EVALUATION OF THE EFFICACY OF TRANSDERMAL DELIVERY OF CHLOROQUINE ON PLASMODIUM BERGHEI-INFECTED MALE SPRAGUE-DAWLEY RATS AND EFFECTS ON BLOOD GLUCOSE AND RENAL ELECTROLYTE HANDLING

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INTRODUCTION

African children with severe Plasmodium falciparum malaria often present with metabolic complications which include impairment of glucose homeostasis, cardiovascular and kidney function partly ascribed to Plasmodium falciparum infection and/or drugs used to manage malaria [1]. The traditional oral chloroquine (CHQ) dose schedule of 4 tablets, equivalent to 300 mg active base, on day 1, followed by 3 tablets, equivalent to 200 mg active base, 6 hours later, and 2 tablets, 300 mg, a day for the next 2 days, giving a total of 1350 mg contributes to high plasma CHQ concentrations. Alternative methods of CHQ delivery have, therefore, been suggested, in an effort to modify the dosing schedule which may improve patient comfort and compliance. Against this background, literature evidence indicates that transdermal drug delivery provides sustained controlled release of drugs directly into the systemic circulation [2].

OBJECTIVES

• To develop a novel CHQ formulation that delivers sustained slow CHQ into the systemic circulation.
• To investigate the ability of the CHQ formulation to clear the malaria parasites in Plasmodium berghei-infected male Sprague-Dawley rats.
• To evaluate the effects of the transdermal delivery of CHQ on renal function and on blood glucose homeostasis in malaria infected rats.

MATERIALS AND METHODS

Patch preparation

Patch was dissolved in deionized water followed by adding CHQ, DMSO and antioxidants. After freezing, CaCl₂ was added for cross linking and patch formation. The patches were then stored in the refrigerator at 4°C until use.

Determination of the amount of CHQ in patch

The amount of CHQ in the CHQ patch was determined spectrophotometrically after dissolving the patch in methanol in a 1:100 solution. The blank contained CHQ free pectin patches in deionized water.

Patch preparation

Malaria was induced in male Sprague-Dawley rats (90-190 g body weight) with a single intraperitoneal injection of P. berghei (16S parasitized red blood cells).

Experimental design

Male Sprague-Dawley rats were divided into two groups (control) and Plasmodium berghei infected groups (n=6 in each group). The groups were further subdivided into those treated orally with CHQ, CHQ hydrogel or transdermally applied CHQ patch formulations for assessment of the effects on parasitaemia, blood glucose and renal functions.

Sub-chronic effects of CHQ

Oral CHQ was administered twice daily 60 mg/kg, p.o. by means of a ball-tipped steel needle whilst the CHQ patch (53 mg/kg) was applied once into the matrix patch was 74%.

The amidated CHQ pectin patches contained 15.9 mg/ml translating into a dose of 53 mg/kg for a 300 g rat. The percentage CHQ incorporation into the matrix patch was 74%.

Statistical analysis

All data presented as means ±SEM. Graph Pad Instat software (version 5) using one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test was used. P values < 0.05 were considered significant.

RESULTS

Chloroquine loading efficiency

The estimated CHQ pectin patches contained 15.8 mg CHQ translating into a dose of 53 mg/kg for a 300 g rat. The percentage CHQ incorporation into the matrix patch was 74%.

Effects of CHQ treatments

Parasitaemia

Figure 1 shows that the peak percentage parasitaemia of control animals was reached at day 14. The control animals were sacrificed on day 14 into the experimental period based on preliminary results. As such, all the subsequent results showing the infected control animals will be having a parasitaemia level of 0.

Topical application of the CHQ hydrogel matrix patch on the skin as well as oral CHQ equally reduced P. berghei parasitaemia to levels that were undetectable by day 5 of treatment (Table 1 and Figure 1).

Table 1: Comparison of P. berghei parasitaemia in animals treated with oral CHQ or transdermal CHQ (n=6 in each group).

<table>
<thead>
<tr>
<th>Group</th>
<th>% parasitaemia</th>
<th>P. berghei (0)</th>
<th>% parasitaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral CHQ</td>
<td>90±14</td>
<td>90±14</td>
<td>0±0</td>
</tr>
<tr>
<td>Transdermal CHQ</td>
<td>90±3</td>
<td>90±3</td>
<td>0±0</td>
</tr>
</tbody>
</table>

Biochemistry

Plasmodium berghei-infected animals (n=6) were sacrificed on the last day of treatment and infected groups (n=6 in each group). The groups were further differentiated from 0.5 to 5 days  and 0.5 to 4 days following oral CHQ or CHQ patch, respectively.

Between groups comparisons indicated that the plasma CHQ concentration vs. time profiles in treated animals could not be statistically differentiated from each other. The plasma CHQ concentrations of animals treated twice daily with oral CHQ were significantly elevated when compared with pectin CHQ matrix patch treated animals (Table 2).

Figure 4 displays the changes in plasma CHQ concentrations of animals treated twice daily with oral CHQ or CHQ patch, respectively.

Table 2: The effects of CHQ treatments on plasma AQP and electrolyte concentrations (n=6 in each group).

<table>
<thead>
<tr>
<th>Group</th>
<th>Measurements</th>
<th>Period</th>
<th>Oral</th>
<th>Patch</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP (pmol/l)</td>
<td>Pre-treatment</td>
<td>5.09±0.33</td>
<td>5.49±0.40</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Na⁺ (mmol/l)</td>
<td>Pre-treatment</td>
<td>140±2</td>
<td>141±2</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>K⁺ (mmol/l)</td>
<td>Pre-treatment</td>
<td>3.5±0.5</td>
<td>3.0±0.5</td>
<td>0.5±0.5</td>
</tr>
<tr>
<td>GFR (ml-min⁻¹·100g⁻¹)</td>
<td>Pre-treatment</td>
<td>100±10</td>
<td>100±10</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSIONS

The current study has demonstrated the sustained controlled release of CHQ from the pectin matrix patch, demonstrating the therapeutic ability to clear the malaria parasites from systemic circulation. The zero-off application of the CHQ patch was able to circumvent the adverse effects of oral CHQ delivery on blood glucose homeostasis and renal function. We conclude that the pectin CHQ matrix patch has the potential to aver the adverse effects that are associated with oral administration of CHQ.

REFERENCES


Figure 1: Comparison of percentage parasitaemia in animals treated twice daily with oral CHQ (50 mg/kg) and those treated with a single topical application of CHQ patch (53 mg/kg) with control animals. *p<0.05 by comparison to control animals.

Figure 2: The effects of oral CHQ (60 mg/kg) treatment (X) and single topical application of CHQ matrix patch (53 mg/kg) (X) on blood glucose concentrations. **p<0.05 by comparison to baseline control animals. *p<0.05 by comparison to control animals.

Figure 3: Comparison of the effects of oral CHQ treatment (OT) and single topical application of CHQ matrix patch (PT) on plasma insulin concentrations with respective control baseline values (Figure 3).

Figure 4: Comparison of AQP concentrations in animals treated with oral CHQ treatment (OT) and single topical application of CHQ matrix patch (PT) (n=6 in each group).

Figure 5: Comparison of effects of oral CHQ treatment (OT) and single topical application of CHQ matrix patch (PT) on renal Na⁺ and K⁺ outputs.

Figure 6: Comparison of effects of oral CHQ treatment (OT) and single topical application of CHQ matrix patch (PT) on renal Na⁺ and K⁺ outputs.