Assessment of pyronaridine activity in vivo and in vitro against the hepatic stages of malaria in laboratory mice

Leonardo K Basco¹, Pascal Ringwald¹, Jean-François Franetich² and Dominique Maziez² ¹Institut de Recherche pour le Développement (IRD) – Laboratoire de Recherche sur le Paludisme, Laboratoire Associé Francophone 352, Organisation de Coordination pour la lutte contre les Épidémies en Afrique Centrale (OCÉAC), BP 288, Yaoundé, Cameroon; ²INSERM Unité 511, Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires, Centre Hospitalo-Universitaire Pitié-Salpêtrière, 91 Boulevard de l’Hôpital, 75013 Paris, France

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Chloroquine-resistant Plasmodium falciparum malaria is now widespread in many parts of the African continent, where a large majority of malaria infections occur. One of the promising new drugs that may replace chloroquine for the first-line treatment of uncomplicated falciparum malaria in Africa is pyronaridine. This synthetic derivative of the acridine-type Mannich bases was developed in China and was found to possess high blood-schizontocidal activity against rodent, simian, and human malaria parasites (Chin et al., 1992). Subsequent clinical studies have confirmed the safety, tolerance, and efficacy of pyronaridine to treat chloroquine-resistant P. falciparum malaria infections (Loosaretuwat et al., 1996; Ringwald et al., 1996). Although its potent action against the asexual blood stages of various malaria species has been demonstrated, the possible activity of pyronaridine against the hepatic stages has not been documented.

We performed experiments in vitro and in vivo to evaluate whether pyronaridine also acts against the liver stages of malaria parasites.

Experiments in vitro were performed using C57/B16 mouse hepatocytes infected with sporozoites of P. yoelii yoelii. In brief, hepatocytes were isolated by collagenase perfusion as previously described (Maziez et al., 1986) and seeded in 8-chamber plastic Lab-Tek slides (Nalge Nunc International, Naperville, IL, USA) at a concentration of 8 x 10⁴ cells per well, in Williams Medium E (Bio Whittaker, Walkersville, ML, USA), supplemented with 10% fetal calf serum, 10 mg/mL of streptomycin. Sporozoites were obtained by dissection of salivary glands of Anopheles stephensi infected with malaria parasites, crushed in a glass grinder and diluted in culture media. Pyronaridine was first diluted in phosphate-buffered saline (PBS) for a 10-nM stock solution and then diluted from 1 nM to 100 µM in culture medium and added with sporozoites (35 000/well) on hepatocyte cultures (24 h old). After 3 h, cultures were washed and the medium was replaced with fresh medium or diluted drug. Forty-eight hours after infection, cultures were fixed with cold ethanol and parasites labelled with an immunofluorescent assay. Schizonts were counted and culture was observed with a fluorescence microscope.

Experiments in vivo were carried out using C57/B16 mice. One hour after intravenous injection of 5000 P. yoelii yoelii sporozoites, mice were infected subcutaneously with 30 mg/kg (0-6 mg per mouse) of pyronaridine diluted in PBS (experimental group) or an equivalent volume of PBS alone (control group). This dose is equivalent to that given to humans over 3 days (Chen et al., 1992). Giemsa-stained peripheral blood smears were observed daily until 14 days after sporozoite inoculation.

In vitro, pyronaridine was hepatotoxic at the concentrations of 100 µM, 10 µM, and 1 μM, as evidenced by morphological features of cell death as well as cell detachment. No hepatotoxic effect was observed at 100 nM, 10 nM, and 1 nM in the hepatocyte culture. However, there was only a slight decrease (P > 0.05) in the number of hepatic schizonts in pyronaridine-treated culture at 100 nM, as compared with untreated culture. While there was no schizontocidal effect at 10 nM and 1 nM (Figure).

In vivo, 5 days post-infection, 4 of 5 control mice were found to be parasitized, whereas the 5 mice treated with pyronaridine were free of blood-stage parasites (Table). From day 6 to day 12, all untreated controls were found to be parasitized, and all treated mice were still negative.

Our experiments in vitro and in vivo suggest that pyronaridine has a blood-schizontocidal, but not hepatic-schizontocidal, action against P. yoelii. Pyronaridine is known to exert a potent blood-schizontocidal action. The growth in vitro of the human malaria parasites P. falciparum, P. ovale, and P. malariae is inhibited at <50 nM during a 48–72-h incubation (Childs et al., 1998; Ringwald et al., 1996, 1997; Fradines et al., 1996; 1997; 1999).

Figure. Activity in vitro of pyronaridine against pre-erythrocytic stages of P. yoelii. Reduction in the number of hepatic schizonts is expressed as mean ± standard deviation of treated cultures compared to untreated cultures (number of schizonts = 77-75 ± 14).

Table. Activity of pyronaridine in vivo against P. yoelii in mice

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>4/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Pyronaridine-treated</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Treated and control group mice (n = 5 in each group) were injected intravenously with 5 x 10⁴ P. yoelii sporozoites in phosphate-buffered saline (PBS). The mouse given 1 h after infection, was with 30 mg/kg of pyronaridine (experimental group) or diluent only (control group). The delay of the appearance of blood parasites was determined from Giemsa-stained blood smear taken daily.

Address for correspondence: Leonardo Basco, OCEAC, B.P. 286, Yaoundé, Cameroon; phone +237 23 00 61, fax +237 23 00 61, e-mail oceac@camnet.cm

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1998). In the present study, no growth inhibition was observed in the infected hepatocytes at 10 nM and 10 nM of pyronaridine. The results of higher drug concentrations were uninterpretable owing to drug toxicity on hepatocytes. Thus, the suppressive effect of pyronaridine observed in vitro in our study was most likely to have been due to its blood-schizontocidal action.

Currently available antimalarial agents and antibiotics used in antimalarial chemotherapy that possess tissue-schizontocidal activity include primaquine, doxycycline, and proguanil (Wernsdorfer, 1997). Like other derivatives belonging to the Mannich bases (amodiaquine, amopyroquin) and 4-aminoquinolines, pyronaridine did not exert any effect on the hepatic stages of malaria parasites. Nonetheless, because of its established blood-schizontocidal activity, even against chloroquine-resistant malarial infections, pyronaridine is a promising drug for antimalarial chemotherapy.

References

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**Book Review**


Coming 25 years after Stanley and Alpers’ classic book on man-made lakes and human health, this book can be seen in some senses as a sequel. The former book had an Australian editor, and so does this, although it is not unduly antipodean; Australians have a significant experience in this area, and their 6 chapters (out of 15) do not seem too many. The area has been expanded significantly through the WHO/FAO/UNEP Panel of Experts on Environmental Modification for Vector Control (PEEM), and this book arose from a PEEM-sponsored conference in Brisbane.

The era of building large dams is over now, except in China, which has half the world’s big dams. Elsewhere, maintenance is the chief concern. The book tells us much about maintenance, but precious little about dams in China, which in the circumstances is a shortcoming. One would also have wished to see more about the research work sponsored by the West African Rice Development Association, which is mentioned only once.

The editor’s avowed aim was to ‘acquaint the reader with a variety of topics without going into too much detail’, and indeed the book is something of a dilettante’s delight (although some of the introductory chapters on overall principles and the policies of international agencies are so general that it is hard to find the interest in them), PEEM’s manuals are more useful to practitioners than these, but the guidelines and checklists do help.

The chapter on ‘Environmental and health impact assessment’ is of interest, as its Australian setting means that stakeholder participation is taken for granted; this is refreshing in view of how often it is neglected in the developing world, and so is Buxley’s re-christening of ‘Health opportunities assessment’ in the following chapter.

Where things get more interesting is in the case-studies; there are 5 chapters on Australia, 2 on the Tennessee Valley Authority (an old example, but the update is worth reading), 1 from Thailand, a fascinating historical chapter on fish culture and malaria in Indonesia, and the editor adds a final overview of urban vector-borne disease problems.

One recurring theme is the unpredictability of many of the health impacts and political forces which can now be documented with hindsight. No one foresaw that Murray Valley encephalitis could be transported over long distances by avian migrants, that wastewater-irrigated wetlands would produce 30 times more mosquitoes, or that villagers downstream of a dam would complain that the low level of dissolved oxygen would make the water unsuitable for bathing or washing. In the circumstances, it is salutary that the case-studies mention a number of ways to monitor some of the health impacts, such as the use of sentinel flocks of chickens to provide early warning of arbovirus epidemics. As the authors of the Thai case-study conclude, ’it would seem rather ambitious to forecast the health impact of water resources development’. It is nevertheless worth the attempt, and this book will be of interest to anyone who tries to do so.

Sandy Cairncross
WELL Resource Centre
London School of Hygiene and Tropical Medicine
Keppel Street
London WC1E 7HT, UK
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Royal Society of Tropical Medicine and Hygiene,
Manson House, 26 Portland Place,
London, W1N 4EY, UK
Telephone: +44 (0)20 7580 2127
Fax: +44 (0)20 7436 1389
e-mail (administration): mail@rstmh.org
e-mail (editorial office): trans@rstmh.org
Web site: http://www.rstmh.org

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