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EFFICACY OF NICLOSAMIDE AS A POTENTIAL TOPICAL ANTIPENETRANT (TAP) AGAINST CERCARIAE OF *SCHISTOSOMA MANSONI* IN MONKEYS

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A 1% (W/V) formulation of Niclosamide (2', 5-Dichloro-4-nitrosalicylanilide) (TAP) was tested on Cebus apella *monkeys as a topical prophylactic against schistosomiasis mansoni. Two experiments were conducted using the same formulation. In the first experiment, the TAP provided complete protection against schistosomiasis for 3 days. Of the 4 monkeys treated with TAP 7 days before exposure to* Schistosoma mansoni *cercariae, 2 were completely protected. The remaining 2 monkeys of the 7 day treatment group had a 78% or greater reduction in adult worm burdens when compared to the placebo treated monkeys. The second experiment was designed to determine the time between day 3 and 7 when the TAP no longer provided complete protection. However, all of the TAP treated monkeys in this experiment were completely protected, even the monkeys treated 7 days earlier. In both experiments, all monkeys used as infection controls and those receiving only the placebo became infected and showed typical experimental schistosomiasis. These results demonstrate that the TAP could provide fast acting, short-term protection to people who must enter cercariae infested water.*

Key words: *Schistosoma mansoni* – cercariae – niclosamide – protection

During the past several decades, many reports have been published concerning the identification and testing of substances administered to experimental animals, either systematically or topically, for the prevention of cercarial penetration of skin (Churchill et al., 1945; Wright et al., 1948; Hunter et al., 1956; Kemp et al., 1956a; Chen & Kwo, 1958; Campbell & Cuckler, 1961; Bruce & Sadun, 1966; Pellegrino, 1967; Van Rensburg, 1972; Fripp & Armstrong, 1973; Austin, 1974; Khalil, et al., 1986; Lambertucci, 1988). None of the above studies yielded results which warranted further studies beyond the preliminary experiments. More recently, three compounds have been studied for their anticercarial properties. The broad spectrum antischistosomal drug, Amoscanate, was studied for its anticercarial activity and found to provide approximately 90% protection to mice when applied to skin at 1 day prior to cercarial exposure (Greene et al., 1983). Hexachlorophene in absolute methanol or 70% isopropanol provided 95% protection when applied to mouse tail skin up to three days before exposure to schistosome cercariae (Grenan et al., 1985). Because neither of these compounds provided complete protection, further testing beyond the mouse model system was not undertaken. However, 21 of 39 salicylanilide analogs tested as topical antipenetrants against cercariae of *Schistosoma mansoni* in mice provided 98% or better protection of 24 h (Miller & Reid, 1986, 1987). One of the most promising of these 21 compounds was the anthelmintic compound Niclosamide.

Niclosamide was tested as a potential topical antipenetrant in rodent test model systems. The compound, when prepared in absolute ethanol, provided complete protection against *S. mansoni* and *S. haematobium* cercariae when applied at 1 day before exposure (Miller & Reid, 1987). Based on these results Niclosamide was prepared as a 1% formulation and studied for its potential as a topical antipenetrant (TAP) in a primate test model system. The results of these studies are presented in this paper.

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MATERIALS AND METHODS

Schistosome strains – An oxamniquine-resistant strain of *S. mansoni* (MAP) obtained from Brazil in 1986, was used to expose monkeys in experiment I (Table I). This strain is maintained in laboratory bred *Biomphalaria glabrata* snails (BH-Belo Horizonte, State of Minas Gerais, Brazil) and outbred CD_1 Albino male mice. A niridazole resistant strain of *S. mansoni* (MNK), obtained from Kenya in 1984, was used for monkey exposures in experiment II (Table II). The Kenyan strain is maintained in laboratory bred *B. sudanica* and outbred CD_1 Albino male mice. The techniques for laboratory maintenance of the schistosome strains and their snail intermediate hosts were those published previously (Liang et al., 1987).

Primates – Twenty-four male and twenty-nine female *Cebus apella* monkeys, with a weight range of 2.5-4.5 kg and estimated age of 4-6 years, were purchased from Worldwide Primates, Inc., Miami, Florida. All primates were housed individually in stainless steel cages in a ventilated ward maintained at an ambient temperature of 25 °C and at a relative humidity of 50-60%. The monkeys were fed a 15% protein primate chow (Ralston Purina Co., St. Louis, Missouri) twice daily, supplemented with fresh apples, bananas, or oranges. Upon arrival at the Center for Tropical Diseases in Massachusetts, the monkeys were placed under quarantine for a 6-8 week period during which time they were tested for tuberculosis and given physical examinations, including weighings. Fecal samples were also taken and examined for parasites and all monkeys with helminth infections were given two oral doses of 200 mg/kg of thiabendazole 10 days apart. At the start of the experiments, all animals were clinically normal.

Throughout the experiments, animals were maintained as outlined by the guide for Care and Use of Laboratory Animals (U. S. Department of Health and Human Services, Vol. 14, No. 8, June 25, 1985).

Drug – Niclosamide (2', 5-Dichloro-4'-nitrosalicylanilide (Bayer)) is a pale yellow crystalline powder which is practically insoluble in water and only moderately soluble in ethanol, chloroform and ether. Its molecular weight is 327.1 (Van den Bossche, 1985). The topical antipenetrant formulation (TAP) containing 1% (W/V) Niclosamide and a placebo were prepared by Miles Pharmaceuticals, Division of Miles Laboratory Inc., West Haven, Connecticut, 06516, U.S.A. Both the TAP and the placebo were prepared in the same vehicle, however Food and Drug Administration approved dyes and citric acid were added to the placebo to adjust the color and pH.

Prophylactic studies – Experiment I (Table I). Twenty-three monkeys were divided into 7 groups. Experimental groups I-VI each treated at different time intervals, contained 4 monkeys which received the TAP and 2 which received the placebo. Group VII contained 5 monkeys which were not treated and served as infection control animals.

TABLE I

Experimental design (No. 1) for *Schistosoma mansoni* topical antipenetrant (TAP) study in *Cebus apella* monkeys

Group No.	Days treated pre-infection	Treatment type	No. of animals
I	7	TAP	4
II	7	Placebo	2
III	3	TAP	4
IV	3	Placebo	2
V	1	TAP	4
VI	1	Placebo	2
VII	0	Infection control	5

Total number of animals = 23.

TABLE II

Experimental design (No. 2) for the *Schistosoma mansoni* topical antipenetrant (TAP) study in *Cebus apella* monkeys

Group No.	Days treated pre-infection	Treatment type	No. of animals
I A	7	TAP	3
I B	7	Placebo	2
II A	6	TAP	3
II B	6	Placebo	2
III A	5	TAP	3
III B	5	Placebo	2
IV A	4	TAP	3
IV B	4	Placebo	2
V A	3	TAP	3
V B	3	Placebo	2
VI	0	Infection control	5

Total number of animals = 30.

Experiment II (Table II). Thirty monkeys were divided into 11 groups. Experimental groups I-X, each treated at different time intervals, contained 3 monkeys which received the TAP and 2 which received the placebo. Group XI contained 5 monkeys which were not treated and which served as infection control animals.

All monkeys were anesthetized with intramuscular injections of ace promazine maleate and ketamine hydrochloride (Aveco Co. Inc., Fort Dodge, Iowa, 50501) to temporarily immobilize them during treatment applications, washings and exposure to cercariae. All monkeys, regardless of treatment, had their right arms clipped free of the coarse hair with barber's clippers. The drug solution was applied using a 4 x 4" gauze pad (clamped by, and wrapped around a hemostat) to the shaven arms of the experimental animals. The entire arm was treated with the drug by wiping it from the shoulder and down the arm to include the hands. Animals receiving the placebo were treated in the same manner. It required approximately 20 ml of either the TAP or placebo to completely cover the arm. Drug solution and placebo were applied at either 1, 3, 4, 5, 6, or 7 days prior to exposure to schistosome cercariae (Tables I and II).

The right arms of all experimental animals, including the infection control animals (those receiving neither the TAP nor placebo), were washed for 30 minutes post-treatment to remove any excess compound. Each arm was placed in a 152 x 450 mm pipet washer and washed with filtered water at one cycle per minute for 30 min. (One cycle is defined as the amount of water filling the pipet washer from the level of the finger tips to the shoulder and draining from the shoulder to the finger tips). The infection control animals, which did not receive drug or placebo, were also washed.

Monkeys were immobilized as previously described, and the right arm of each one was immersed in a container with 1.4 l of water containing *S. mansoni* cercariae. The water level in the container was such that it was below the uppermost washed area of the forearm. Exposure was for a period of 45 min, after which the arm was withdrawn and allowed to dry before monkeys were returned to their respective quarters.

To determine the viability of cercariae, 10 mice (Experiment I) and 20 hamsters (Experiment II) were exposed to shistosome cercariae from the same pool used to expose the monkeys. Five mice or 10 hamsters were exposed just prior to the exposure of the first group of monkeys and 5 mice or 10 hamsters were exposed after the last group of monkeys was exposed.

Following exposure of the monkeys, 60 ml aliquots of formalin were added to the exposure solution. The contents of the exposure containers were concentrated by pouring the infection solution through a 7.6 cm diameter, 20 μ sieve. The trapped cercariae were washed off the sieve with filtered water into a petri dish, stained with 4% Lugol's iodine, and counted. Cercariae remaining in mouse exposure containers were also collected, stained, and counted.

Throughout the experiments, monkeys were observed for any clinical signs of the schistosomiasis as well as toxic or abnormal manifestations following treatment with the TAP.

Beginning on day 28 post-exposure and continuing twice per week until the termination of the experiments, fecal samples from each monkey were examined for schistosome eggs using the AMS-III quantitative method (Hunter et al., 1948).

Necropsies were performed approximately 49-54 days after treatment. Each monkey was anesthetised with ketamine, exsanguinated by cardiac puncture. Euthanasia was completed by the intravenous administration of heparinized pentabarbitol sodium. Worms were recovered from the portal and mesenteric circulation using a modified Perf-O-Suction method (Radde et al., 1961) with citrated saline (0.85% sodium chloride, 0.75% sodium citrate). Following perfusion, the intestines and liver were removed and examined for the presence of worms. The number, sex, condition (normal, stunted, or immature) of the worms were determined.

Therapeutic efficacy of the TAP was based on the number of live worms recovered from treated animals as compared to the average number of live worms recovered from untreated animals according to the following equation (Kemp et al., 1956)

$$\frac{x - y}{x} \times 100 = \text{relative protection}$$

TABLE III

Results of examination of exposure containers for cercariae after exposure of monkeys (Experiment I)

Control/ Drug group	Animal		Cercarial count in exposure containers (Intact and Non-Intact)	% of cercariae in exposure containers (Intact and Non-Intact)
	No.	Sex		
Infection control	24	F	102	26
	25	M	96	26
	30	F	117	29
	31	F	81	20
	32	F	113	28
Placebo 1 day	20	F	192	48
	23	F	83	21
Placebo 3 days	9	M	66	17
	14	F	105	26
Placebo 7 days	3	M	132	33
	6	M	140	35
TAP 1 day	15	F	241	60
	19	M	303	76
	21	F	367	92
	22	F	336	84
TAP 3 days	7	M	215	54
	8	F	186	47
	11	M	266	67
	12	F	231	58
TAP 7 days	1	M	190	48
	2	M	274	69
	4	F	100	25
	5	M	180	45

where x = average number of live worms recovered from untreated animals and y = number of live worms from protected animals.

RESULTS

Animals which became patent with schistosomiasis infection displayed overt signs typical of the disease including: hemorrhagic diarrhea, mucus in the feces, anorexia and mild lethargy.

The gross pathology observed in the infected animals was consistent with experimental schistosomiasis. The livers showed granular and fibrotic texture and appearance, as well as surface spotting and some discoloration (Sadun et al., 1966).

Mice and hamsters used to test the viability of the cercariae employed in monkey exposures also presented symptoms of the schistosome infection including diarrhea, anorexia and lethargy. Most of the mice died from complications of the infection prior to termination of the experiment.

Experiment I – The percentage of cercariae (intact and non-intact) remaining in the water of the exposure chambers after 45 min of contact with subject monkey skin ranged from 17% to 92% respectively depending on the particular group of monkeys. The percentage of cercariae remaining in the exposure chambers of the infection control group of monkeys, which did not receive any drug application to their skin, was very similar (20% to 29%) to that of the placebo group of monkeys (17% to 38%) (Table III). The number of cercariae remaining in the viability control mouse chambers was very low as compared to the numbers of cercariae remaining in monkey exposure chambers.

The treated monkeys showed no obvious signs of drug toxicity. The prophylactic efficacy of the TAP formulation against drug-resistant (oxamniquine-hycanthone-resistant) cercariae of Brazilian (MAP-strain) origin is presented in Table IV. At necropsy, 49-55 days after exposure to cercariae, complete protection was observed in animals receiving drug application to their skin at 1 and 3 days before

TABLE IV

Prophylactic effect of the topical antipenetrant (TAP) against *Schistosoma mansoni* (Brazilian strain) in *Cebus apella* exposed experimentally to 400 cercariae

Control/ drug group	Animal		Worm burdens after perfusion: Collection filter[a]				Tissue examination[b]				Total # worms	Mean value	Efficacy (%)
	No.	Sex	M[c]	F[d]	SM[e]	SF[f]	M	F	SM	SF			
	24	F	38	24	–	–	15	16	–	–	93		–
Infection	25	M	19	11	–	–	16	15	–	–	61		–
	30	F	7	6	–	1	21	22	–	2	59	67.2	–
Control	31	F	6	6	–	–	3	3	–	–	18		–
	32	F	39	43	–	–	10	13	–	–	105		–
Placebo	20	F	10	8	–	–	–	–	–	–	18		–
1 day	23	F	9	3	–	–	5	5	–	–	22	20	–
Placebo	9	M	52	58	–	–	12	12	–	–	134		–
3 days	14	F	16	26	–	–	6	5	–	–	53	93.5	–
Placebo	3	M	13	12	–	–	1	1	–	–	27		–
7 days	6	M	37	38	–	–	15	16	–	–	106	66.5	–
TAP	15	F	–	–	–	–	–	–	–	–	0		100
1 day	19	M	–	–	–	–	–	–	–	–	0		100
	21	F	–	–	–	–	–	–	–	–	0	0	100
	22	F	–	–	–	–	–	–	–	–	0		100
TAP	7	M	–	–	–	–	–	–	–	–	0		100
3 days	8	F	–	–	–	–	–	–	–	–	0		100
	11	M	–	–	–	–	–	–	–	–	0	0	100
	12	F	–	–	–	–	–	–	–	–	0		100
TAP	1	M	–	–	–	–	–	–	–	–	0		100
7 days	2	M	–	–	–	–	–	–	–	–	0		100
	4	F	8	6	–	–	–	–	–	–	14	5	78.9
	5	M	1	3	–	–	1	1	–	–	6		91.0

a: filter used to trap worms perfused from the liver and mesenteric veins.
b: examinations of liver, mesenteric veins, and adipose tissue containing veins for lodged worms after perfusion.
c: male worms.
d: female worms.
e: stunted male worms.
f: stunted female worms.

exposure to schistosome cercariae. Of the group of animals receiving the drug application at 7 days before parasite exposure, 2 were completely protected, while the remaining 2 had levels of protection at 78.9% and 91% respectively (Table IV). At necropsy, mature worms of both sexes (14 and 6 respectively) were recovered and viable eggs were obtained in feces (30-35 days after exposure) and organ tissues from the 2 monkeys which were not protected.

All monkeys of the infection control and placebo groups were infected. Schistosome eggs appeared in their feces at 30-35 days post-exposure. The worm burden levels observed at perfusion for both infection and placebo groups of animals were very similar in individual ranges and overall group averages (Table IV). Each of these animals had viable eggs in their organs and worms were mostly mature adults of both sexes.

Experiment II – In Experiment I, complete protection against schistosomiasis was observed up to and including 3 days after application of the TAP, but only partial protection occurred 7 days after treatment. Thus, for Experiment II a protocol was designed to determine the exact time in which a breakthrough would occur among groups of monkeys treated at 3, 4, 5, 6 or 7 days prior to exposure to cercariae. The drug treatment and cercarial exposure methods used in Experiment II were the same as those

TABLE V

Results of examination of exposure containers for cercariae after exposure of monkeys (Experiment II)

Control/ Drug group	Animal		Cercarial count in exposure containers (Intact and Non-Intact)	% of cercariae in exposure containers (Intact and Non-Intact)
	No.	Sex		
Infection control	18	F	188	47
	26	M	303	76
	27	M	241	60
	29	F	220	54
	30	M	211	53
Placebo 3 days	23	M	241	60
3 days	25	M	230	58
4 days	20	F	196	49
4 days	28	F	258	65
5 days	13	F	219	55
5 days	15	F	250	63
6 days	8	F	266	67
6 days	10	M	207	52
7 days	3	M	239	60
7 days	5	F	237	59
TAP 3 days	21	M	290	73
3 days	22	M	312	78
3 days	24	M	275	69
4 days	16	F	378	95
4 days	17	M	340	85
4 days	19	M	279	70
5 days	11	F	332	83
5 days	12	M	282	71
5 days	14	F	284	71
6 days	6	M	251	63
6 days	7	F	303	76
6 days	9	F	243	61
7 days	1	F	315	79
7 days	2	F	237	59
7 days	4	F	280	70

used in Experiment I except that additional groups of monkeys were treated at 4, 5 and 6 days before exposure to cercariae.

The percentage of cercariae (intact and non-intact) remaining in the water of exposure chambers after 45 min of contact with treated monkeys skin ranged from 47% to 95% respectively, depending on the particular group of monkeys. The percentage of cercariae remaining in the exposure chambers of the infection control group of monkeys, which did not receive any drug application to their skin, was very similar (47% to 76%) to that of the placebo group of monkeys (49% to 67%) (Table V).

Again, no toxic manifestations were observed in the TAP-treated monkeys. The prophylactic efficacy of the TAP formulation against a drug-resistant (niridazole-resistant) strain of Kenyan (MNK-strain) origin is presented in Table VI. At necropsy, 49-55 days after exposure, complete protection was observed for all animals receiving the TAP application to their skin.

All monkeys of the infection control and placebo groups were found to be infected. Schistosome eggs were found in their feces at 30-35 days after exposure to cercariae. The worm burden levels observed at perfusion for animals of the infection control and placebo groups were very similar both in individual ranges and in overall group averages (Table VI). Each of the animals harbored viable eggs in their organs and worms were mostly mature adults of both sexes.

Twenty hamsters used to monitor the viability of cercariae used in exposure of monkeys in this experiment were found to be infected. The percentage of adult schistosomes recovered at perfusion ranged from 22% to

TABLE VI

Prophylactic effect of the topical antipenetrant (TAP) against *Schistosoma mansoni* (Kenyan strain) in *Cebus apella* exposed experimentally to 400 cercariae

Control/ drug group	Animal		Worm burdens after perfusion: Collection filter[a]				Tissue examination[b]				Total # worms	Mean value	Efficacy (%)
	No.	Sex	M[c]	F[d]	SM[e]	SF[f]	M	F	SM	SF			
Infection control	18	F	17	13	–	–	5	5	–	–	40		–
	26	M	10	8	2	1	7	3	–	–	31		–
	27	M	26	5	–	–	6	7	–	–	44	35.2	–
	29	F	9	8	–	–	6	6	1	–	30		–
	30	M	4	4	2	1	10	10	–	–	31		–
Placebo													
3 days	23	M	8	4	–	–	4	4	–	–	20		–
3 days	25	M	3	5	1	–	5	8	–	–	22	21	–
4 days	20	F	7	5	–	–	5	5	–	–	28		–
4 days	28	F	15	5	–	–	3	3	–	–	26	27	–
5 days	13	F	13	6	–	–	2	2	–	–	23		–
5 days	15	F	32	14	–	–	6	6	–	–	58	40.5	–
6 days	8	F	8	7	–	–	7	6	–	–	28		–
6 days	10	M	6	6	–	–	9	11	–	–	22	25	–
7 days	3	M	24	15	–	–	5	5	–	–	49		–
7 days	5	F	9	9	–	–	13	13	–	–	44	46.5	–
TAP													
3 days	21	M	–	–	–	–	–	–	–	–	0		100
3 days	22	M	–	–	–	–	–	–	–	–	0	0	100
3 days	24	M	–	–	–	–	–	–	–	–	0		100
4 days	16	F	–	–	–	–	–	–	–	–	0		100
4 days	17	M	–	–	–	–	–	–	–	–	0	0	100
4 days	19	M	–	–	–	–	–	–	–	–	0		100
5 days	11	F	–	–	–	–	–	–	–	–	0		100
5 days	12	M	–	–	–	–	–	–	–	–	0	0	100
5 days	14	F	–	–	–	–	–	–	–	–	0		100
6 days	6	M	–	–	–	–	–	–	–	–	0		100
6 days	7	F	–	–	–	–	–	–	–	–	0	0	100
6 days	9	F	–	–	–	–	–	–	–	–	0		100
7 days	1	F	–	–	–	–	–	–	–	–	0		100
7 days	2	F	–	–	–	–	–	–	–	–	0	0	100
7 days	4	F	–	–	–	–	–	–	–	–	0		100

a: filter used to trap worms perfused from the liver and mesenteric veins.
b: examinations of liver, mesenteric veins, and adipose tissue containing veins for lodged worms after perfusion.
c: male worms.
d: female worms.
e: stunted male worms.
f: stunted female worms.

89%. All hamsters showed gross liver, spleen and intestinal pathology.

DISCUSSION

Previous attempts to identify chemicals which could be used as topical antipenetrants against schistosome cercariae have been only partially successful. This was due either to the limited amount of protection afforded or because of the toxicity of the compound tested. Ideally, a successful topical antipenetrant should be able to stop invading cercariae at the skin-water boundary and should be absorbed and/or bound by the skin so that water-washing of the treated surface generally will not reduce the original level of protection afforded by the compound. In addition, the topical antipenetrant should not generate toxic serum levels of the active compound ingredient and should provide protection against cercarial penetration for reasonable periods of time. Niclosamide, formulated as a 1% solution, appears to have met all of the above criteria.

The data predsented in Tables IV and VI clearly demonstrates that the TAP provides protection for up to 7 days in primates. The 2 monkeys becoming infected when exposed to cercariae at 7 days after application of the TAP in Experiment I indicates either that the upper limit of protection occurs about this time or that the application of the chemical was not complete and therefore a breakthrough occurred. The number of intact and non-intact cercariae observed remaining in exposure containers (Tables III and V) indicates that the invading cercariae did not penetrate at the skin-water boundary. This may be the result of the absorption and binding of Niclosamide into the skin. Further studies concerning the mode of action of Niclosamide are needed to validate this hypothesis. Water-washing of the treated arms for 30 min after application of the TAP did not reduce its effectiveness. Whether or not washing of the treated skin surface area with soap during bathing will reduce the level of protection afforded was not determined. This aspect of the effectiveness of TAP should also be investigated.

No toxic manifestations such as skin rash or other gross abnormalities were observed in any of the TAP-treated monkeys. Nor were any toxic manifestations observed for monkeys receiving the placevo. Currently, Niclosamide has been used extensively to treat several species of tapeworm infections in both man and animals and no toxic effects are known. The compound is very poorly absorbed through the intestinal wall of the host and no systemic pharmacological effects have been observed (Hecht & Gloxhuber, 1960; Gonner et al., 1963; Van Den Bossche, 1985).

The results of this study demonstrate that a topically applied formulation containing 1% Niclosamide protects non-human primates from schistosomiasis mansoni for at least 7 days. Such a formulation may prove useful for providing fast-acting, short-term protection for those individuals who must enter water harboring shistosome cercariae.

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