

## Hypersensitivity (PCA and DTH) Response in *H. diminuta* Infected Mice, Treated with Praziquantel and Ivermectin

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**Abstract:** The objective of this study was to investigate the immunomodulatory efficacy of drugs (Praziquantel and Ivermectin) in *Hymenolepis diminuta* infected mice. The drugs were administered to the infected mice on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> post infection days. The immunomodulation due to test drugs was observed on PCA and DTH response. PCA and DTH reactions were found to be directly proportional to the dose of drugs. PCA response was maximum (23.4 mm) in praziquantel treated mice and minimum (20.2 mm) in ivermectin treated mice. DTH response was maximum (16.4 mm) in praziquantel treated mice and minimum (10.4mm) in ivermectin treated mice. Significant increase in PCA and DTH responses in the infected mice indicates stimulated cell mediated as well as humoral immunity. Obtained results indicate that both studied drugs can be good cestocidal agent and may boost the immune response of the host but Praziquantel is more effective immunomodulatory agent than Ivermectin.

**Key words:** Praziquantel • Ivermectin • Immunomodulation • PCA • DTH And *H. Diminuta*

### INTRODUCTION

*Hymenolepis diminuta* belongs to Phylum-Platyhelminthes and is commonly known as the rat, mice and man's tapeworm. It is the best known of the tapeworms as it has been subject of numerous veterinary or biological studies.

Praziquantel is a pyrazino-iso-quinoline derivative which is most effective cestocidal agent [1]. It is an effective drug for cestodes and trematodes. Praziquantel induces a rapid contraction of helminth parasites by a specific effect on the permeability of cell membrane. Praziquantel has been reported to be a very effective cestocidal agent against larvae and adults of *H. nana* [2]. Cestocidal action of praziquantel is attributed to its interference in calcium transport [3], Tegumental damage [4] and contraction of the muscles by membrane depolarization [5].

Ivermectin is a unique new chemical entity [6]. It was introduced to the market-place as an antiparasitic drug in 1981. Its worldwide acceptance in livestock production and in the health care of companion animals has made it a major commercial success. Ivermectin was developed against the arachnids and ectoparasitic arthropods [7].

It has also been found to be highly effective against several nematode spp. Ivermectins are produced by the actinomycete *Streptomyces avermitilis*. Ivermectin paralyzes and ultimately kills the parasitic nematodes, arachnids and insects by a unique effect on the nervous system. Larvicidal efficacy of ivermectin has been screened against a variety of nematodes by several workers [8- 11].

The main objective of this study was to investigate the immunomodulatory efficacy of drugs (Praziquantel and Ivermectin) in *H. diminuta* infected mice on basis of PCA and DTH response.

### MATERIALS AND METHODS

**Experimental Animal:** The mice were obtained from the College of Veterinary Science and Animal Husbandry, Mhow (M.P.) and were kept in the animal house under local conditions of light, temperature and ventilation. Inbred Swiss albino mice, *Mus musculus albinus*, 7-9 weeks old and 18-20 gms in weight were selected as the experimental animals. Only those animals which were not having any kind of helminthic infection were selected for the present study. It was confirmed by examining the

stool. These were kept in sterilized cages with dry husk padding and were fed daily with standard balanced diet and along with water ad-lib.

**Maintenance of *H. Diminuta*:** *Hymenolepis diminuta* was selected as a test parasite. It was obtained from Helminthology laboratory, Department of Zoology, Govt. Model Autonomous Holkar Science College, Indore. *H. diminuta* is being maintained in the helminthology laboratory by serial passage of 100 viable infective cysticercoid larvae in healthy mice. The worms were recovered from these infected mice after every 16<sup>th</sup> day post infection

**Experimental Design:** Experiments were carried out in the following groups of mice:

- Control Group I: Non infected and non treated.
- Control Group II: Infected and non treated.
- Experimental Groups: Infected and treated with the test drugs.

Total 20 mice were used. Five mice were used for positive control, 5 mice used for negative control and 10 mice used for experiment

**Test Drugs:** Praziquantel and ivermectin were selected as the test drugs. The different doses of proposed drugs were administrated in powdered form to the infected mice to assess their immunomodulatory efficacy in experimental *H. diminuta* infection. The drug treatment was given on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> post infection days.

**Pca and Dth Response:** The estimation of passive cutaneous anaphylaxis (PCA) was done by Ovary, [12] method and delayed hypersensitivity (DTH) was done by Talwar [13].

## RESULTS

Results of PCA and DTH reactions in mice infected and treated with different doses of drugs are summarized in Table 1 and 2 and presented in Figure 1 and 2. PCA and DTH reactions were found to be directly proportional to the dose of drug.

Minimum PCA 21.3 mm was observed in ITPZQ<sub>1</sub>, which increased to 22.6 mm in ITPZQ<sub>2</sub> and maximum 23.4 mm in ITPZQ<sub>3</sub>.

Minimum DTH reaction 11.4 mm was observed in ITPZQ<sub>1</sub>, which increased to 13.9 mm in ITPZQ<sub>2</sub> and maximum in ITPZQ<sub>3</sub> 16.4 mm.

Table 1: PCA and DTH responses in *H. diminuta* infected mice, treated with Praziquantel

Group No.	Groups	Dose	On day 16 <sup>th</sup> post infection	
			PCA (mm)	DTH (mm)
1	NINTC1	-	-	-
2	INTC2	-	15.2 ± 0.135	6.2 ± 0.260
3	ITPZQ <sub>1</sub>	15 mg/kg	21.3 ± 0.202	11.4 ± 0.247
4	ITPZQ <sub>2</sub>	20 mg/kg	22.6 ± 0.220	13.9 ± 0.249
5	ITPZQ <sub>3</sub>	25 mg/kg	23.4 ± 0.235	16.4 ± 0.268

Skin reaction greater than 5 mm in diameter are considered significant.

Table 2: PCA and DTH responses in *H. diminuta* infected mice, treated with Ivermectin

Group No.	Groups	Dose	On day 16 <sup>th</sup> post infection	
			PCA (mm)	DTH (mm)
1	NINTC1	-	-	-
2	INTC2	-	15.2 ± 0.135	6.2 ± 0.260
3	ITIVR <sub>1</sub>	0.025 mg/kg	20.2 ± 0.221	10.4 ± 0.250
4	ITIVR <sub>2</sub>	0.05 mg/kg	21.2 ± 0.221	13.6 ± 0.251
5	ITIVR <sub>3</sub>	0.1 mg/kg	22.3 ± 0.236	15.3 ± 0.269

Skin reaction greater than 5 mm in diameter are considered significant.

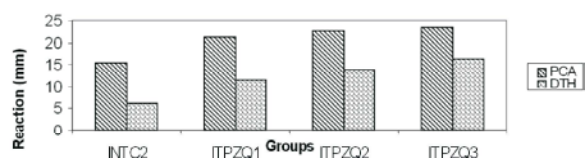


Fig. 1: PCA and DTH responses in *H. diminuta* Infected Mice, Treated with Praziquantel

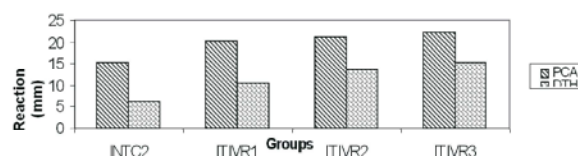


Fig. 2: PCA and DTH Responses in *H. diminuta* Infected Mice, Treated with Ivermectin.

NINTC1- Non infected non treated control<sub>1</sub>.

INTC2- Infected non treated control<sub>2</sub>.

ITPZQ<sub>1</sub>- Infected and treated with 15 mg/kg praziquantel on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> p.i. days.

ITPZQ<sub>2</sub>- Infected and treated with 20 mg/kg praziquantel on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> p.i. days.

ITPZQ<sub>3</sub>- Infected and treated with 25 mg/kg praziquantel on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> p.i. days.

ITIVR<sub>1</sub>- Infected and treated with 0.025 mg/kg ivermectin on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> p.i. days.

ITIVR<sub>2</sub>- Infected and treated with 0.05 mg/kg ivermectin on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> p.i. days.

ITIVR<sub>3</sub>- Infected and treated with 0.1 mg/kg ivermectin on 13<sup>th</sup>, 14<sup>th</sup> and 15<sup>th</sup> p.i. days.

Minimum PCA reaction 20.2 mm was in ITIVR<sub>1</sub>, which increased to 21.2 mm in ITIVR<sub>2</sub> and maximum 23.3 mm in ITIVR<sub>3</sub>.

Minimum DTH reaction 10.4 mm in ITIVR<sub>1</sub>, which increased to 13.6 mm in ITIVR<sub>2</sub> and maximum in ITIVR<sub>3</sub> 15.3 mm were observed.

## DISCUSSION

Increase in PCA reactions after chemotherapy indicates the stimulation of reagenic (IgE) response by the test drugs as these are the only type of antibodies which are involved in anaphylactic reactions. Increased levels of IgE are responsible for mortality of the parasites. The role of reagenic antibodies in killing/expulsion of helminthic parasites conferring protection to the host is well known [14] and their increased levels after specific chemotherapy clearly indicate the synergism between drug's cestocidal activity and reagenic response. Curative doses of PZQ may stimulate IL-4 production thereby inducing synthesis of IgE. Treatment of helminthic infections with therapeutic doses of PZQ thus provides the most potent stimulus for the biosynthesis of IgE. Probably PZQ treated worms produce a factor which acting via T-cells has the capacity to stimulate those B-cells which have been programmed for IgE production. The maximum PCA reaction was shown by praziquantel and least by ivermectin. Increased PCA values in treated mice suggest the role of IgE in providing protection [15] observed that the effect of PZQ on 21 day old schistosomules is dependent on the presence of specific antibodies and their increased levels after specific chemotherapy clearly indicate the synergism between drug's cestocidal activity and antibody response. Curative doses of PZQ may stimulate IL-4 production thereby inducing synthesis of IgE.

Increase in DTH response after chemotherapy with PZQ/IVR indicates the synergistic anthelmintic action of these drugs and CMI through macrophage activation. In cell mediated hypersensitivity CD8 cytotoxic T-cell and CD4 helper T-cells recognize either intracellular or extra cellular synthesized antigen. Macrophages function as antigen presenting cells and release interleukin-1, which promotes the proliferation of helper T-cells which release interferon-gamma interleukin-2, which together regulate delayed hypersensitivity reactions centered on macrophage activation and T-cell mediated immune response. Activated macrophages express increased phagocytic activity when confronted with intracellular pathogens which undergo granulomatous transformation

into epithelioid and multinucleated giant cells. In DTH responses, values showed significant increase in the experimental mice which indicated the enhanced cellular immune response due to effective drug treatment.

According to the concept of cell mediated immunity [16] T-cells (usually of the Th1 (D4 or CD8<sup>+</sup> phenotype) are stimulated by antigen to secrete lymphokines (notably IFN- $\gamma$  and TNF- $\alpha$ ) which activate effector cells to mediate immune killing. The most important cytokine-activated cells for parasite killing appear to be macrophages. The production of reactive nitrogen oxide (NO) appears to be the mechanism by which these cells kill parasites or inhibit their growth [17].

Increase in PCA as well as DTH values indicates that in *H. diminuta* infection both the humeral and cell-mediated immunity play vital roles and thus increase markedly after drug treatment. Obtained results indicate that both studied drugs can be good cestocidal agent and may boost the immune response of the host but Praziquantel is more effective immunomodulatory agent than Ivermectin. The protective role of cell mediated immunity as indicated by the increase of eosinophil and mast cell has also been demonstrated by histopathological experiments by [18].

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