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A comparison of topical formulations for the prevention of human schistosomiasis

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Abstract

Recently, a dimeticone formulation has been shown to be effective at preventing *Schistosoma* cercariae infecting skin, while DEET (*N*,*N*-diethyl-*m*-toluamide), a highly effective insecticide, has been shown to have activity against cercariae. Seven formulations, 3 containing DEET, were prepared and applied to excised human skin in Franz cells for 1 h. *Schistosoma* cercariae were applied for 30 min at 1 and 24 h, and the number that penetrated the skin calculated (n = 9). DEET could not be incorporated into the dimeticone formulation, yet it remained the most effective at preventing cercarial penetration, both 1 and 24 h after application. The ointments that contained DEET did prevent penetration but their mode of action was due to the toxicity of DEET against the cercariae. The persistence of the protection afforded by the dimeticone formulation after washing suggests that the formulation may be interacting with the stratum corneum to prevent cercarial recognition of skin.

Introduction

Digenean worms of the genus *Schistosoma* inhabit the blood vessels of their host, causing disease (schistosomiasis) in both man and non-domesticated animals. In the 76 countries of the tropics and sub-tropics where the disease is endemic, it is estimated that 193 million people are infected and a further 652 million people are at risk (Engels et al 2002). In 1999 it was estimated that 14 000 deaths were attributable to and 1.932 million DALYS (disability-adjusted life years) were lost due to schistosomiasis (Pruss et al 2002), indicating that the disease also has both social and economic consequences as the illness means that people are unable to study or work. As a result of the rise in eco- and adventure-tourism, an increasing number of people who visit such endemic zones are also open to exposure (Corachan 2002). There are two types of schistosomiasis — urinary, caused by *S. haematobium* and intestinal, caused by several species including *S. mansoni* and *S. japonicum*. An initial acute phase of the disease can be followed by a much longer chronic, sub-clinical phase (von Lichtenberg 1987). If left untreated, *S. haematobium* infections may result in haematuria, renal failure and calcification of the bladder or ureters (Corachan 2002). Intestinal schistosomiasis is characterised initially by bloody diarrhoea, developing into chronic colitis and eventually, if untreated, portal hypertension and liver failure (Corachan 2002). In some severe cases there may also be CNS involvement.

Infection occurs when free-swimming cercarial larvae, which emerge from freshwater snails, detect physical and chemical cues from a host. A cercaria (ca. 300–600 μm in length) is composed of a head and a tail and when skin is encountered, the head probes the surface, pushing in between the cells. At some point during penetration the tail detaches and the tail-less cercaria, now known as a schistosomulum, continues to move through the skin to deeper tissue and eventually enters the systemic circulation, a journey that takes approximately 10 h (Wilson 1987). The schistosomula then migrate through the body to the preferred site, where maturation occurs and in-copulo adults produce eggs. These are released into the environment in urine or faeces and, on contact with water, eggs hatch to release miracidial larvae, which are infective to freshwater snails. In the snail the larvae reproduce asexually giving rise to a large population of cercariae.

A range of control and treatment measures can be used for the containment of this globally important disease, targeting the various life-cycle stages of the parasite.

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Improving sanitation prevents eggs reaching water, the use of molluscicides, such as niclosamide, against freshwater snails removes the intermediate host and providing piped water reduces human contact with cercariae. Praziquantel, which kills adult worms, is the drug of choice, being effective against all species and also being readily available. Recently, artemisinins, successful antimalarials, have been shown to be effective against juvenile schistosomes (Shuhua et al 2002). Combinations of these methods and health education can reduce disease prevalence. They all require, however, long-term financial and infra-structural commitment (Li et al 2001).

The prevention of infection by inhibition of cercarial penetration has been studied intermittently since Hunter et al (1956) tested a range of compounds thought to have protective abilities. Much of the work has been concerned with chemicals with putative cercaricidal activity and the development of methods to enable their safe application to the skin. When used in this way, niclosamide, a salicylanilide, appeared to be effective in the laboratory (Miller & Reid 1986). Field trials carried out in Egypt, however, using 1% of the drug in an alcoholic lotion, indicated that although infection rates were reduced, the results were not good enough to recommend it as a method of control (Abu-Elyazeed et al 1993).

Some insect repellents have also been shown to be cercaricides. 1-(3-Cyclohexen-1-yl-carbonyl)-2-methylpiperidine (AI3-37220) confers 100% protection against *S. mansoni* cercariae (Secor et al 1999). *N*,*N*-diethyl-*m*-toluamide (DEET), the active ingredient in most insect repellents, kills cercariae at concentrations as low as 7.5% (Salafsky et al 1998). It has been estimated that DEET-containing products are used at least once a year by 25% of the UK population (Goodyer & Behrens 1998) and few cases of adverse reactions have been reported (Gupta & Rutledge 1994). Salafsky et al (1999) produced a vehicle for DEET that enhanced its protective ability against *S. mansoni* cercariae and reduced dermal absorption. By incorporating the insect repellent into liposomes it was retained in the superficial layers of the skin, offering protection for 48 h.

Formulations that inhibit attachment or penetration, without the addition of cercaricides, have also been identified (Lim et al 1999). A propylene glycol–isopropyl alcohol (3:1) formulation prevented cercarial penetration even when the putative active ingredients (enzyme inhibitors that blocked cercarial serine proteases) were omitted (Lim et al 1999). Recently, a barrier cream containing dimeticone and composed entirely of pharmacopoeial grade listed excipients was developed in our laboratory. Previous in-vitro studies have shown that topical application prevented up to 95% of cercariae from penetrating human skin after a single application and continued to do so for 48 h after the initial application (Ingram et al 2002). Interestingly, Vaseline, liquid paraffin and dimeticone alone were also tested and they were all found not to significantly inhibit attachment (Ingram et al 2002).

The precise mode of action of the dimeticone formulation remains to be elucidated. However, it has been hypothesised that the formulation prevents cercarial penetration of the skin by either formation of a physical barrier on the skin surface or by blocking the transepidermal pathways through which endogenous chemicals pass and attract the cercariae. Thus, the aim of this study was to gain a more fundamental insight into the mode of action of the dimeticone formulation by comparing its inhibitory effect with other silicone-based formulations and a simple ointment. In addition, given the effectiveness of DEET as a cercaricide (Salafsky et al 1998) and as an insect repellent, its possible incorporation into the formulations and resultant effect on the cercariae were also investigated.

Materials and Methods

Topical formulations

All topical formulations were prepared 24 h before use and stored at room temperature. Table 1 shows a list of the formulations used and their constituents.

Silicone-based formulations

Elastomer 10 and DEET (if present) were mixed with a spatula for 5 min. At this time isopropyl myristate, cyclomethicone and emulsifier 10 were individually added and the formulation mixed continuously for 3 min, until the resultant mixture became homogenous. The formulation was then placed on a minishaker (MS2 minishaker, IKA) for a further 1 min at 2500 rev min^{-1}.

Cetomacrogol-based formulations

Cetomacrogol emulsifying ointment (CMO) was melted by heating in a water bath for 10 min at 60°C. DEET was then slowly incorporated into the ointment, which was then mixed for 3 min using a spatula until it was homogenous. The formulation was then removed from the water bath and the contents were allowed to solidify for approximately 20–30 min at room temperature. CMO that had been melted and cooled without addition of DEET was used as a control.

Dimeticone formulation

Dimeticone 350, liquid paraffin and cetostearyl alcohol were warmed to 50°C and mixed mechanically using a magnetic flea for 15 min, until completely homogenous. The aqueous phase was prepared by dissolving cetrimide in distilled water warmed to 60°C. The two phases were then mixed together and stirred using a hand-held mechanical whisk until the temperature of the formulation had fallen to 25°C and it appeared homogenous upon visual inspection. Upon addition of DEET the formulation cracked indicating an obvious incompatibility. A stable formulation could not be prepared without a fundamental change in the excipients used, which might have affected the anti-cercarial properties of the formulation. Hence no DEET–dimeticone formulation could be included in this study.

Parasites

A Puerto Rican strain of *S. mansoni* has been maintained in the laboratory since 1989 when infected snails were received

Table 1 The percentage w/w of components for each topical formulation

Composition (% w/w)	Source	S1 + 20% DEET	S1	S2 + 40% DEET	S2	CMO + 20% DEET	CMO	Dimeticone
Isopropyl myristate	Merck	2	2	1	1	—	—	—
Cyclomethicone	Dow Corning	2	2	—	—	—	—	—
Elastomer 10	Dow Corning	74	94	55	95	—	—	—
Emulsifier 10	Dow Corning	2	2	4	4	—	—	—
Cetomacrogol Ointment BP	Thornton & Ross	—	—	—	—	80	100	—
DEET	Nomad Medical	20	—	40	—	20	—	—
Dimeticone 350	Dow Corning	—	—	—	—	—	—	10
Liquid paraffin	Sigma	—	—	—	—	—	—	40
Cetostearyl alcohol	Crook chemicals	—	—	—	—	—	—	5
Cetrimide	Sigma	—	—	—	—	—	—	0.5
De-ionised water		—	—	—	—	—	—	44.5

S, silicone; CMO, cetomacrogol ointment (cetostearyl alcohol–cetomacrogol, 4:1).

from the Liverpool School of Tropical Medicine and Hygiene (Home Office licence 70/4776). Female NMRI mice have been used throughout as the definitive host and *Biomphalaria glabrata* snails as the intermediate host, essentially as described by Standen (1949). All water used with the cercariae was filtered to remove heavy metals and chlorine (Carbon-resin filter; Prosep Filter Systems Ltd). Cercariae were stimulated to emerge by incubating snails in bright light at 28°C and they were used within 1 h of emergence. The density of the cercarial suspension was determined by counting the number present in 5×1-mL samples of suspension, after fixation with Lugol's iodine, under a low power ($\times 20$) microscope.

Human skin

Human skin, obtained from female patients undergoing elective abdominoplasty, was frozen until required for use (the procedures for the collection of human skin were approved by the College Research Ethics Committee). The skin was prepared and mounted into Franz cells as described by Bartlett et al (2000). Briefly, the subcutaneous fat was dissected from the dermis (being careful not to puncture the surface) and the skin surface washed with warm water and dried. A circular piece of skin, diameter 3.5 cm, was cut and mounted between the two chambers of each Franz cell. The receptor well was filled with tissue culture medium (CO_2-independent medium, GIBCO, containing 1% antibiotic/antimycotic solution, Invitrogen). Negative controls were set up using a latex rubber membrane above a Teflon O ring in place of skin.

Testing the formulations

1 h after application

Twenty-four Franz cells were prepared with skin and 3 with an artificial latex rubber membrane. Groups of 3 skin Franz cells were treated with each formulation by applying sufficient formulation (ca. 30 mg cm^{-2}) to cover the skin surface uniformly. One group of 3 Franz cells with skin was not treated with formulation (positive controls). All Franz cells were equilibrated for 1 h at 37°C before use, by standing the receptor well in a water bath, which allowed the formulation to spread into all skin creases and the skin to become fully hydrated.

Cercariae were harvested as described above and 2.5–3 mL of cercarial suspension with a known density of cercariae (ca. 150) was applied to the donor well of each Franz cell. After a 30-min exposure period the cercarial suspension from each Franz cell was removed and transferred to a counting dish; the skin surface was washed twice by vigorously pipetting filtered water (2×2.5 mL) and the washings added to the counting dish. The behaviour of whole cercariae and detached cercarial heads was noted, then they were fixed and stained with Lugol's iodine and counted.

24 h after application

All Franz cells used on day 1 were capped and stored at 4°C for 24 h then the skin surfaces or artificial latex rubber membranes were washed extensively with distilled water. They were equilibrated for 1 h in a water bath at 37°C before freshly shed cercariae were applied, recovered and counted as described above. No additional formulation was applied, therefore the effectiveness of a single application was being tested. The experiments were repeated 3 times to give a final replication value of $n = 9$.

Statistical analysis of results

The number of cercariae recovered from each Franz cell was expressed as a percentage of the inoculum to determine by subtraction the percentage that had become irretrievably attached or penetrated. These data were analysed (after arcsine transformation) for statistically significant differences by analysis of variance (Tukey's test). Previous experience with the Franz cell system (Bartlett et al 2000) has shown that cercariae do not penetrate an artificial membrane but a small fraction of applied cercariae cannot

be recovered. It has been assumed that this disparity results from mechanical losses in the apparatus. Equally, with unprotected skin, less than 100% of applied cercariae attach to the target surface. This means that raw experimental results are best expressed by considering the recovery from the artificial membrane to represent 100% protection and the attachment to unprotected skin to represent 0% protection. In analysing the results of the experiments described above, formulation protective power (FPP) has been normalised in this way.

Results and Discussion

Figure 1 (formulations without DEET) and Figure 2 (formulations with DEET incorporated) illustrate the results of Franz cell experiments to assess the protective power of a range of topical formulations against cercarial attachment and penetration of human skin. The experiments included untreated skin and latex artificial membrane as controls.

About 88% of cercariae attached to, or penetrated, untreated skin; about 12% could not be recovered from the artificial membrane. These results were similar to those observed previously (Bartlett et al 2000), supporting the validity of the methods used. The dimeticone cream (Figure 1) was effective at both 1 and 24 h after application, with recoveries of cercariae showing no significant differences from those obtained with the artificial membrane control (Tukey's test, $P < 0.05$). Figure 1 also shows that none of the other formulations (without DEET) had the same pattern of efficacy as dimeticone. S1, S2 and CMO all prevented cercarial attachment or penetration to a degree but they had all lost some activity 24 h after application, unlike dimeticone where efficacy remained constant.

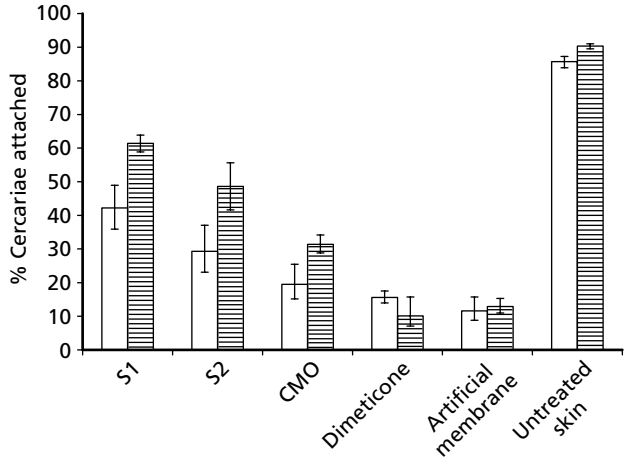

Figure 1 The percentage of the cercarial inoculum that had attached to, or penetrated, human skin after 20 min incubation with formulations allowed to penetrate for 1 h (open box) and formulations left a further 24 h (striped box). The values shown are the mean ± s.e., n = 9.

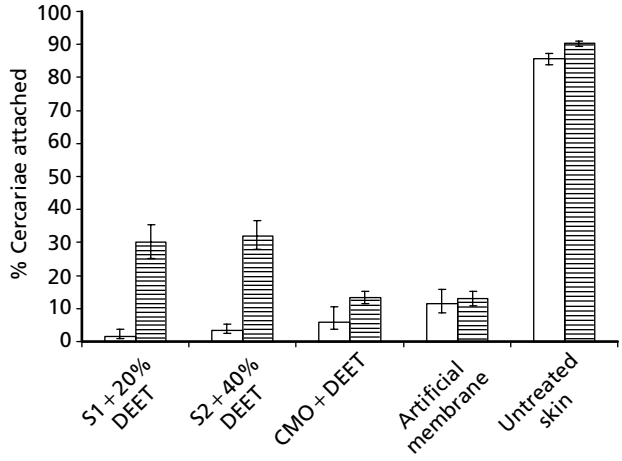

Figure 2 The percentage of the cercarial inoculum that had attached to, or penetrated, human skin after 20 min incubation with formulations containing DEET allowed to penetrate for 1 h (open box) and formulations containing DEET left a further 24 h (striped box). The values shown are the mean ± s.e., n = 9. (There are no histogram columns in this figure corresponding to dimeticone cream in Figure 1, as DEET could not be incorporated into the dimeticone formulation).

Liquid paraffin and dimeticone alone have previously been tested, in the Franz cell system, to study their effectiveness in preventing cercarial attachment (Ingram et al 2002). Neither was found to inhibit cercarial attachment significantly, when compared with untreated skin, indicating that protection may be due to the combined effect of all the excipients in the formulation. The apparent persistence of the dimeticone formulation both after 24 h and washing, which was not demonstrated by S1, S2 or CMO, may provide an insight into how the formulation is functioning to prevent cercarial penetration. Such results suggest that S1, S2 and CMO, which are oleaginous formulations containing no water, are acting as a superficial physical barrier that is lost upon washing and over time. However, the fact that the anti-penetration ability of the water-in-oil dimeticone formulation persists under similar conditions indicates that it is not simply acting as a barrier and that its site of action probably lies within the stratum corneum. It has previously been reported that silicones interact with the lipids of the skin and that this interaction is most apparent with lower-molecular-weight dimeticones (Laugel et al 2000). Thus, it is possible that the fact that the silicone formulation is a water-in-oil emulsion containing dimeticone 350 may facilitate its interaction with the stratum corneum. Such an effect could mean that the formulation is creating a barrier in the stratum corneum and possibly preventing the chemical cues required for cercarial attachment or penetration, such as linoleic acid (Shiff & Graczyk 1994), ceramides, acylglycerols or L-arginine (Haas et al 2002), reaching the cercariae.

Incorporation of DEET in formulations S1 and S2 significantly changed the effectiveness of the formulations at both 1 h and 24 h after application (Tukey's test, $P < 0.05$).

Table 2 Observed cercarial behaviour after exposure to formulation or to untreated controls

Formulation	Cercarial behaviour	
	1 h	24 h
S1	Cercariae moving, some swimming	Cercariae moving, some swimming
S2	Cercariae moving	Cercariae moving
CMO	Cercariae moving	Cercariae swimming
Dimeticone	Cercariae swimming and moving even when separated	Cercariae swimming and moving even when separated
S1 + 20% DEET	No movement, heads all separated from tails	All moving, some separated
S2 + 40% DEET	No movement, heads all separated from tails	No movement, heads all separated from tails
CMO + 20% DEET	No movement, heads all separated from tails	Cercariae swimming
Untreated skin	Cercariae swimming	Cercariae swimming
Artificial membrane	Cercariae swimming	Cercariae swimming

Attachment/penetration was less than 5% for both S1 + 20% DEET and S2 + 40% DEET after 1 h and these values were statistically significantly different from the values obtained with basic S1 and S2 alone (Tukey's test, $P < 0.05$). However, this difference was not as noticeable at 24 h.

Observations made on the behaviour of cercariae, after they were recovered but before fixation for counting, are summarised in Table 2. At 1 h it was clear that the DEET within the formulation was toxic, since the recovered cercariae were dead and most had shed their tails. Tail loss can be an indicator of attempted penetration; however, in this situation it was likely that the DEET had stimulated a stress response, resulting in tail loss and death. Such morphological changes were also observed by Salafsky et al (1998), who suggested that cercariae exposed to DEET displayed ultrastructural changes associated with transformation into schistosomula. In contrast, cercariae recovered from the same formulations without DEET were whole and actively swimming or moving across the observation chamber — behaviour that was entirely normal, indicating that none of the formulations were cercaricidal. At 24 h the formulations containing 20% DEET (S1 and CMO) did not show toxic activity, whereas that with 40% DEET (S2) had killed the cercariae.

The enhancement of FPP values of S1, S2 and CMO with added DEET, particularly at 1 h, seems to be due to the direct cercaricidal properties of DEET rather than the underlying barrier properties of the formulation. Twfeek (1999) confirmed that it is the toxicity of DEET that prevents cercarial penetration, and even when some cercariae penetrate they do not successfully establish within the host. The toxic effects could be caused by direct contact with the DEET-containing formulation or by leaching of DEET from the formulation into the liquid in the donor wells of the Franz cells. The latter is likely to be the principal mechanism as such leaching would also explain why dead cercariae were not seen at 24 h in S1 + 20% DEET and CMO + 20% DEET. It is assumed that DEET leaching losses over the period between 1 and 24 h reduced DEET levels sufficiently to produce this effect. Interestingly and in contrast, with S2 + 40% DEET, all cercariae were dead when the test was carried out over 24 h. Perhaps at this higher concentration there was still sufficient DEET present to cause cercarial mortality.

The comparisons of formulation activity made above have been based on raw attachment data. As explained previously, it is also appropriate to compare the activities normalised with respect to the protective power of a latex membrane (100% protection) and of unprotected human skin (regarded as 0% protection). Table 3 shows the formulation protective power (FPP) of the different treatments in this way.

Analysis of protective power in this standardised manner emphasises the comparative efficacy of the dimeticone formulation over that of the other non-DEET containing formulations, demonstrating as it does, unsurpassed FPP values of 95 and 100 at 1 h and 24 h, respectively. Table 3 also shows that DEET added to S1, S2 and CMO induces maximal FPP values at 1 h but that these have dropped to less than 80 for S1 + 20% DEET and S2 + 40% DEET at 24 h. Only CMO + 20% DEET has a maximal 100 FPP value at both 1 h and 24 h. However, this ointment is highly viscous and therefore difficult to apply properly and is aesthetically unpleasant.

Table 3 Normalised formulation protective power (FPP) of the formulations and controls

Formulation	Formulation protective power (FPP)	
	1 h	24 h
S1	59 ± 33	37 ± 9
S2	76 ± 25	54 ± 24
CMO	89 ± 14	76 ± 5
Dimeticone	95 ± 4	100 ± 3
S1 + 20% DEET	100 ± 1	78 ± 16
S2 + 40% DEET	100 ± 13	75 ± 12
CMO + 20%DEET	100 ± 13	100 ± 4
Untreated skin	0	0
Artificial membrane	100	100

Normalisation limits: untreated skin 0%, artificial membrane 100%. The values shown are the mean ± s.d., n = 9.

Conclusion

Of all the non-DEET-containing formulations studied, the dimeticone cream was shown to be the most effective at preventing *Schistosoma* cercarial penetration, both 1 and 24 h after application and washing. Such apparent persistence indicates that the formulation may not be simply acting as a physical barrier but may be interacting with the stratum corneum, preventing the chemical cues required for cercarial attachment or penetration reaching the cercariae. However, unlike the ointment formulations, S1, S2 and CMO, it was not possible to incorporate DEET into this dimeticone cream and thus its use to prevent other tropical diseases is limited. A dual function formulation could be of use in the areas of the tropics where insect-transmitted diseases like malaria, yellow fever, dengue fever and lymphatic filariasis co-exist with endemic schistosomiasis. In 1996 alone, 240 000 UK residents visited African countries where the latter disease is endemic (Whitty et al 2000). The incidence of disease in returnees is likely to be underrecorded, and thus a dual combination product would have obvious benefits.

References

Abu-Elyazeed, R. R., Podgore, J. K., Mansour, N. S., Kilpatrick, M. E. (1993) Field trial of 1% niclosamide as a topical antipenetrant to *Schistosoma mansoni* cercariae. *Am. J. Trop. Med. Hyg*. **49**: 403–409

Bartlett, A., Brown, M., Marriott, C., Whitfield, P. J. (2000) The infection of human skin by schistosome cercariae: studies using Franz cells. *Parasitology* **121**: 49–54

Corachan, M. (2002) Schistosomiasis and international travel. *Clin. Infect. Dis*. **35**: 446–450

Engels, D., Chitsulo, L., Montresor, A., Savioli, L. (2002) The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Trop*. **82**: 139–146

Goodyer, L., Behrens, R. H. (1998) Short report: the safety and toxicity of insect repellents. *Am. J. Trop. Med. Hyg*. **59**: 323–324

Gupta, R. K., Rutledge, L. C. (1994) Role of repellents in vector control and disease prevention. *Am. J. Trop. Med. Hyg*. **50**: 82–86

Haas, W., Grabe, K., Geis, C., Pach, T., Stoll, K., Fuchs, M., Haberl, B., Loy, C. (2002) Recognition and invasion of human skin by *Schistosoma mansoni* cercariae: the key-role of L-arginine. *Parasitology* **124**: 153–167

Hunter, G., Kemp, H., Smalley, H., Wilkins, O., Dixon, C. (1956) Studies on schistosomiasis. XII. Some ointments protecting mice against the cercariae of *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg*. **5**: 713–736

Ingram, R. J., Bartlett, A., Brown, M. B., Marriott, C., Whitfield, P. J. (2002) Dimethicone barrier cream prevents infection of human skin by schistosome cercariae: evidence from Franz cell studies. *J. Parasitol*. **88**: 399–402

Laugel, C., Rafidison, P., Potard, G., Aguadisch, L., Baillet, A. (2000) Modulated release of triterpenic compounds from a O/W/O multiple emulsion formulated with dimethicones: infrared spectrophotometric and differential calorimetric approaches. *J. Control. Release* **63**: 7–17

Li, Y., Kusel, J., Kennedy, M., Ruppel, A. (2001) Diversity among schistosomes: perspectives for control. *Trends Parasitol*. **17**: 113–115

Lim, K. C., Sun, E., Bahgat, M., Bucks, D., Guy, R., Hinz, R. S., Cullander, C., McKerrow, J. H. (1999) Blockage of skin invasion by schistosome cercariae by serine protease inhibitors. *Am. J. Trop. Med. Hyg*. **60**: 487–492

Miller, R. E., Reid, W. A. (1986) *Schistosoma mansoni*: salicylanilides as topical prophylactic against cercarial penetration of mice. *Exp. Parasitol*. **61**: 359–368

Pruss, A., Kay, D., Fewtrell, L., Bartram, J. (2002) Estimating the burden of disease from water, sanitation, and hygiene at a global level. *Environ. Health Perspect*. **110**: 537–542

Salafsky, B., Ramaswamy, K., He, Y. X., Anderson, G. L., Nowicki, D. K., Shibuya, T. (1998) Evaluation of N,N-diethyl-m-toluamide (DEET) as a topical agent for preventing skin penetration by cercariae of *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg*. **58**: 828–834

Salafsky, B., Ramaswamy, K., He, Y. X., Li, J., Shibuya, T. (1999) Development and evaluation of LIPODEET, a new long-acting formulation of N, N-diethyl-m-toluamide (DEET) for the prevention of schistosomiasis. *Am. J. Trop. Med. Hyg*. **61**: 743–750

Secor, W. E., Freeman, G. L., Wirtz, R. A. (1999) Short report: prevention of *Schistosoma mansoni* infections in mice by the insect repellents AI3-37220 and N,N-diethyl-3-methylbenzamide. *Am. J. Trop. Med. Hyg*. **60**: 1061–1062

Shiff, C. J., Graczyk, T. K. (1994) A chemokinetic response in *Schistosoma mansoni* cercariae. *J. Parasitol*. **80**: 879–883

Shuhua, X., Tanner, M., N'Goran, E. K., Utzinger, J., Chollet, J., Bergquist, R., Minggang, C., Jiang, Z. (2002) Recent investigations of artemether, a novel agent for the prevention of Schistosomiasis japonicum, mansoni and haematobium. *Acta Trop*. **82**: 175–181

Standen, O. D. (1949) Experimental schistosomiasis II: maintenance of *Schistosoma mansoni* in the laboratory, with some notes on experimental infection with *S. haematobium*. *Exp. Parasitol*. **43**: 268–283

Twfeek, G. M. (1999) The potential use of N,N-diethyl-*m*-toluamide (DEET) as a prophylactic agent in the control of schistosomiasis. *J. Egypt. Soc. Parasitol*. **29**: 763–776

von Lichtenberg, F. (1987) Consequences of infections with schistosomes. In: Rollinson, D., Simpson, A. J. G. (eds) *The biology of schistosomes: from genes to latrines*. Academic Press Ltd, London, pp 185–224

Whitty, C. J., Mabey, D. C., Armstrong, M., Wright, S. G., Chiodini, P. L. (2000) Presentation and outcome of 1107 cases of schistosomiasis from Africa diagnosed in a non-endemic country. *Trans. R. Soc. Trop. Med. Hyg*. **94**: 531–534

Wilson, R. A. (1987) Cercariae to liver worms: development and migration in the mammalian host. In: Rollinson, D., Simpson, A. J. G. (eds) *The biology of schistosomes: from genes to latrines*. Academic Press Ltd, London, pp 115–146