

Short Report

Therapeutic effect of praziquantel enantiomers in mice infected with *Schistosoma mansoni*

S. Xiao^{1,2}, J. Chollet², M. Booth², N. A. Weiss² and M. Tanner^{2*} ¹Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai, China; ²Swiss Tropical Institute, Socinstrasse 57, 4051 Basel, Switzerland

Keywords: schistosomiasis, mice, *Schistosoma mansoni*, chemotherapy, praziquantel, laevo-praziquantel, dextro-praziquantel, hepatic shifting, worm burdens

Praziquantel, an effective drug for the treatment of schistosomiasis, is a racemic compound containing equal parts of its optical isomers, laevo-praziquantel (L-Pra), and dextro-praziquantel (D-Pra). L-Pra has been identified as the active component against *Schistosoma japonicum* infections (ANDREWS, 1985; TANAKA *et al.*, 1989), and has been demonstrated to have greater efficacy and safety than the racemic compound in field trials (WU *et al.*, 1991). L-Pra has also been identified as the probable active compound against *S. mansoni* infection from studies *in vitro* (ANDREWS, 1985; STAUDT *et al.*, 1992), and a study *in vivo* against adult male worms (XIAO & CATO, 1989). The purpose of the present study was to extend previous work, by assessing whether L-Pra is also the active component of praziquantel when used against juvenile *S. mansoni* infections, through a series of experiments *in vivo* on mice. Attention was given to the effects of each enantiomer on female worms, since these are the source of pathology and transmission.

Praziquantel for each experiment was supplied by the Shanghai No. 6 Pharmaceutical Factory, China. L-Pra and D-Pra were synthesized by the Institute of Parasitic Diseases, Shanghai. In ethanol at 11°C, the optical rotation of L-Pra was -132.43 , and that of D-Pra was $+133.21$. The optical specificity of the enantiomers was confirmed by high-performance liquid chromatography, which revealed that the L-Pra was pure, whereas the D-Pra was slightly contaminated (<2%) by L-Pra.

In each experiment, groups of 2–5 female MORO mice (SPF) were inoculated with 60 cercariae of the Liberia strain of *S. mansoni* by subcutaneous injection in the root of the tail. Six weeks later the mice were treated

intra-gastrically, sacrificed at a defined time point after medication, and the liver and intestines were removed and separated. The liver was placed in a 20 × 30-cm plastic folder and compressed between 2 glass plates until the parenchyma was a thin transparent layer. Worms were then easily counted and sexed at ×10 magnification. The mesenteric veins were placed in a Petri dish and examined with a stereoscopic microscope.

In preliminary experiments, we determined the relationship between the dose of each enantiomer and the extent of hepatic shifting. Mice were treated with either L-Pra or D-Pra at a single dose of 2.5 mg/kg, 5.0 mg/kg or 10 mg/kg, sacrificed 1 h after treatment, and the numbers of worms in the mesenteric veins and livers were compared. Although the numbers of mice in each group were too small to allow statistical analysis, considerably more worms were found in the livers of mice treated with L-Pra than either control mice or mice given D-Pra (Table 1). There were more than twice as many male worms recovered from mice than female worms, partly owing to an imbalance in the sex ratio of cercariae. We also observed an apparent disproportionate effect of D-Pra on male worms, for unknown reasons.

The results of these experiments indicated that hepatic shifting was largely attributable to the activity of L-Pra. In further experiments, we then tested the hypothesis that D-Pra would cause shifting at higher doses. In these experiments, L-Pra was given intra-gastrically to infected mice at a dose of 10 mg/kg, whereas D-Pra was given at higher doses of 50 mg/kg or 100 mg/kg. All schistosomes in mice given L-Pra were shifted to the livers, whereas this occurred among only 25% and 80% of the worms in mice treated with 50 mg/kg or 100 mg/kg of D-Pra. In the control group, only 7.9% of the worms had shifted. The hepatic shift of the worms in the D-Pra high-dose group was most likely due to the small amount of L-Pra contamination in the D-Pra compound, and/or a disproportionate effect on male worms which formed the majority of the worm population.

Finally, we investigated the comparative efficacy of racemic praziquantel (Pra) and each isomer in mice infected with *S. mansoni* for 7 weeks and sacrificed 4 weeks after treatment (Table 2). ANOVA was used to test for differences in worm burdens after treatment. When infected mice were treated intra-gastrically with L-Pra 150 mg/kg or Pra 300 mg/kg, the mean worm burdens in these groups were significantly lower than in the control group (L-Pra vs control: $F = 17.12$, $P = 0.0005$; Pra vs control: $F = 53.37$, $P < 0.0001$), and the mean worm burden was significantly lower in the Pra group than in the 50 mg/kg L-Pra group ($F = 13.79$, $P = 0.0012$). Increasing the dose of L-Pra to 300 mg/kg resulted in worm burden reductions that were signifi-

Table 1. Effects of praziquantel enantiomers on numbers and distribution of *S. mansoni* in mice

Drug and dose (mg/kg)	n	Liver			Mesenteric veins		
		Male	Female	Overall	Male	Female	Overall
Control	5	3.8	0	3.8	9.2	5.6	14.8
L-Pra							
2.5	5	8.8	2.4	11.2	5.2	3.0	8.2
5	4	11.0	5.8	16.7	3.3	1.0	4.3
10	4	17.3	5.8	23.5	1.0	0.5	1.5
D-Pra							
2.5	2	11.0	0	11.0	14.0	9.0	23.0
5	4	5.7	0.3	6.0	7.3	5.5	12.8
10	3	4.7	0	4.7	7.0	6.3	13.3

Mean worm burdens of each sex (1 h after treatment) in the livers and mesenteric veins of mice were calculated for each treatment group. n, number of mice in each group.

*Author for correspondence: phone +41 61 2848 287, fax +41 61 271 7951, e-mail tanner@ubaclu.unibas.ch

Table 2. Efficacy of praziquantel enantiomers against 7-week infections with *S. mansoni* in mice

Drug and dose (mg/kg)	<i>n</i>	Mice without female worms	Mean worm burden (\pm SD)
Control			
—	10	0	41.2 \pm 11.6
Pra			
300	9	2	7.9 \pm 7.6
L-Pra			
50	9	0	21.7 \pm 8.5
150	10	3	2.6 \pm 1.3
300	10	7	0.7 \pm 0.9
D-Pra			
150	10	0	37.2 \pm 9.4
300	10	0	38.0 \pm 11.7
600	10	0	36.8 \pm 10.1

The arithmetic mean worm burden was recorded for each group 4 weeks after treatment, together with the number of animals free of female worms. *n*, number of mice in each group.

cantly greater than those gained with 50 mg/kg of L-Pra ($F = 20.7$, $P = 0.0014$), or 150 mg/kg of L-Pra ($F = 14.4$, $P = 0.001$). Worm burdens were lower in the L-Pra group given 300 mg/kg than in the Pra group given 300 mg/kg, but the difference was not significant, presumably because of the low sample size ($F = 2.8$, $P = 0.1314$). In further comparing these groups, we also observed that the L-Pra group had more mice without female worms than the Pra group. The lack of efficacy of D-Pra was clearly shown in this experiment—none of the 3 doses given to different groups of mice reduced the worm burdens significantly when compared to the control or any other treatment group.

Our main conclusion from these experiments is that L-Pra is indeed the active enantiomer of praziquantel when the drug is used to treat juvenile *S. mansoni* infections. Pure L-Pra may also be more efficacious than the racemic mixture, but larger-scale studies are required to test this hypothesis further. There was no evidence of a disproportionate effect of L-Pra on female worms in terms of hepatic shifting, which suggests that the relatively high number of mice without female worms in the L-Pra 300 mg/kg group is due to an equally proportionate effect of the enantiomer on both worm sexes, and a high male-to-female sex ratio in the cercariae. Overall, these results lend weight to the idea that treatment with L-Pra may be equally efficacious at lower doses than are recommended for the racemic mixture. This could

reduce the risk of side-effects in treated patients, provided that side-effects are associated with D-Pra, or at least not disproportionately associated with L-Pra.

References

- Andrews, P. (1985). Praziquantel: mechanisms of anti-schistosomal activity. *Pharmacology and Therapeutics*, **29**, 129–156.
- Staudt, U., Schmahl, G., Blaschke, G. & Mehlhorn, H. (1992). Light and scanning electron microscopy studies on the effects of the enantiomers of praziquantel and its main metabolite on *Schistosoma mansoni* *in vitro*. *Parasitological Research*, **78**, 392–397.
- Tanaka, M., Ohmae, H., Utsunomiya, H., Nara, T., Irie, Y. & Yasuraoka, K. (1989). A comparison of the antischistosomal effect of levo- and dextro-praziquantel on *Schistosoma japonicum* and *S. mansoni* in mice. *American Journal of Tropical Medicine and Hygiene*, **41**, 198–203.
- Wu, M. H., Wei, C. C., Xu, Z. Y., Yuan, H. C., Lian, W. N., Yang, Q. J., Chen, M., Jiang, Q. W., Wang, C. Z. & Zhang, S. J. (1991). Comparison of the therapeutic efficacy and side effects of a single dose of levo-praziquantel with mixed isomer praziquantel in 278 cases of schistosomiasis japonica. *American Journal of Tropical Medicine and Hygiene*, **45**, 345–349.
- Xiao, S. H. & Catto, B. A. (1989). Comparative *in vitro* and *in vivo* activity of racemic praziquantel and its levorotated isomer on *Schistosoma mansoni*. *Journal of Infectious Diseases*, **159**, 589–592.

Received 18 January 1999; revised 25 March 1999; accepted for publication 26 March 1999