Research on the use of surface active substances in the protection against "Schistosoma" cercariae

Autor(en): **Combes, C. / Arnaudis, J.**

Objekttyp: **Article**

Zeitschrift: **Acta Tropica**

Band (Jahr): **39 (1982)**

Heft 1

PDF erstellt am: **12.07.2024**

Persistenter Link: https://doi.org/10.5169/seals-312964

Acta Tropica *39*, 79–84 (1982)

Département de Biologie animale, Université de Perpignan, 66025 Perpignan, France
Produits chimiques de la Montagne Noire, 81100 Castres, France

Research on the use of surface active substances in the protection against *Schistosoma* cercariae

C. COMBES, J. ARNAUDIS

Summary

Cercariae immobilisation and *Schistosoma mansoni* adult recovery tests in the mouse show that tensides can greatly reduce the infectivity of cercariae. In the case of two amphoteric products, Laurylbetain and Laurylamidopropylbetain, this action is obtained with very low (0.1–5 ppm) concentrations.

Key words: *Schistosoma; Biomphalaria glabrata;* cercariae; cercaricides; surface active agents; tensides.

Introduction

A high proportion of infections by schistosome cercariae occur in washing and bathing areas. This observation has led several researchers to take an interest in the possibility of using cercaricidal products in addition to other methods which reduce the incidence of the disease.

As early as 1938, Witenberg and Yofe made a comparative study of the different methods which could be used to destroy cercariae, and carried out detailed tests on the cercaricidal properties of several chlorine based agents (gaseous chlorine, sodium hypochlorite and chloramine); of these, chloramine appears to be the most effective since it kills *Schistosoma mansoni* and *Schistosoma haematobium* cercariae in just over an hour at a concentration of 0.36 ppm. Similar results were obtained by Jones and Brady (1947) with chloramine and by Fripp et al. (1972) with various commercial hypochlorite preparations.

Correspondence: Dr. C. Combes, Département de Biologie animale, Université de Perpignan, Avenue de Villeneuve, 66025 Perpignan Cedex, France

The effect of pH on the results was studied by Frick and Hillyer (1966) who demonstrated that the chlorine concentration necessary to inactivate the cercariae must be increased considerably when the pH becomes basic.

Pellegrino (1967) thought that the action of chlorine based compounds could complement non-chemical methods such as storing water, heating water to 55° C for a few minutes, or filtration through diatomaceous earths.

As well as chlorine compounds, some iodine based compounds tested by Jones and Brady (1947) were shown to be effective. These authors demonstrated that some detergents also had cercaricidal properties; they note that alkyl-aryl sodium sulfonate is the active component of a commercial detergent which kills cercariae in 3–5 min at a concentration of 1 g per liter and that alkyl dimethyl ammonium chloride quickly destroys them at concentrations of 1/5,000 and 1/10,000.

In this paper, we study the effect of several groups of surface active substances on *Schistosoma* cercariae, in order to determine if it would be possible to use them in transmission sites.

Material and Methods

1. *Products tested:* Surface active agents or tensioactive products are molecules with two parts, a lipophilic and a hydrophilic. The hydrophilic part can be an anion, a cation or a «zwitter-ion» in which case, the surface active agent is amphoteric, or can be built up by hydrophilic group, e.g. polyethers, in which case the substance is non ionic.

We tested:

- as anionic: sodium dodecylbenzenesulfonate = dodecyl 3 benzenesulfonic acid salt:
  $C_{12}H_{25}-C_6H_4-SO_3Na$
  and the sodium laurylethersulfate = sodium dodecyldiethoxyethylethersulfate:
  $C_{12}H_{25}-O-CH_2-CH_2-O-CH_2-O-SO_3Na$

- as cationic: lauryldimethylbenzylammonium bromide = N dodecyl dimethyl benzyl ammonium bromide:

$$C_{12}H_{25}-\underset{\displaystyle CH_3}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{N}}}}-CH_2-C_6H_5,\ Br$$

- as non ionic: octylphenyldecaethoxyethylether:
  $C_8H_{17}-C_6H_4-O-[CH_2-CH_2-O]_9-CH_2-CH_2OH$
- as amphoteric: laurylbetain sodium salt of N dodecyldimethylbetain

$$C_{12}H_{25}-\underset{\displaystyle CH_3}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{N}}}}-CH_2-COO\,Na$$

and laurylamidopropylbetain sodium salt of N dimethyl N propyldodecanamide betain

$$C_{12}H_{25}-CO-NH-CH_2-CH_2-CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{N}}}}-CH_2-COONa$$

2. *Preparation of the solutions:* All concentrations were obtained by successively diluting a stem solution by half with a 1 ml syringe graduated every 100th. The last dilution was obtained by adding water containing the cercariae. All experiments were made at 24° C and at a pH of 7.3.

3. *Schistosome strain:* The experiments were carried out with a *Schistosoma mansoni* strain maintained on a *Biomphalaria glabrata* strain. These strains, both originating from Brazil (Recife), have been kept in the laboratory for several years.

4. *Techniques used to appreciate the mobility of the cercariae:* A first series of tests consisted of observing the changes in cercarial activity in the presence of tensides under a binocular magnifying glass. After a certain length of time, depending on the concentration, the cercariae become inactive and fall to the bottom of the container.

The tests were carried out by placing batches of 10 cercariae in alveoli containing 1 ml of solution. The time necessary for at least 5 cercariae to become totally inactive was noted.

5. *Mice infection techniques:* A second series of tests was performed with some of the products. Mice were infected with cercariae in contact with different concentrations, and the number of adult schistosomes recovered by perfusion was counted.

The mice were infected according to the Smithers and Terry technique (1965) for anaesthesia and to the Erickson technique (1974) for the cercariae/mouse contact. The mice, with shaved bellies, were placed on dishes filled with 20 ml of solution containing 150 cercariae. The cercariae, which came from *B. glabrata* infected over two months before, were collected in the three hours following the acrophase of issue and emerged for less than one hour.

The adult schistosomes were recovered during the fifth week according to the technique of Duwall and Dewitt (1967).

The egg density in the liver was evaluated by selective action of potassium at 5% on weighed fragments of the organ, at a temperature of 60° C, followed by an egg count under the binocular magnifying glass.

Results

Cercariae immobilization tests were used to compare the activity of different tenside groups.

Although these tests, by their very nature, cannot be absolutely precise, they clearly show that all the products used are capable of inactivating cercariae and that amphoteric agents are the most effective, whatever their concentration (Fig. 1, left).

As an exemple, Table 1 gives an estimate of the concentrations of amphoteric agents which immobilize cercariae in 30, 15 and 10 min.

In view of these results, we continued research on amphoteric products with infection tests on mice. We studied the effect of the time the cercariae were in contact with surface active substances, the effect of the concentration of surface active agents and the fertility of the schistosomes which had been in contact with surface active agents.

Contact time

A series of adult schistosome recovery tests was carried out with each of the two amphoteric agents, with a concentration of 1 ppm and a contact time of 5, 10, 15, 20, 30, 45 and 60 min.

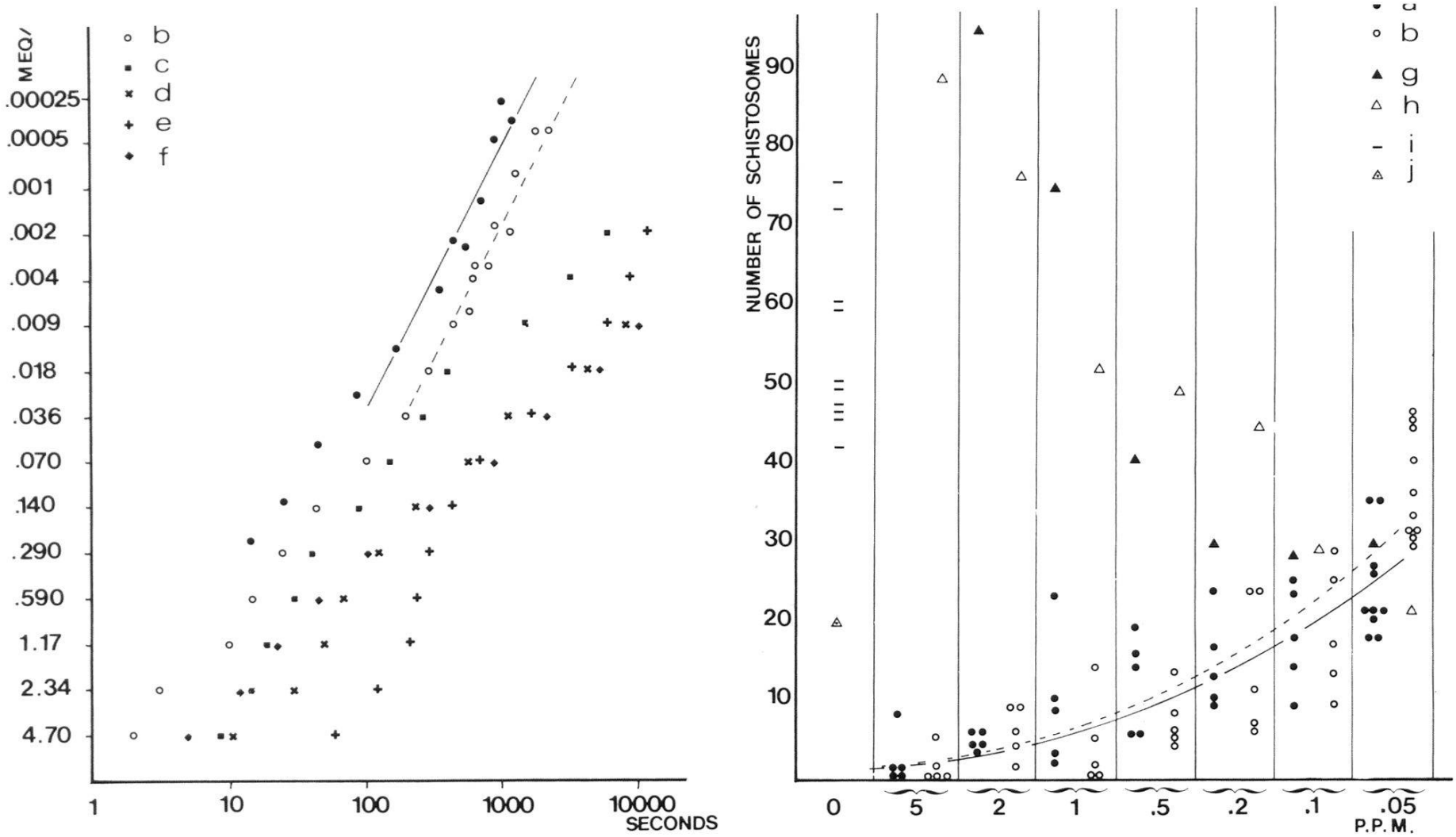

Fig. 1. *On the left:* Cercariae immobilisation tests showing the activity of various surface active agents. Each point represents an experiment with 10 cercariae; concentrations are plotted against time. *On the right:* Schistosome recovery tests for a 30 min contact time and various concentrations. Triangles show the number of cercariae which have been found inactive in the vessel after mouse exposure.

a, g = Laurylamidopropylbetain
b, h = Laurylbetain
c = Sodium laurylethersulfate
d = Sodium dodecylbenzenesulfonate
e = Octylphenyldecaethoxylether
f = Lauryldimethylbenzylammonium bromide
i, j = control

Table 1. Approximate concentrations of amphoteric surfactants leading to the immobilisation in 30, 15 and 10 min time (from a series of 10 experiments using 10 cercariae each, for every concentration)

	Time (min)	Concentration (ppm)	Concentration (meq/l)
Laurylbetain	30	0.1	0.0004
	15	0.5	0.0018
	10	1.0	0.0036
Laurylamidopropylbetain	30	0.1	0.0003
	15	0.2	0.0005
	10	0.7	0.0009

Table 2. Number of eggs/100 mg of liver/couple of schistosomes, 5 weeks post-infection, for series of 5 mice. The results are given for concentrations of 0.5 ppm and contact time of 30 min

Laurylbetain	15	36	38	48	52
Laurylamidopropylbetain	24	26	30	45	70
Control	20	22	25	32	61

The results of these tests seem to indicate that the contact time has no significant effect. We can therefore conclude that the "incapacitating" action on cercariae is obtained almost immediately when the parasite is immersed in the product solution, although the time necessary for immobilization to occur depends on the concentration, as we have already seen.

Concentration

A series of tests similar to the previous experiment was performed, but with 30 min contact and concentrations of 5, 2, 1, 0.5, 0.2, 0.1, 0.05 ppm.

Fig. 1 (right) shows the results of these tests. In this case a clear variation is observed depending on the concentration; the experiment confirms that the products are active even at a very low dosage; it shows that the concentrations which give interesting results are those between 5 and 0.1 ppm; when dilution is as low as 0.05 ppm the result obtained is similar to that of the control batch.

Fertility of schistosomes

The egg count in the mice livers showed that the fertility of the schistosomes which developed after being in contact with the tensides was not very different from that of the control batches, as shown in Table 2.

Discussion

Our results demonstrate that certain synthetic surface active substances prevent penetration by schistosome cercariae to an appreciable extent; this action is obtained, in the case of amphoteric agents, with extremely low concentrations; the efficiency of laurylbetain and laurylamidopropylbetain is much greater than that obtained previously with tensides; the active concentrations are more or less the same as those of chlorine based compounds.

It can be concluded that the surface active substance acts on a process directly related to the percutaneous passage of the cercariae; however, no other lesion is caused since the cercariae which manage to penetrate develop normally into fertile adults.

It is interesting to note that surface active agents have the same effect on *Schistosoma* cercariae as hydrocarbon chains, which also have a hydrophilic and a lipophilic part. Haas (1978) showed that these substances, including certain human skin lipids, stimulate cercariae skin penetration and also that they quickly kill cercariae when they are added to water. Haas assumes that these substances act as receptors, the stimulation of which would provoke a modification of the tegument, comparable to the changes observed after cercariae have penetrated the host's skin. This might result in osmotic disturbances for the water dwelling cercaria. Haas stresses: «Perhaps this mechanism can be used in a specific schistosomiasis control by adding penetration stimulating substances to infected waters».

It would seem that amphoteric surface active agents are of particular interest in this connection since they are considered to be biodegradable, of very low toxicity as far as the environment is concerned, and because, being detergents, they could be incorporated into washing powders.

Duwall R. H., Dewitt W. B.: An improved perfusion technique for recovering adult schistosomes from laboratory animals. Amer. J. trop. Med. Hyg. *16*, 483–486 (1967).

Erickson D. G.: An efficient technique for exposure of rodents to *Schistosoma mansoni* and *Schistosoma haematobium*. J. Parasit. *60*, 553–554 (1974).

Frick L. P., Hillyer G. V.: The influence of pH and temperature on the cercaricidal activity of chlorine. Milit. Med. *131*, 372–378 (1966).

Fripp P. J., Armstrong F. A., Jaskulla E.: The cercaricidal properties of commercial hypochlorite preparations. S. Afr. med. J. *46*, 1819–1822 (1972).

Haas W.: Invasion stimuli for *Schistosoma mansoni* cercariae. Proc. 4th Int. Congr. Parasit., Warszawa, sect. C, 110–111 (1978).

Jones M. F., Brady F. J.: Effects of water treatment processes on schistosome cercariae. Nat. Inst. Hlth Bull. *189*, 109–130 (1947).

Pellegrino J.: Protection against human schistosome cercariae. Exp. Parasit. *21*, 112–131 (1967).

Smithers S. R., Terry R. J.: The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. Parasitology *55*, 695–700 (1965).

Witenberg G., Yofe J.: Investigation on the purification of water with respect to schistosome cercariae. Trans. roy. Soc. trop. Med. Hyg. *31*, 549–570 (1938).