter of values in our patients (Table 3). The main difference between our data and those of Hartmann et al. [12] was in the delay in mobilization of the RP from its intramuscular depot. Hartmann et al. [12] modelled this using a Weibull-type function, and so comparison of their parameter ‘Tₜ’ (the time to reach half the lower limit of bioavailability) with our delay constant is problematic. Nevertheless, serum RP peaked between 12 and 20 h in their series, whereas the peak occurred later in our patients (between 24 and 72 h). As RP is transported in non-chylomicron as well as chylomicron lipoprotein fractions, it is possible that this difference reflects the depressed serum cholesterol concentrations in our severely ill patients. This relationship requires further study.

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Assessment of the change in serum retinol itself after RP administration had to take into account the rise in serum retinol exhibited in unsupplemented patients [20]. Nevertheless, there was good evidence that RP increased circulating retinol levels in our Group 2 patients, even though they started from a higher baseline than those in Group 1. AUC values for the change in serum retinol from baseline in both absolute and percentage terms were significantly greater in Group 2 patients. The maximal serum retinol achieved in Group 2 patients exceeded the upper limit of the reference range (\(> 2.7 \, \text{mol/l}\)) in five out of six cases (83\%) during the first 96 h, whereas in only two of the larger Group 1 series (7\%) was there such an ‘overshoot’. Consistent with this observation, Group 1 patients with the lowest baseline serum retinol concentrations had the greatest proportionate increase over 96 h, whereas there was no such correlation in Group 2 patients. Maximal separation of group serum retinol curves occurred at \(> 48\) h, paralleling RP kinetics in supplemented patients (Figures 2 and 4).

Although our patients were unselected, the significant between-group differences in serum retinol responses might reflect, in part, the fact that Group 1 patients were more severely ill than those in Group 2, as indicated by lower GCS scores, higher peripheral parasitaemias and lower baseline serum retinol concentrations \(\text{per se}\). We were, however, primarily concerned that the groups were well matched for hepatic and renal function, factors that could alter retinol metabolism during treatment. This matching was achieved. We included serial measurements of vitamin E and cholesterol as other metabolic markers of clinical status. Serum vitamin E and cholesterol concentrations were similar in the two groups at baseline. In addition, fever and parasite clearance times, and days in hospital, the major prospective indices of severity of illness, were similar in the two groups. A variety of other surrogate markers of severity at presentation and during treatment were also comparable, including pre-admission duration of fever, duration of intravenous therapy, and requirement for blood transfusion and other adjunctive treatments such as antibiotics (results not shown). The numbers of patients in each group who were oliguric and required dialysis were also similar, suggesting that urinary losses were unlikely to have contributed to the differences in group serum retinol profiles.

An alternative explanation for the different serum retinol responses in the two groups of patients is that pre-existing socio-economic, rather than infection-specific, differences were present. However, there were no obvious differences in occupation, place of residence or ethnicity between the groups. In addition, serum retinol concentrations in available Group 1 patients beyond 96 h showed a continued increase, such that they would have exceeded 1.0 \(\text{mol/l}\) in all such patients by 7–10 days. This suggests that the reference range was appropriate for both groups of patients.

Because the present study was primarily a pharmacokinetic one and involved a limited number of subjects, we were not able to assess the benefits of adjunctive RP on outcome. In particular, the relationship between serum retinol and parasitaemia could not be evaluated. Although we have shown previously in an \(\text{in vitro}\) study that retinol inhibits the growth of \(P. \text{falciparum}\) at assayed concentrations in culture media that are close to those in normal human serum [22], most of our patients had received prior anti-malarial therapy. As a result, admission parasite densities were generally low for severe malaria in both groups, and parasite clearance times were short.

Retinol has antioxidant properties, and malaria parasites are vulnerable to oxidant stress [28]. Artemisinin drugs are thought to act through free radical generation within the parasitized erythrocyte [29], and thus retinol may be antagonistic to this. We have found evidence of such an interaction \(\text{in vitro}\) [30]. However, given that binding of retinol to RBP and transthyretin can attenuate its antioxidant effects, significant retinol–artemisinin antagonism may not occur \(\text{in vivo}\). In any case, artemisinin derivatives such as artesunate typically have a short half-life, and most parasites are cleared within 48 h with these agents [31], suggesting that the delay in RP mobilization and consequently increased serum retinol concentrations in severe malaria would limit such an interaction. In fact, the most beneficial effect of adjunctive RP may be to reduce the likelihood of recrudescence through the intrinsic anti-malarial activity of retinol. Recrudescences are relatively common after a course of an artemisinin drug given as sole therapy for malaria [32].

Instead of increasing in parallel with serum retinol during recovery, as would be expected in unsupplemented patients [20], serum vitamin E remained depressed in Group 2 subjects. Uptake and utilization of retinol and vitamin E are interrelated [11]. High-dose vitamin A induces vitamin E deficiency [33] which, in turn, increases retinol storage [34]. Consistent with animal studies [11], administration of RP and its conversion into retinol tended to keep vitamin E levels low in our patients. This would counteract the antioxidant effects of relatively high serum retinol levels in supplemented patients and so the net inhibitory effect of RP administration on artemisinin anti-malarial activity after several days of therapy may not differ from that in unsupplemented patients. Whether continued low serum vitamin E levels in RP-supplemented patients might themselves inhibit retinol release by the liver, as in animal models [34], is unknown.

The present study has shown that the bioavailability of intramuscular RP is not reduced by severe malaria. However, there is delayed mobilization of RP, such that the subsequent increase in retinol is most evident beyond 48 h. This suggests that multiple doses of RP in the first few days of treatment are unlikely to be any more
beneficial than a single injection of 400000 i.u. Oral administration of RP results in peak concentrations that occur typically after 3–7 h in healthy adults [35], suggesting a more rapid availability than after intramuscular injection. Nevertheless, most of our patients were nauseated or vomiting during initial treatment, and altered gastrointestinal function in severe illnesses such as malaria [17] would also influence the absorption of RP given orally or by nasogastric tube. The delayed mobilization of intramuscular RP will substantially reduce the potential benefit of retinol with regard to complications such as secondary bacterial infection occurring during the early phase of treatment. In the case of malaria, any antagonism between retinol and the artemisinin derivatives will be minimized initially, while there may be a later contribution of at least normal serum retinol concentrations to the prevention of the recrudescence of parasitaemia which is often seen with this group of anti-malarial drugs.

Our preliminary data suggest that further pharmacokinetic studies are needed, especially in children, in whom retinol supplementation is most often used [36] and in whom prophylactic vitamin A reduces morbidity due to P. falciparum [37]. In addition, although we were primarily concerned with the relationship between changes in RP and retinol, measurement of serum RBP and lipoprotein-associated RP may assist in the analysis and interpretation of future studies. Mortality due to severe malaria remains high. The safety, simplicity and availability of retinol supplementation, together with the present results and those of others, suggest that a controlled trial of retinol as an acute adjunctive treatment for severe falciparum malaria should also be considered.

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