Toxicity of cadmium and zinc to miracidia of *Schistosoma mansoni*

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SUMMARY

The specific objectives of this study were to elucidate metal toxicity to hatching, survival and avoidance behaviour of *Schistosoma mansoni* miracidia. The toxicity of cadmium, zinc, and cadmium/zinc mixtures at concentrations ranging from 10000 to 10 μg/l was investigated. Metal mixture toxicity investigation was undertaken with equal concentrations of the metals. The hatching of miracidia from eggs was inhibited by concentrations of 1000–10000 μg/l of single metals. Metal mixtures had no effect on egg hatching. Survival of miracidia was reduced by increasing metal concentration except at concentrations of 10 μg/l for single metal toxicity where survival was increased above the control. Miracidia demonstrated a rapid avoidance behaviour when briefly exposed to heavy metals. The mechanisms of metal toxicity to miracidia are briefly discussed.

Key words: cadmium, zinc, miracidia, survival, *Schistosoma mansoni*.

INTRODUCTION

In recent years there has been an increasing interest in the relationship between aquatic parasites and toxic pollutants (e.g. Paperna, 1997). Due to agricultural and industrial activities organic and inorganic pollutants are released into the aquatic environment where they may have considerable effects on indigenous wild animal populations. However, little information is available on the potential toxicity to parasites, especially the free-living stages of many common, or medically important, digeneans. Almost all published work has focused on the cercarial stage (e.g. Evans, 1982; Abd Allah, Wannas & Thompson, 1996) with only a limited number of studies on the miracidia or egg stages (e.g. Wolmarans, Yssel & Hamilton-Attwell, 1988). Surprisingly, when one considers its medical importance, no study has investigated pollutant toxicity to the miracidia or eggs of *Schistosoma mansoni*, especially as heavy metal pollutants have become cause for serious concern in areas endemic with schistosomiasis e.g. The Nile Delta (Abdel-Aal *et al*. 1988).

In the present study the toxicity caused by the heavy metals cadmium and zinc to *S. mansoni* egg and miracidia is investigated. Cadmium and zinc are related metals used widely in industry, and both are major pollutants of aquatic systems (Depledge *et al*. 1994), concentrations chronically polluting the environment have been recorded as high as 700 μg/l for cadmium (Fergusson, 1990) and 800 μg/l for zinc (Kronfeld & Navrot, 1974). Zinc is omnipresent in the environment and, although it is almost always accompanied by cadmium in pollution incidents (Attar & Maly, 1982), most toxicity studies only consider the individual toxicity of these two metals. The present study investigated both individual and combined toxicity of cadmium and zinc, an experimental approach not previously attempted in parasite ecotoxicology. The specific objectives of this study were to elucidate metal toxicity to hatching, survival and avoidance behaviour of *S. mansoni* miracidia.

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MATERIALS AND METHODS

Test solutions

Stock solutions of 100 mg/l metal concentration of cadmium and zinc were prepared by dissolving either cadmium chloride ($CdCl_2.5/2H_2O$) or zinc chloride ($ZnCl_2$) (Sigma Chemicals) in distilled water. Test solutions of 10, 100, 1000, and 10000 μg/l metal concentration were obtained by diluting stock solutions in distilled water. Synthetic 'soft' water (25 mg/l $CaCO_3$, pH 7·85) was prepared using the procedure described by HMSO (1969).

Source of parasites

Small pieces of liver of mice infected with *S. mansoni* (Richard Toll strain) were dissected out and homogenized in 0·85% saline. The suspension was repeatedly centrifuged and the supernatant removed and replaced with fresh saline until relatively clear. The supernatant was finally removed and replaced

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with fresh 'soft' water and the suspension agitated. Miracidia were hatched and collected according to the method of Rau, Bourns & Ellis (1972).

Water analysis

Samples of all test solutions were analysed for decay over time at 0·5, 5 and 24 h as a single experiment. Solutions were analysed on a Perkin Elmer Optima 3300 Inductively Coupled Plasma-Atomic Emission Spectrometer which was calibrated with a 1 % nitric acid blank and a standard which consisted of 1000 μg/l of cadmium and zinc in 1 % nitric acid.

Chronic toxicity tests with miracidia

Recently hatched miracidia (max. age 20 min) were individually exposed in round bottomed 96-well microtitre plates (Life Sciences International) containing 150 μl of soft water and the appropriate heavy metal concentration (10000, 1000, 100, 10 μg/l). Mixtures of cadmium and zinc were added to give concentrations of 10000 μg/l Cd + 10000 μg/l Zn, 1000 μg/l Cd + 1000 μg/l Zn, 100 μg/l Cd + 100 μg/l Zn, 10 μg/l Cd + 10 μg/l Zn. Three replicates of 36 miracidia were exposed at each concentration and incubated at 25 °C under artificial light. Survival was monitored at appropriate time-intervals dependent on the metal concentration (at 0·5 h intervals–10000 μg/l, at 1 h intervals–1000 μg/l – 10 μg/l). Death was determined when miracidia failed to respond to the touch of a blunt needle. Results were analysed using the Lee–Desu Comparison test (Unistat software computer package)

Avoidance behaviour of miracidia

A 'phi-shaped test chamber' was constructed according to the technique described by Roberts *et al.* (1978). Twenty miracidia were placed in 1 ml of soft water and 100 μl of heavy metal solution or distilled water for control, were then added to one side of the chamber. After 30 sec a dam was inserted to bisect the chamber and the number of miracidia on the inoculated side was counted. Six replicates of this procedure were undertaken at each of the 4 metal concentrations, all tests were conducted at the same time. The chamber was briefly rinsed after each treatment with 10 % nitric acid followed by distilled water. Statistical analysis of results was undertaken using one-way ANOVA (SPSS software computer package).

Egg hatch inhibition assay

Eggs of *S. mansoni* were isolated from mouse tissue using the sieving method of Dresden & Payne (1981). The pure egg solution produced by sieving in saline was kept on ice at all times until the commencement of each experiment and was concentrated by centrifugation until an approximate egg concentration of 20–25 eggs per ml was achieved. A final centrifugation replaced the saline with soft water. One ml of egg solution was added to 9 ml of soft water and toxin in 35 mm plastic Petri dishes to give a final metal concentration of 100, 1000 or 10000 μg/l. Three replicates of each concentration were prepared, all tests were conducted at the same time. Eggs were then incubated at 25 °C for 3 h to induce hatching. The experiment was suspended with the addition of alcohol–formalin–acetic acid. The proportion of hatched to unhatched eggs was determined, immature eggs being discarded, and the results analysed using one-way ANOVA (SPSS software computer package).

RESULTS

Water analysis

Loss of dissolved metals from the test solutions occurred within 0·5 h in most cases and generally continued up to 24 h. The initial rate of loss was greatest from the highest metal concentration (10000 μg/l, 4·1 % for Cd after 5 h, 8·1 % for Zn after 5 h), although over the 24 h period there was no more than a 16 % loss of concentration from any of the test solutions; however, the lowest metal concentration (10 μg/l) showed no concentration loss over this period.

Survival of miracidia

Miracidia exposed to either cadmium, zinc, or a mixture of both metals showed similar patterns in reduced survival with increasing metal concentration (Fig. 1). These results were significantly different from controls at single metal concentrations of 1000 μg/l or higher ($P < 0{\cdot}001$) and at cadmium/zinc mixtures of 10 μg/l or higher ($P \leqslant 0{\cdot}002$). When miracidia were exposed to cadmium or zinc at the lowest concentration (10 μg/l) survival was increased beyond that set by the controls, although statistical analysis showed that only cadmium at 10 μg/l was significantly different ($P < 0{\cdot}005$). Additional analysis after 4 h, which represented the lower limit of the time of maximum miracidia infectivity (Sturrock, 1993), demonstrated that only concentrations of 10000 and 1000 μg/l significantly reduced survival with cadmium or zinc ($P \leqslant 0{\cdot}037$), and that only at 10000 μg/l was survival significantly affected for mixtures of cadmium and zinc ($P < 0{\cdot}001$).

Behaviour of miracidia

Rapid avoidance behaviour was only significant at 1000 and 10000 μg/l for individual exposures of cadmium and zinc ($P \leqslant 0{\cdot}032$) (Fig. 2). However, significant avoidance occurred at the lower concentration of 100 μg/l for mixtures of both metals

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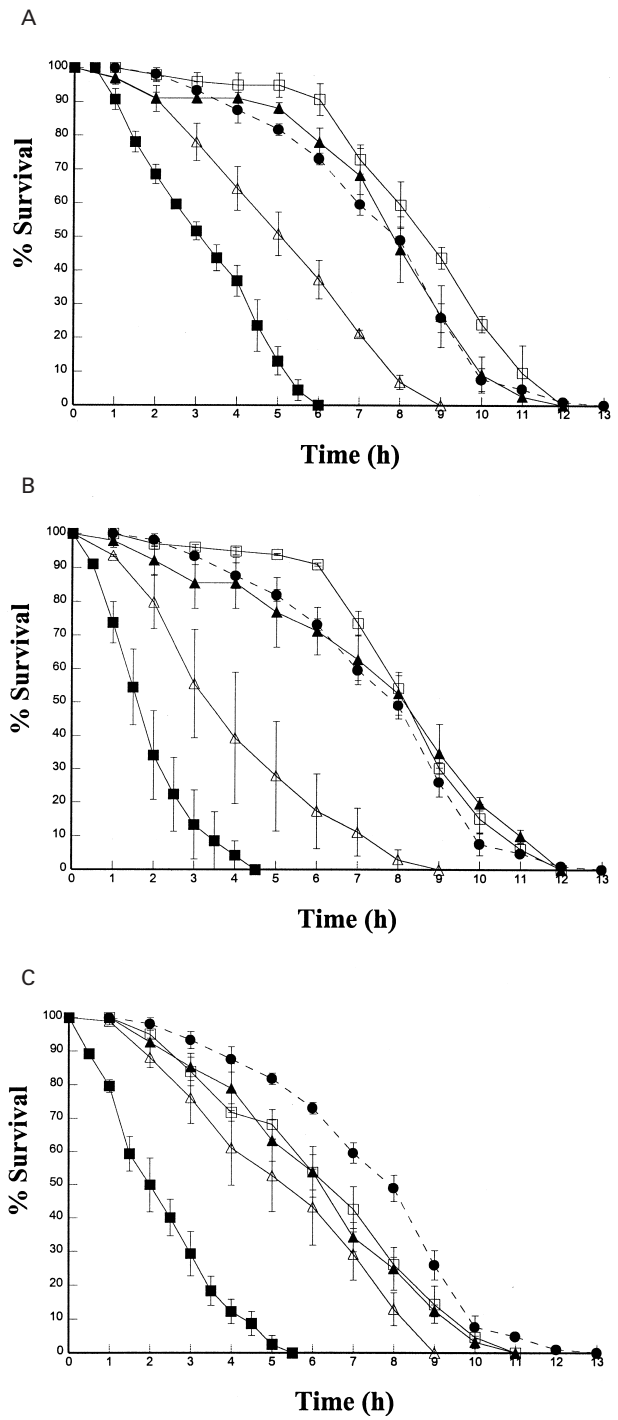

Fig. 1. Survival of miracidia of *Schistosoma mansoni* in (A) cadmium, (B) zinc, and (C) Cd/Zn mixture. Error bars are standard error. (●) Control; (□) 10 μg/l; (▲) 100 μg/l; (△) 1000 μg/l; (■) 10000 μg/l.

($P \leqslant 0{\cdot}025$) (Fig. 2). Nevertheless there were no significant differences in toxicity between the 3 exposure media at any concentration apart from 10 μg/l where cadmium was found to be different from zinc ($P = 0{\cdot}042$).

Inhibition of egg hatching

There was no consistent pattern in inhibition of egg hatching in the 3 toxic media tested. Exposure to

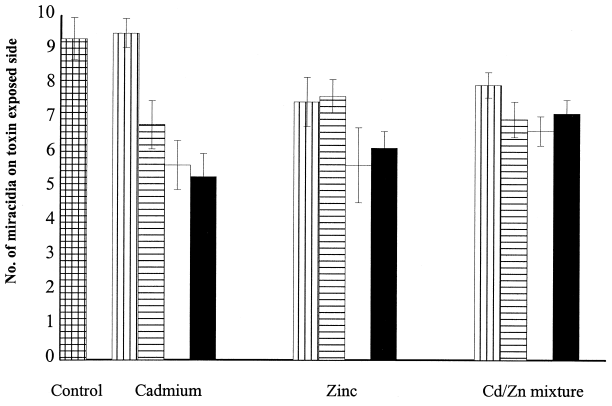

Fig. 2. Avoidance behaviour of miracidia of *Schistosoma mansoni* in cadmium, zinc, and a Cd/Zn mixture. Error bars are standard error. (▦) Control; (▥) 10 μg/l; (▤) 100 μg/l; (□) 1000 μg/l; (■) 10000 μg/l.

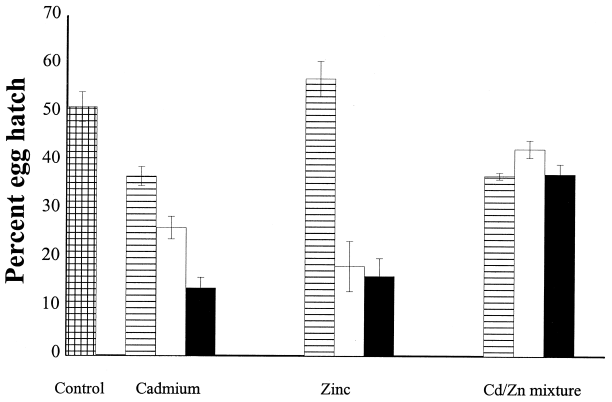

Fig. 3. Hatching of the eggs of *Schistosoma mansoni* in cadmium, zinc, and a Cd/Zn mixture. Error bars are standard error. (▦) Control; (▤) 100 μg/l; (□) 1000 μg/l; (■) 10000 μg/l.

cadmium led to a decrease in hatching with increased metal concentration (Fig. 3), although only 1000 and 10000 μg/l were significantly different from the controls ($P < 0{\cdot}001$). Exposure to zinc also resulted in a significant reduction in hatching at 1000 and 10000 μg/l ($P < 0{\cdot}001$) (Fig. 3). Mixtures of cadmium and zinc showed no significant differences in hatching compared with controls ($P \geqslant 0{\cdot}073$) (Fig. 3).

DISCUSSION

The survival of control miracidia declined with age and showed a life-expectancy comparable with previous studies (Anderson *et al.* 1982). Both cadmium and zinc exhibited a substantial toxicity to the survival of *Schistosoma mansoni* miracidia, but comparisons with other toxicity studies are limited. Wolmarans *et al.* (1988) examined the effects of the heavy metal chromium on miracidia of *S. haematobium* and reported that at 4·5 μM (234 μg/l) no mortalities were observed within 1 h. However, Siddall & des Clers (1994) found that 0·1 % sewage

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sludge, containing a mixture of 8 heavy metals, caused an earlier onset of mortality to the miracidia of the marine digenean *Zoogonoides viviparus*.

In the present study the enhanced survival observed at 10 μg/l may be the result of a retardation in miracidial activity leading to a reduced rate of utilization of the finite glycogen reserves. Reduced activity in the presence of low heavy metal concentrations occurs in cercariae of *Diplostomum spathaceum* (unpublished observations) and other invertebrates (Ortel, 1996). Indeed Lyddiard *et al*. (1998) showed that sublethal concentrations of plant secondary compounds significantly reduced the activity of *S. mansoni* miracidia.

The mechanisms of impairment of these previous studies and the present results may be a result of inhibition of enzymes involved in glycogen synthesis. Cadmium is known as a potent enzyme inhibitor (Ortel, 1996) which could explain its greater effects at low concentrations when compared with zinc in the present study. These results suggest the phenomenon of hormesis, where a low toxin dose appears to have a beneficial effect on an organism (Sanders *et al*. 1983). However, it is unlikely that in miracidial survival this effect is the product of an overcompensation of homeostatically regulated processes in response to stress, as is found in many invertebrates (Sanders *et al*. 1983), but more likely to be a by-product of the inhibition of glycogen synthesis. Interestingly, the combined effects of a cadmium/zinc mixture had an increased toxic effect when compared with the individual toxicities of these metals which negated this effect at low concentrations. Zinc can reduce many of the toxic effects of cadmium (Sharma, Mukherjee & Talukder, 1985), which may be indicative of different modes of toxicity for the 2 metals, possibly related to different cellular binding sites.

A demonstration of rapid avoidance behaviour to heavy metals has been shown for miracidia and, although the simple nature of the present experiments restricts any major interpretation of parasite behaviour in the presence of toxic substances, it is likely that a negative chemokinetic response is initially occurring. Previous studies utilizing this technique (Roberts *et al*. 1978; Plorin & Gilbertson, 1985) have concentrated on chemicals that stimulate a positive response in miracidia associated with host location. Nevertheless, Roberts *et al*. (1978) demonstrated that a number of neuro-inhibitors, such as Mecamylamine, incorporated into the miracidia suspension reduced the effectiveness of many known chemo-stimulants. Whether other toxic substances, such as heavy metals, can be shown to inhibit chemotaxis in this way remains to be seen.

Inhibition of egg hatching by short-term exposure to high concentrations of cadmium and zinc has also been shown in the present study. Although previous work by De-Carvalho *et al*. (1998) failed to demonstrate toxicity by plant secondary compounds, a number of other studies have successfully shown the toxic effects of pesticides on hatching of *Fasciola hepatica* miracidia (Guttowa & Bonieka, 1975, 1977). This suggests that the egg stage may be particularly vulnerable to certain pollution effects, especially as a large amount of water uptake is necessary to activate miracidia within the egg-shell to facilitate hatching (Becker, 1977). The mechanism of metal toxicity in the present investigation, potentially occurring in a different manner to these other studies, may be related to the inhibition of aminopeptidase, which functions as an initiator of hatching (Xu & Dresden, 1986). Work by Auriault *et al*. (1982) has shown that *S. mansoni* aminopeptidase is metal dependent, indicating that cadmium and zinc may be inhibiting the egg's enzymatic hatching function. Potential metal toxicity to transmission to the snail host has been beyond the scope of the present study. However, work by Morley (unpublished observations) on histochemical staining of *S. mansoni* miracidia after a short-term exposure (30 min) to 10000 μg/l Zn revealed that this metal selectively binds to areas dense in sensory receptors on the parasite surface. Such initial attraction by a subchronic exposure of a heavy metal to such sensitive and important areas may have wide-reaching consequences for transmission to the next host. Indeed previous studies by Wolmarans *et al*. (1988) on toxic effects of chromium to *S. haematobium* miracidia found that a 1 h exposure to 4·5 μM (234 μg/l) caused a reduction in the percentage sporocyst formation *in vitro*, miracidial penetration of the snail host, and the number of snails that eventually shed cercariae. Other heavy metals, including cadmium and zinc, may also have a wide-ranging effect on the transmission of *S. mansoni* in its molluscan host.

The present results have demonstrated wide-ranging metal toxicity to *S. mansoni* miracidia. In particular, mixtures of heavy metals have been shown to have a significant, if variably, different impact on miracidia compared to single metal toxicity. As metal mixture pollution incidents are more likely to be experienced in natural systems (Howell, 1985) their potential synergistic or antagonistic relationship and its influence on helminth free-living stages requires further investigation.

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