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Short Report

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

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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 (CHEN *et al.*, 1992). Subsequent clinical studies have confirmed the safety, tolerance, and efficacy of pyronaridine to treat chloroquine-resistant *P. falciparum* malaria infections (LOOAREESUWAN *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 (MAZIER *et al.*, 1986) and seeded in 8-chamber plastic Lab-Teck slides (Nalge Nunc International, Naperville, IL, USA) at a concentration of 8×10^4 cells per well, in Williams Medium E (Bio Whittaker, Walkersville, ML, USA), supplemented with 10% fetal calf serum, 100 units/mL of penicillin and 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-mM 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 methanol and parasites labelled with an immunofluorescent assay. Schizonts were counted and culture was observed with a fluorescence microscope.

Experiments *in vivo* were carried out in C57/B16 mice. One hour after intravenous injection of 5000 *P. yoelii yoelii* sporozoites, mice were injected 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.*, 1988; RINGWALD *et al.*, 1996, 1997; PRADINES *et al.*,

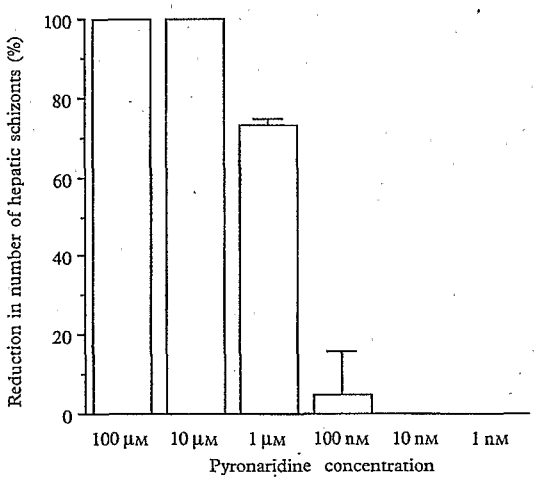

Figure. Activity *in vitro* of pyronaridine against pre-erythrocytic stages of *P. yoelii*. Reduction in the number of hepatic schizonts was estimated by counting schizonts in 48-h cultures. Results are expressed as mean ± standard deviation of treated cultures compared to untreated cultures (number of schizonts = 77·75 ± 14).

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

	Number of parasitized mice		
Mouse group	Day 5	Day 6	Day 12
Untreated controls	4/5	5/5	5/5
Pyronaridine-treated	0/5	0/5	0/5

Treated and control group mice ($n = 5$ in each group) were injected intravenously with 5×10^3 *P. yoelii* sporozoites in phosphate-buffered saline (PBS). Treatment, 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 smears taken daily.

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1998). In the present study, no growth inhibition was observed in the infected hepatocytes at 100 nM and 10 nM of pyronaridine. The results of higher drug concentrations were uninterpretable owing to drug toxicity on hepatic cells. Thus, the suppressive effect of pyronaridine observed *in vivo* 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.

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Book Review

Water Resources: Health, Environment and Development. B. H. Kay (editor). London: E & F N Spon: 1999. xviii + 250pp. Price £55.00. ISBN 0-419-22290-1.

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 to share 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 Birley's re-christening of 'Health opportunities assessment' in the following chapter.

Where things get more interesting is in the case-studies; there are 3 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 viraemic birds, 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.

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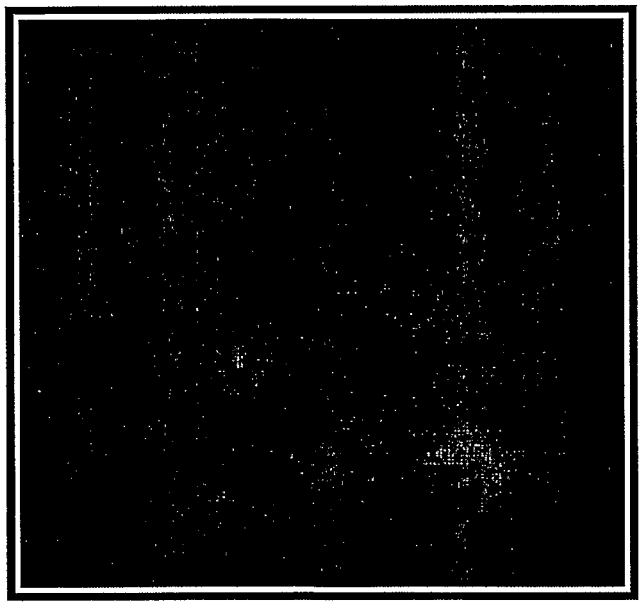

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