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HEXACHLOROPHENE AS A TOPICALLY APPLIED CHEMICAL FOR PROPHYLAXIS AGAINST SCHISTOSOMA MANSONI INFECTIONS IN MICE

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SUMMARY

Treatment of mouse tail skins with hexachlorophene (1.25% w/v) in absolute methanol or 70% isopropanol suppressed **Schistosoma mansoni** infections by more than 95% even when the application was performed up to three days prior to exposure to cercarial suspensions by tail immersion. Treatment with concentrations of 0.313% or higher one day prior to exposure provided at least 98% protection when the treated surface was not subjected to water washes of greater duration than 1/2 hour. Tail immersion application of 1.25% hexachlorophene one day prior to exposure still provided 87-92% protection after 3 hours water wash. Wipe application of 1.25% hexachlorophene three days prior to exposure still provided 93% protection following 3 hours water wash. High cercarial recoveries from exposure tubes at the end of exposure periods indicated high antipenetrant activity for hexachlorophene. Sufficient hexachlorophene leached from treated tail skins into the surrounding water to affect subsequently added cercariae so that they were no longer infective to untreated mice.

INTRODUCTION

Since World War II many laboratories have screened chemicals for their abilities to prevent schistosome cercarial penetration following application to skin. Hexachlorophene has emerged as one of the most active and persistent chemicals. Ointments consisting of hexachlorophene (5%) in wool fat (lanolin) [4], hexachlorophene (3%) in Pro-derna [4] and hexachlorophene (1.0%) in lanolin[1] have been recommended for further evaluation as they were highly effective and well tolerated by mice. PHisoHex [12] and Schistopel [2,13] two hexachlorophene containing cleansers, have been shown to provide protection to rodents when applied to skin prior to cercarial exposure. Dial soap, which contained hexachlorophene, did not, however, provide useful protection [4].

For several years our laboratory has been testing chemicals for topical, prophylactic, antischistosomal activities [3]. In this paper, we report prophylactic activities for alcoholic solutions of hexachlorophene applied to mouse tail skins.

MATERIALS AND METHODS

Treatments

Four to nine-week-old male and female mice (*) (WRAIR colony, outbred Charles Ri-

(*) In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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ver, ICR strain) were used. Hexachlorophene from ICN Pharmaceuticals, Inc., K and K Labs Division, was used in studies presented in Tables I through IV and Table VI. Hexachlorophene from Sigma Chemical Company was used in studies presented in Table V. Absolute methanol was the solvent in studies presented in Tables I through III and Table VI. Isopropanol (70%) was the solvent in studies presented in Table IV and Table V. Treatments were by tail immersion in the prepared solutions for 5 minutes in the studies presented in Tables I through III, and groups 1, 2 and 5 in Table IV. Treatments were by 5 wipes of the tail skin with a gauze pad saturated with the appropriate solutions in groups 3, 4 and 6 in Table IV and all groups in Table V. Treatments were performed one day prior to cercarial exposure except where specified otherwise in Table I, Table II and Table V.

The ability of treatment protection to withstand elution by various water wash durations was examined. Persistence of 90% or better protection following a minimal water wash (1/2 hour duration) has been used in our laboratory as a criterion for identifying treatments with useful prophylactic activities. As indicated in individual tables of this paper, the persistence of protection following longer water washes (1-5 hours) has also been examined. Water washes were performed by the immersion of mouse tails in pans filled with flowing tap water.

Infections

Mice were individually exposed to WRAIR, Puerto Rican strain, **Schistosoma mansoni** by immersion of the mouse tails for one hour in aged tap water containing cercariae. Exposure dosages for the various trials ranged from 96 to 151 cercariae per mouse.

Cercaria penetration studies

At the end of the one-hour exposure period, the mouse tails were withdrawn from their exposure vessels. The cercariae remaining in the vessels were killed and fixed by adding buffered stock formalin to achieve a final formalin concentration of 10%. Cercariae were then flushed from the exposure vessels and counted. The percentages of non-penetrating cercariae were calculated from these counts and the estimated cercarial exposure dosages. The mean percent non-penetrating cercarial recoveries for hexachlorophene treated and alcohol treated mouse groups were compared.

Seventh week post-exposure worm burdens

Mice were sacrificed by injections of heparinized sodium pentobarbital solution. While their hepatoportal circulations were perfused with citrated saline, their worms were collected on filter paper discs [10,11]. The recovered worms were counted and the percent recoveries as adult worms calculated on the basis of the estimated cercarial exposure dosages.

Hexachlorophene leaching study

Mouse tails used for pre-conditioning the exposure fluid were first treated by 5 minutes immersion in the appropriate solutions. Some tails were subsequently water washed. The treated tails were then immersed in the exposure fluid. These tails were removed and freshly shed cercariae were added. Shortly thereafter, one untreated mouse tail was added to each exposure vessel for the one hour exposure period. Subsequently, the mice and the unpenetrating cercariae were processed as in the other exposure studies.

Toxicity studies

Male ICR mice were treated by tail immersion for 5 minutes in methanolic hexachlorophene (1.25% w/v) (K and K Labs Division) or, in the case of control mice, methanol alone. Mouse mortality was recorded during subsequent days. Deaths within two days of treatment were considered to be due to treatment toxicity.

Data analyses

Ninety-five percent confidence intervals for mean percent cercarial recoveries and mean percent adult worm recoveries were calculated using the Student-t distribution.

RESULTS

Topical treatment with 1.25% (w/v) hexachlorophene provided better than 95% protection when mice were challenged three days post-treatment (Table I). By the sixth day, however, residual protection decreased to slightly better than 60%. The protective effect was associated with antipenetrant activity (Table I). 61.7 ± 11.3% of the cercarial dosage was recovered from the exposure vessels at the end of the exposure period. Under simpler conditions in which cercariae were added to exposure tubes without mouse tails, fixed and then counted, a recovery efficiency of 82 ± 5.0% was achieved.

TABLE I

Persistence of antipenetrant activity and protection upon aging against adult worm infections by methanolic hexachlorophene (1.25% w/v) treatment (*)

Treatment/treatment age	Percent recovered as non-penetrating cercariae	Percent recovered as Day 49 adult worms	Percent protection
Hexachlorophene + 1/2 hour water wash/three days	61.7 ± 11.3 (09)**	0.0 ± 0.0 (09)**	100
Methanol + 1/2 hour water wash/three days	1.1 ± 1.2 (10)	39.4 ± 9.8 (10)	—
Hexachlorophene + 1/2 hour water wash/six days	28.1 ± 9.7 (10)	11.2 ± 11.2 (09)	62
Methanol + 1/2 hour water wash six days	0.9 ± 0.7 (10)	29.4 ± 5.2 (09)	—
Hexachlorophene + 1/2 hour water wash/seven days	18.6 ± 13.1 (08)	10.6 ± 12.9 (08)	67
Methanol + 1/2 hour water wash/seven days	2.4 ± 1.8 (10)	31.7 ± 6.7 (10)	—

* Exposure dosage of 99.0 cercariae per mouse.
** Mean Percent ± 95% confidence interval (number of mice)

The protection observed after four 0.078% hexachlorophene applications spaced over a one week interval was not significantly greater than after a single application one day before cercarial exposure (Table II). The mean worm burdens following cercarial exposures were 12.9 ± 6.0 and 23.4 ± 8.6, respectively. The percent cercarial recoveries were 12.9 ± 8.3 and 5.4 ± 4.4, also not significantly different. The protection from the four treatments was, however, significantly less than from a single treatment of the four fold higher concentration (0.312%), administered one day pre-exposure (72% versus 99%, respectively) (Table II).

1.25% Hexachlorophene, applied one day before cercarial exposure, provided complete protection when treated surfaces were subjected to water washes of one hour duration (Table III). Protection declined appreciably following water washes of four to five hours. This decline was observed as both reduced non-penetrating cercarial recoveries and increased adult worm recoveries (Table III).

Wipe-on application of 1.25% hexachlorophene provided protection similar to that achieved by immersion (Table IV). Antipenetrant activities, as measured by non-penetrating cercarial recoveries, were not significantly different (76.6 ± 5.6 versus 71.5 ± 7.1 for unwashed groups, and 69.6 ± 7.5 versus 69.9 ± 7.3 for washed groups). Better than 99% protection was calculated on the basis of the worm burdens (Table IV). Wipe-on application of 1.25% hexachlorophene provided better than 90% protection when treatments were performed as long as three days before cercarial exposure, and after washes of up to three hours duration (Table V).

Within five minutes, the amounts of hexachlorophene, which leached from treated tail skins into the 4 ml exposure fluids, were sufficient to kill virtually all cercariae added subsequently (Table VI). Even when the treated surfaces had been water washed for five hours, hexachlorophene concentrations in the exposure fluids were sufficient to reduce cercarial penetration by 60% as seen both by a 40% reduc-

T A B L E II

Efficacies of multiple, low concentration hexachlorophene applications versus single application ***

Treatment	Percent recovered as non-penetrating cercariae	Percent recovered as Day 47 adult worms	Percent protection
0.078% (w/v) hexachlorophene 8 days, 6 days, 3 days and 1 day pre-exposure (4 treatments)	12.9 ± 8.3 (10)***	12.9 ± 6.0 (10)***	72
0.078% (w/v) hexachlorophene 6 days, 3 days and 1 day pre-exposure (3 treatments)	13.5 ± 9.0 (10)	19.1 ± 9.0 (09)	63
0.078% (w/v) hexachlorophene 3 days and 1 day pre-exposure (2 treatments)	7.5 ± 7.1 (10)	14.8 ± 7.6 (10)	70
0.078% (w/v) hexachlorophene 1 day pre-exposure (1 treatment)	5.4 ± 4.4 (10)	23.4 ± 8.6 (10)	56
0.312% (w/v) hexachlorophene 1 day pre-exposure (1 treatment)	58.5 ± 11.5 (10)	0.4 ± 0.6 (10)	99
Methanol 8 days, 6 days, 3 days and 1 day pre-exposure	1.2 ± 0.9 (10)	45.4 ± 9.1 (10)	—
Methanol 6 days, 3 days and 1 day pre-exposure	1.6 ± 1.6 (10)	52.0 ± 12.8 (09)	—
Methanol 3 days and 1 day pre-exposure	0.7 ± 0.7 (10)	49.1 ± 13.8 (08)	—
Methanol 1 day pre-exposure	0.4 ± 0.7 (10)	53.4 ± 13.3 (09)	—

* Exposure dosage of 114 cercariae per mouse
** All groups water washed for 1/2 hour
*** Mean percent ± 95% confidence interval (number of mice)

T A B L E III

Persistence following various water wash durations of antipenetrant activity and protection from infection provided by treatment with methanolic hexachlorophene (1.25% w/v) solution (*)

Duration of water wash	Percent recovered as non-penetrating cercariae	Percent recovered as Day 49 adult worms	Percent protection
One hour	58.9 ± 12.9 (10)** ***	0.0 ± 0.0 (10)**	100
Two hours	60.4 ± 13.3 (10)	0.3 ± 0.5 (10)	99
Three hours	42.3 ± 11.7 (10)	2.8 ± 2.4 (10)	92
Four hours	38.4 ± 13.6 (09)	10.7 ± 7.7 (10)	70
Five hours	17.1 ± 10.1 (10)	19.7 ± 11.3 (10)	49

* Exposure dosage of 96 cercariae per mouse
** Mean percent ± 95% confidence interval (number of mice)
*** Corresponding values for the methanol controls were less than 2.3%

T A B L E IV

Comparison of the prophylactic activities from treatment with isopropanolic hexachlorophene (1.25% w/v) solution by tail immersion and by tail wiping with a saturated gauze pad (*)

Treatment	Percent recovered as non-penetrating cercariae	Percent recovered as Day 49 adult worms	Percent protection
Hexachlorophene by tail immersion	76.6 ± 5.6 (15)**	0.0 ± 0.0 (14)**	100
Hexachlorophene by tail immersion + 1/2 hour water wash	69.6 ± 7.5 (15)	0.0 ± 0.0 (13)	100
Hexachlorophene by tail wiping 5 times with a saturated gauze pad	71.5 ± 7.1 (15)	0.0 ± 0.0 (15)	100
Hexachlorophene by tail wiping 5 times with a saturated gauze pad + 1/2 hour water wash	69.9 ± 7.3 (15)	0.1 ± 0.2 (15)	99+
70% isopropanol by tail immersion	2.2 ± 1.1 (15)	43.0 ± 4.9 (15)	—
70% isopropanol by tail wiping 5 times with a saturated gauze pad	2.9 ± 2.6 (15)	43.9 ± 3.9 (13)	—

* Exposure dosage of 151 cercariae per mouse
** Mean percent ± 95% confidence interval (number of mice)

TABLE V

Combined effects of variations of treatment age and post-treatment water wash duration on residual protection afforded mice by wipe application of isopropanolic hexachlorophene (1.25% w/v) (*)

Treatment age	Residual protection following water washes of various durations		
	0 hours	1 hour	3 hours
1 day	99+ (15)**	99 (14)	100 (15)
3 days	99+ (15)	99 (15)	93 (15)
6 days	54 (13)	43 (15)	33 (11)

* Exposure dosage of 104 cercariae per mouse
** Mean percent (number of mice)

tion in the non-penetrating cercarial recovery (43.2% down from 72.4%) and the 62% reduction in the resultant adult worm burdens (16.9% versus 44.2%).

Hexachlorophene (1.25% w/v) treatment was sometimes associated with paralysis or death. No deaths were observed in Trial 1 of a toxicity study in which 15 male mice of body weights ranging from 25 to 35 grams (mean 32.9) were treated with hexachlorophene but were not washed or exposed to schistosome cercariae. However, in Trials 2, 3 and 4, conducted similarly except that mouse weights ranged from 14 to 35 grams (group means ranging

TABLE VI

Cercaricidal effects of hexachlorophene leaching from treated tail skins into the cercarial exposure media (*)

Exposure medium pre-conditioned by 5 minutes immersion of mouse tail treated with:	Percent recovered as non-penetrating cercariae from pre-conditioned medium following exposure to untreated mouse tails	Percent recovered as adult worms in untreated mice	Percent reduction
Methanolic hexachlorophene (1.25% w/v)	72.4 ± 4.9 (15)**	0.1 ± 0.1 (15)**	99+
Methanolic hexachlorophene (1.25% w/v) + 5 hours water wash	43.2 ± 14.6 (15)	16.9 ± 11.8 (14)	62
Methanol	1.9 ± 1.1 (15)	44.9 ± 6.0 (12)	—
Methanol + 5 hours water wash	2.6 ± 2.2 (15)	44.2 ± 6.9 (14)	—

* Exposure dosage of 142 cercariae per mouse
** Mean percent ± 95% confidence interval (number of mice)

from 19.6 to 21.8), 12 of 15, 11 of 15 and 12 of 15 mice died by Day 2 post-treatment. No toxic deaths were observed in mice treated with methanol alone. Few toxic deaths were observed in our studies where seven to nine week old female mice were used.

DISCUSSION

Immersion or wipe treatment of tail skins with 1.25% (w/v) hexachlorophene provided 95% or better protection for up to three days post-treatment (Tables I and V). This high level of protection could be extremely valuable in reducing the likelihood of detectable symptoms from acute schistosomiasis, even with massive cercarial exposures. The level of protection after a single alcoholic hexachlorophene treatment was similar to that reported for Schistopel from six treatments spaced over the previous week (97% protection at Day 2 post-treatment) but superior to that reported for a single Schistopel treatment [2].

Preliminarily, 70% isopropanol would appear to have been as effective a solvent as absolute methanol. Toxicological factors, however, would have to be considered in selecting an optimal solvent for human application.

Protection of 92-100% was provided by treatment with alcoholic solutions of hexachlorophene (1.25% w/v) following a three hours water wash (Tables III and V). After four hours of water wash, however, protection had sometimes decreased to 70%, which is lower than the complete protection reportedly available from treatment with hexachlorophene (5%) in wool fat (lanolin) or hexachlorophene (3%) in Pro derna [4]. The level of protection available from treatment with Schistopel following a four hours water wash is unknown.

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Few published studies have evaluated changes in protection available from treatments when both treatment age and duration of pre-exposure water wash are varied. In this study, wipe applied hexachlorophene (1.25% w/v) in 70% isopropanol appeared to provide very useful protection up to a combination of three days age and three hours water wash.

Results of the hexachlorophene leaching study (Table VI) suggested that toxicity rather than repellency or skin lipid extraction was the cause of the high rate of penetration failures by cercariae. If mere repellency was responsible, cercariae attempting to escape from the exposure fluid might have penetrated the untreated tail skins and thus infected these mice. Because cercariae were in proximity to untreated mouse tails, normal penetration stimuli (lipids, etc.) were available. The protective effects of hexachlorophene leached from treated skins into infectious water may, however, only be observed in static water situations. Hence, where bare, treated skin is passed through continuously renewed infectious stream water, less protection may be observed. By stopping the larvae at the skin surface, hexachlorophene may prevent the skin rashes, fevers and coughs associated with "swimmers' itch" of schistosome cercarial origin [5-8].

Unfortunately, alcoholic hexachlorophene treatments can be very toxic to mice. An individual mouse's age, size and sex may determine its sensitivity to hexachlorophene. The toxicity of alcoholic, topical hexachlorophene treatments for humans is unknown. Wipe application may prove less toxic than immersion application. Concern over hexachlorophene's toxicity has, however, already limited some human use applications [9]. Toxicological studies on serum hexachlorophene levels and toxic reactions following topical alcoholic hexachlorophene treatments should be started with rodents and non-human primates.

These studies also suggest that (whether or not hexachlorophene is safe enough for human use) the strategy of utilizing organic vehicles to dissolve water insoluble compounds for skin application may be a useful procedure for providing durable, cosmetically acceptable and water resistant protection.

RESUMO

Hexaclorofeno como substância de aplicação tópica na profilaxia de infecção por Schistosoma mansoni **em camundongo**

O tratamento da pele da cauda de camundongos com hexaclorofeno (1,25% p/v), dissolvido em metanol absoluto ou isopropanol a 70% reduziu a infecção por **Schistosoma mansoni** em mais de 95%, mesmo quando a aplicação foi feita até três dias antes da exposição da cauda à suspensão de cercárias. O tratamento com concentrações de 0,313% ou mais, um dia antes da exposição, resultou em proteção de 98%, quando a superfície tratada não foi submetida a lavagem com água por tempo superior a 30 minutos. O tratamento com hexaclorofeno a 1,25% por imersão, um dia antes da exposição, conferiu proteção de 87% a 92%, após três horas de lavagem com água. A aplicação de hexaclorofeno a 1,25% com gaze, três dias antes da exposição, ainda resultou em proteção de 93%, após lavagem semelhante. A recuperação de grandes quantidades de cercárias, ao fim do período de exposição, demonstrou a elevada capacidade do hexaclorofeno para impedir sua penetração. Ao mesmo tempo, quantidade suficiente de hexaclorofeno desprendeu-se da pele da cauda dos camundongos, dissolvendo-se na água circundante e exercendo efeito sobre as cercárias, tornando-as não infectantes para camundongos não submetidos a tratamento prévio com hexaclorofeno.

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