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Transdermal Device of a Substituted Benzimidazole Carbamate and its Efficacy Against Helminth Parasites

Suman GUPTA1, J.K. SRIVASTAVA1, J.C. KATIYAR1, G.K. JAIN2, S. SINGH2 and J.P.S. SARIN2

1Division of Parasitology, and 2Division of Pharmaceutics, Central Drug Research Institute, Lucknow, 226 001, India

Abstract: The anthelmintic efficacy of a substituted benzimidazole carbamate (methyl-5-[4-(2-pyridinyl)-1-piperazinyl]-1H-benzimidazole-2yl-carbamate) in an adhesive diffusion controlled matrix was assessed against adult and larval forms of Ancylostoma ceylanicum (hookworm), Nippostrongylus brasiliensis (trichostrongylid) and Hymenolepis nana (tapeworm). Tapes impregnated with 5 mg of compound and applied on the dorsum of hamster resulted in complete elimination of adult A. ceylanicum. Against the metamorphic forms of this parasite, the reduction rate was 66.9%, 90.4% and 92.6% against L3, L4 and L5 stages respectively. Remarkable efficacy through this delivery system was also noticed against N. brasiliensis and H. nana. The transdermal drug delivery is the first attempt in the helminth chemotherapy and could beneficially be extended to clinical conditions.

Key words: Anthelmintic efficacy, Benzimidazole carbamate, Ancylostoma ceylanicum, Nippostrongylus brasiliensis, Hymenolepsis nana

INTRODUCTION

Promising wide-spectrum of anthelmintic efficacy was reported in Methyl-5-[4-(2-pyridinyl)-1-piperazinyl]-1H-benzimidazole-2yl-carbamate (CDRI Comp. 81-470). The compound possesses strong adulticidal and larvicidal activity, besides, having long prophylactic action (Katiyar et al., 1984; 1987; Srivastava et al., 1988). Like other benzimidazoles comp. 81-470 is poorly soluble in water and other organic solvents and is poorly absorbed through GI tract. A major portion of this drug is excreted unchanged following an oral administration which is also associated with ill effects like GI disturbances, nausea, vomiting and headache, etc. In addition, helminth parasites elicit weak immunogenic response (Wakelin, 1986) and therefore, more often lead to reinfection and polyparasitism. Since helminthiasis need repeat drug administration, the hazard of multiple dose therapy signals caution and calls for improved drug administration systems.

Comp. 81-470 is currently under final stages of clinical development at Central Drug Research Institute, Lucknow. Its detailed biopharmaceutical parameters have been worked
out by Moinf et al. (1991). Katiyar et al. (1984, 1988) have demonstrated its anthelmintic action by oral as well as by topical route. This property prompted us to develop transdermal tape of compound and single application of which will provide drug for 5 to 7 days i.e. for total duration of therapy. Transdermal delivery of comp. 81-470 will provide other pharmacotherapeutic benefits like:
1 elimination of gastro-intestinal vagaries
2 improved patient compliance
3 controlled drug administration for prolonged period of time
4 ability to easily terminate the medication in case of toxic manifestations.

The present study is an attempt to establish anthelmintic potential of comp. 81-470 by an adhesive diffusion controlled transdermal system against representatives of commonly occurring helminth parasites in experimental animals.

MATERIALS AND METHODS

Test Parasites and Drug Testing

The efficacy of comp. 81-470 in transdermal tapes was tested against adult and larval forms of Ancylostoma ceylanicum in hamsters and Nippostrongylus brasiliensis, Hymenolepis nana in rats.

Animals

Laboratory bred male hamsters (40-60 g) and male rats of UF strain (35-40 g) served as experimental hosts. They were housed in plastic cages in a climatically controlled animal room (25–28°C) and had free access to standard rodent food pellets (Lipton India Ltd.) and water.

Preparation of Transdermal Tape

Transdermal tapes (Jain et al., 1991) of 1cm² containing 1.25, 2.5, 3.5, 5.0 and 10.0 mg/cm² of comp. 81-470 were prepared by incorporating 63.5 parts of polyvinyl pyrrolidone (PVP), 18.5 parts of propylene glycol, 16.5 parts of polyethylene glycol 400 (PEG-400), 1 part of n-octanol, 0.5 parts of urea and the required amount of drug.

The required amount of drug and urea was first dissolved or finely dispersed in PEG-400 by sonication. This was added to a homogenous mixture of PVP, n-octanol and propylene glycol with homogenous mixing. The resulting residue was deaerated under vacuum for two hours, left overnight, spread over a plastic backing sheet and cut into pieces of 1 cm² with the help of a circular die.

Anthelmintic Assay

1. Ancylostoma ceylanicum (hookworm)

Adulcicidal Action:

The drug testing was carried out by the procedure described earlier by Ray et al.
Hamsters were orally infected with \(60\pm5\) infective larvae (L3) of *A. ceylanicum*. The hamsters found positive by ovoscopic examination on day 17 were used in therapeutic trials. The dorsum of hamsters was depilated and the animals were randomized in control and experimental group each with 5 hamsters. The tapes containing test compound were fixed on the shaven area to ensure intimate contact with skin. A strip of adhesive tape was wrapped around the belly. The control animals received only placebo tape. The animals were kept for 5 days in individual cages. On day 6 the treated and untreated hamsters were sacrificed under deep ether anaesthesia and the worms in the intestine were counted (Katiyar et al., 1984). The efficacy was expressed in terms of percent worm reduction and absolute host clearance compared to untreated controls (Katiyar et al., 1984, 1987).

**Larvicidal:**

To assess efficacy against various metamorphic forms, different groups of hamsters were infected with \(60\pm5\) L3, L4 and L5. Two hours later, the medicated tapes were applied and restored there for 5 days. Similar protocol was followed for control groups. The treated and untreated groups infected with L3 were autopsied on day 18, those with L4 on day 12 and with L5 on day 9 of infection (Ray et al., 1972). The adult worms were counted and efficacy was evaluated as described earlier.

2. *Nippostrongylus brasiliensis* (trichostrongylid)

**Adulticidal:**

The rats were infected with 500 L3 subcutaneously (Misra et al., 1981). The therapeutic trials were initiated on day 9 post-infection. The application of tapes and efficacy evaluation were done in the same manner as described for *A. ceylanicum* (adulticidal).

**Larvicidal:**

To study the action against L3, L4 and L5, the medicated tapes were applied on day 0, day 2 and day 3 (Haley, 1962; Ogilvie and James, 1971) respectively and kept there for 5 days.

Experimental animals were autopsied on day 9 p.i., the adult worms enumerated and efficacy assessment made as described above.

3. *Hymenolepis nana* (tape worm)

**Adulticidal:**

Male albino rats (35-40 g) of UF strain were given 200 eggs of *H. nana* by gavage (Gupta et al., 1979). The adults mature in the intestine on day 16-17 p.i. (Grassi, 1887; Hunnin, 1935).

To study effect on adult worms, the tapes were applied on day 16 p.i. and left there for 5 days. On day 22 p.i. the experimental and control rats were sacrificed and the adult worms, if any, were counted. The criterion of activity was the absolute clearance of parasites from the host.
RESULTS

Efficacy Profile:

The efficacy of comp. 81-470 administered through transdermal tapes has been presented in Tables 1 and 2.

A. ceylanicum

Adulticidal Activity:

Tapes containing 5 mg of comp. 81-470 resulted in complete elimination of parasites. Lower amounts yielded dose dependent efficacy (Table 1).

Larvicidal Activity:

Against L₃ the tapes containing 5 mg of compound were only partially effective (66.9%). Reduction in dose had proportionate efficacy. Similar dose regimen tried against L₄ and L₅ stages yielded higher efficacy than that recorded against L₃ stage.

N. brasiliensis

Adulticidal Activity:

The animals applied with 20 mg of the compound in tape showed 92.7% worm reduction in comparison to untreated controls. 10 mg of compound yielded 53.4% worm reduction.

Larvicidal Activity:

As in case of A. ceylanicum, the test compound exerted better action against L₄ and L₅ stages, compared to L₃ stage. Two doses 10 mg and 5 mg/tape were tried against L₃, L₄ and L₅ stages. The respective clearance at 10 mg dose was 33.1%, 68.1% and 80.9%. 5 mg dose

Table 1. Anthelmintic efficacy of comp. 81-470 in transdermal tape against adult helminths

<table>
<thead>
<tr>
<th>Parasite (Host)</th>
<th>Dose* mg/tape</th>
<th>Animal cured/ Treated (Replicate)</th>
<th>Per cent cure</th>
<th>worm recovery mean (range)</th>
<th>Per cent worm reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ceylanicum</td>
<td>5.0</td>
<td>18/18 (5)</td>
<td>100.0</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>6/10 (3)</td>
<td>60.0</td>
<td>3.9 (0—13)</td>
<td>85.4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0/ 6 (2)</td>
<td>0.0</td>
<td>11.7 (7—18)</td>
<td>56.6</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0/ 6 (2)</td>
<td>0.0</td>
<td>28.3 (15—43)</td>
<td>0.0</td>
</tr>
<tr>
<td>N. brasiliensis</td>
<td>20.0</td>
<td>0/ 6 (2)</td>
<td>0.0</td>
<td>18.7 (9—28)</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>0/12 (4)</td>
<td>0.0</td>
<td>50.3 (8—96)</td>
<td>80.4</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0/ 6 (2)</td>
<td>0.0</td>
<td>119.2 (35—195)</td>
<td>53.4</td>
</tr>
<tr>
<td>H. nana</td>
<td>10</td>
<td>14/20 (4)</td>
<td>70.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1/ 5 (2)</td>
<td>20.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pooled control</td>
<td>Parasite</td>
<td>Mean (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. ceylanicum</td>
<td>26.6 (17—42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N. brasiliensis</td>
<td>255.8 (108—405)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H. nana</td>
<td>12.8 (8—22)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Tapes applied for 5 days
Table 2. Anthelmintic efficacy of comp. 81-470 in transdermal tapes against developing forms

<table>
<thead>
<tr>
<th>Parasite (Host)</th>
<th>Dose* (mg/tape)</th>
<th>Stage of parasite</th>
<th>Animal cured/ Treated (Replicate)</th>
<th>Per cent cure</th>
<th>Per cent worm reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ceylanicum</td>
<td>5</td>
<td>L₃</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>10.3 (8-12)</td>
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<tr>
<td></td>
<td></td>
<td>L₄</td>
<td>2/6 (2)</td>
<td>33.3</td>
<td>3.0 (0-8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L₅</td>
<td>3/6 (2)</td>
<td>50.0</td>
<td>2.3 (0-8)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>L₃</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>11.5 (4-18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L₄</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>6.0 (2-10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L₅</td>
<td>1/6 (2)</td>
<td>16.7</td>
<td>2.5 (0-15)</td>
</tr>
<tr>
<td>N. brasiliensis</td>
<td>10</td>
<td>L₃</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>130.0 (82-159)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L₄</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>62.2 (36-130)</td>
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<tr>
<td></td>
<td></td>
<td>L₅</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>37.3 (21-61)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>L₃</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>180.0 (77-236)</td>
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<tr>
<td></td>
<td></td>
<td>L₄</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>104.0 (71-156)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L₅</td>
<td>0/6 (2)</td>
<td>0.0</td>
<td>69.3 (46-98)</td>
</tr>
<tr>
<td>Pooled control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Tapes applied for 5 days

showed dose dependent efficacy.

H. nana

Of the two doses used, 10 mg exhibited 70% host clearance. The lower amount (5 mg) could cure only 20% of the treated animals.

DISCUSSION

Any system with equal bio-efficacy and demanding single application is likely to get wide recognition. Such devices will be of particular importance in the management of ailments in infants and in comatose, allergic and mentally retarded patients. This may also be of immense use in pets.

While medication through transdermal tapes has many overt advantages, this has not yet been adopted in clinical helminth chemotherapy though certain reports are on record of drug delivery by topical application (Taylor et al., 1980; Greene et al., 1983).

Comp. 81-470 by transdermal application has been found quite effective against various helminths, although this device demanded slightly higher amount of drug than by oral administration (Katiyar et al., 1984; 1987) to achieve comparable efficacy.

The results of the present study suggest that the compound could be beneficially delivered by transdermal route overcoming the problems of repeat medication. However, the
study being of preliminary nature needs further exploration to establish its clinical utility. In the event of success, the system is likely to open new vistas in the field of helminth chemotherapy.

ACKNOWLEDGEMENTS

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