ORIGINAL PAPER

Penetration of cercariae into the living human skin: *Schistosoma mansoni* vs. *Trichobilharzia szidati*

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Abstract We studied the skin invasion of *Schistosoma mansoni* cercariae by placing gamma-irradiated and non-irradiated cercariae onto the living human skin and timing the behavior of 53 individuals. The skin invasion of *S. mansoni* was less efficient compared to the bird schistosome *Trichobilharzia szidati*. *S. mansoni* cercariae crept longer on the skin after attachment until they started penetration movements (median of 43 s [range of 15 s–6.58 min]; *T. szidati*, median of 8 s [range of 0–80 s]). Subsequent to this longer exploratory phase, 74% penetrated into wrinkles (*T. szidati* 84%), 22% into the smooth skin surface (*T. szidati* 0%), and 4% into hair follicles (*T. szidati* 16%). The *S. mansoni* cercariae needed, on average, 6.58 min (range of 1.57–13.13 min) for full entry, while *T. szidati* needed 4.0 min (range of 1.38–13.34 min); the fastest *S. mansoni* cercaria entered the skin within 94 s, while *T. szidati* entered within 83 s. Sixty percent of the *S. mansoni* cercariae had the tails still attached when the bodies disappeared in the skin whereas all *T. szidati* cercariae shed their tails within 0–105 s after the onset of penetration movements. The faster invasion of *T. szidati* may result from the more sophisticated host-finding mechanisms of this species. Regarding *S. mansoni*,
cercarial dermatitis, as immediate skin response, developed after a sensitization period of 19 days.

Schistosome cercariae actively penetrate the skin of their vertebrate hosts and much is known about the behavioral mechanisms used by the cercariae to find, recognize, and invade the host's skin. When the cercariae are in close proximity of the skin surface, they respond to very different host cues with a sequence of behavior patterns: chemo-orientation towards the skin (Brachs and Haas 2008; Haeberlein and Haas 2008), attachment to the skin, enduring contact with the skin, creeping to entry sites, penetration, shedding of the tail, secretion of acetabular gland contents, and orientation within host tissues (reviewed by Haas and Haberl 1997; Haas 2003). However, a simple question on schistosome host invasion remains mainly unanswered: how fast can schistosome cercariae enter the skin of humans? This is of practical importance, e.g., when contact with schistosome-contaminated water occurs or when the effect of protecting cream formulations is studied (Wulff et al. 2007).

Work with the bird parasite and agent of human cercarial dermatitis *Trichobilharzia szidati* revealed that the cercariae can enter living human skin within a period of time as short as 82 s (Haas and van de Roemer 1998). Whether the cercariae of human schistosomes are similar fast invaders of the human skin is not clear. Studies with various laboratory mammal models and experiments with excised human skin and biopsy skin specimens (Bartlett et al. 2000; Whitfield et al. 2003) gave indirect evidence for a fast entry. Only one study dealt especially with the timing of the schistosome cercarial behavior patterns when entering human skin (Stirewalt and Hackey 1956). The cercariae of *Schistosoma mansoni* needed an average of 7.2 min for full entry, and

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the fastest entry time measured was 3.1 min. However, the data were obtained by timing only 21 cercariae, and the skin samples were partly excised postmortem and some were frozen and thawed. It is well possible that the cercariae may show a different behavior when they enter living skin, which is circulated by blood and whose proteins are not coagulated. In this study, we placed gamma-irradiated and nonirradiated *S. mansoni* cercariae on living skin, recorded the time needed for the host invasion phases, and compared the data with those formerly obtained with *T. szidati* by similar methods (Haas and van de Roemer 1998). As cercariae of *T. szidati* entered living skin faster than did *S. mansoni*, we compared the sensitivity of both species to the typical penetration stimulus linolenic acid (Haas and Schmitt 1982a; Haas and van de Roemer 1998). To our knowledge, this is the first study investigating the crucial process of how fast the human-pathogenic cercariae of *S. mansoni* penetrate into living human skin.

Materials and methods

The strain of the human parasite *S. mansoni* and its intermediate host *Biomphalaria glabrata* were isolated in Belo Horizonte, Brazil. The parasite was maintained in white mice as final host, infected by the paddling method. The data obtained with *S. mansoni* cercariae were compared with those formerly obtained with *T. szidati* (Haas and van de Roemer 1998). This strain of *T. szidati* is synonymous with *Trichobilharzia ocellata* (La Valette 1855) as used by various European laboratories (Odening 1996; Kock 2001; Rudolfová et al. 2005). The stock was isolated from its host snail *Lymnaea stagnalis* taken from fish ponds near Höchstadt/Aisch (Germany) and maintained using laboratory-reared *L. stagnalis* as intermediate hosts and domestic ducks as final hosts.

In order to avoid infections of the authors, four experimental series were conducted with gamma-irradiated cercariae. Freshly shed cercariae were irradiated in an Oncor Linear Accelerator (Siemens, Germany) with a dose of 300 Gy (30 krd). In addition, six experimental series were conducted with nonirradiated cercariae to exclude effects due to irradiation.

Penetration of the cercariae into living skin of the authors was studied by placing three to ten cercariae (less than 4 h post emergence age) in 50 μL of tap water on the skin of the back of the hand and observing the behavior of the cercariae under a dissecting microscope (magnification ×50). The cercariae were illuminated with cold light sources (KL 1500, Schott, Wiesbaden, Germany). The exposed skin area was marked with waterproof ink, and in experiments with nonirradiated cercariae, it was disinfected by adding a layer of ethanol or 10% povidone iodine (Betaisodona®)

for at least 5 min. This could not prevent schistosomiasis infection, which then was treated with praziquantel. The behavior of individual cercariae until complete penetration was more difficult to observe than in *T. szidati* cercariae due to the smaller size of *S. mansoni* and the lack of the distinctive ocelli. Therefore, a total of 715 cercariae had to be applied on the skin within 133 trials to observe the whole sequence of behaviors of 53 cercariae. It should be noted that both the previously performed experiments with *T. szidati* (Haas and van de Roemer 1998) and most of the experiments with *S. mansoni* were performed by the same experimenter and thus imply comparable skin conditions.

The sensitivity of cercariae of *S. mansoni* and *T. szidati* to linolenic acid as penetration stimulus was studied using modifications of the methods described by Haas and Schmitt (1982a) and Haas et al. (1987). Briefly, linolenic acid was incorporated into 2.5% agar (pH 7.0, 5 mM phosphate buffer) and layered into flat-bottom wells (7 mm diameter) of tissue culture plates (experiments with *T. szidati*) or into Petri dishes (30 mm diameter, experiments with *S. mansoni*). The cercariae (20–70 per replicate) were added in tap water on the agar and the percentage of penetrating individuals recorded after incubation for 1 h at 35°C.

The duration of the behavioral phases was statistically compared using the Kruskal–Wallis test and a time to event analysis with the log rank test. The penetration of cercariae into agar substrates was analyzed with the Tukey multiple *t* test after arcsine–square root transformation of the relative abundances.

Results

S. mansoni cercariae readily attached and crept on the skin surface and they penetrated into the skin with the known behavior patterns (Stirewalt and Hackey 1956) which did not differ from those of *T. szidati* (Haas and van de Roemer 1998). All behavioral phases of skin invasion of *S. mansoni* lasted significantly longer ($P<0.001$) than those of *T. szidati* (Fig. 1). After attachment, *S. mansoni* cercariae crept on the skin for a median of 43 s (range of 15 s–6.58 min) before they started penetration movements (Fig. 1a) (*T. szidati*, median of 8 s [range of 0–80 s]). After this exploratory phase, 74% of 54 cercariae penetrated into wrinkles (*T. szidati* 84%), 22% into the smooth skin surface (*T. szidati* 0%), and 4% into hair follicles (*T. szidati* 16%). The *S. mansoni* cercariae needed 6.58 min (range of 1.57–13.13 min) for full entry of the body (*T. szidati* 4.0 min [range of 1.38–13.34 min]; Fig. 1c); the fastest cercaria entered the skin within 94 s (*T. szidati* 83 s). Only 40% of the *S. mansoni* cercariae shed their tails when penetrating
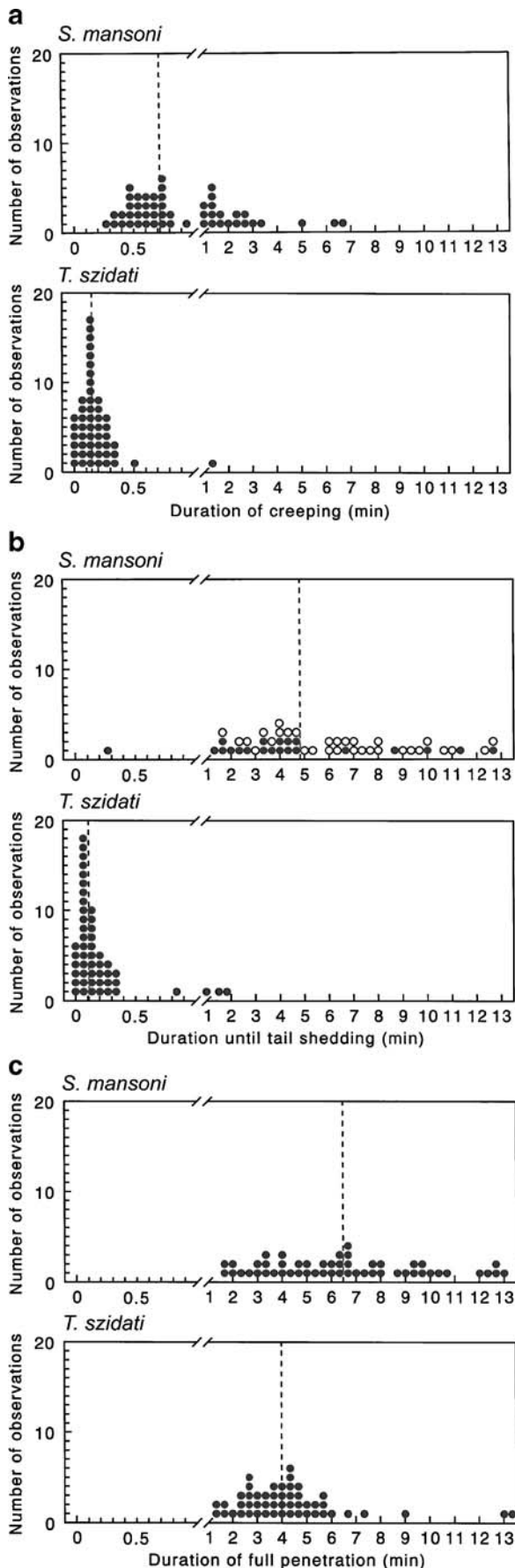
Fig. 1 a–c Duration of behavioral phases during invasion of the living human skin by cercariae of *S. mansoni* and comparison with the data for *T. szidati* (from Haas and van de Roemer 1998). **a** Duration of creeping between attachment and start of penetration movements. **b** Duration between start of penetration and shedding of the tail; *open symbols* represent cases in which the tail was still attached when the body entered the skin. **c** Time required for full penetration, i.e., until the hindbody disappeared in the penetration pore. *Vertical lines* represent median values

(3.93 min [range of 16 s–12.70 min] after onset of penetration movements), 60% had the tails still attached when the bodies disappeared into the skin (Fig. 1b). However, we did not observe how far the tails were pulled into the entry pores. This was different in *T. szidati* cercariae, which all shed their tails 6.5 s (0–105 s) after the onset of penetration movements. The time needed for full entry of the *S. mansoni* cercariae did not differ, whether the cercariae were irradiated or not (*P*=0.92), as well as whether they entered the skin of the junior or senior author (*P*=0.50; data not shown).

We started our experimental series with irradiated cercariae and many cercariae penetrated the skin. However, no immediate skin response could be observed, such as it occurred in experimental infections of the authors with *T. szidati* cercariae. But typical papules appeared 17–22 days after the infections with irradiated cercariae (19+8+3 papules 17 days post infection [p.i.], one papule 19 days p.i., and 3+1 papules 22 days p.i.; Fig. 2). Later in the course of the experiments (19 days after the first infection experiments and after eight infection series), a skin response developed also directly after the infections. The papules appeared in the night after the infections; they itched heavily for 3 days and were still visible 6 days p.i.

Fig. 2 Itching papules appeared 17 days after infection with gamma-irradiated *S. mansoni* cercariae

The sensitivity of *S. mansoni* and *T. szidati* cercariae to penetration stimuli was studied by exposing the cercariae to agar containing linolenic acid as penetration stimulus. *T. ocellata* cercariae penetrated into the agar at concentrations as low as 1 μM (penetration rate 12.0%, control 3.3%; $P=$ 0.01 vs. control; 31 replicates with 660 cercariae). However, *S. mansoni* cercariae did not respond to concentrations of 1 and 3 μM, but 10 μM linolenic acid stimulated them to penetrate (penetration rate 20.8%, control 0%; $P<$ 0.001 vs. control; five replicates with 331 cercariae).

Discussion

We have shown that *S. mansoni* cercariae can already penetrate the skin within 1.5 min and that a disinfection of the skin immediately after the exposure to the cercariae could not prevent schistosomiasis. This indicates that even a short contact with cercariae-contaminated water bears a high risk of infection.

The bird schistosome *T. szidati* invaded the skin of its abnormal human host with higher efficiency than did *S. mansoni* whose main hosts are humans. The *T. szidati* cercariae found their entry sites much faster than did *S. mansoni* cercariae (mean duration of creeping 8 vs. 43 s) and also the penetration process was faster (a mean of 4.0 vs. 6.6 min). This may result from the different host-finding and host-invasion behavior of the two species. In fact, *T. szidati* has more sophisticated behavioral mechanisms. They respond more sensitively to visual, mechanical, and thermal stimuli within their more complex swimming behavior (Feiler and Haas 1988a, b) than *S. mansoni* (Brachs and Haas 2008; Haeberlein and Haas 2008) and attach to the skin in response to more host-specific stimuli (ceramides, cholesterol, shadowing, water currents, and warmth; Feiler and Haas 1988a, b) than *S. mansoni* (arginine, low response to warmth, and water currents; Haas 1976; Haas et al. 2002). It is not yet known which host cues guide *T. szidati* cercariae towards their entry sites, but our observations show that they are also in this respect more successful. *T. szidati* cercariae explored the skin surface in a fivefold shorter time and all individuals preferred wrinkles and hair follicles as suitable entry sites, whereas 22% of *S. mansoni* cercariae penetrated into smooth skin areas. This suggests that they use other host cues when searching for entry sites than *S. mansoni* cercariae, which are attracted by arginine and warmth (Haas et al. 1994, 2002). Penetration of the skin is stimulated in both species by fatty acids with the same chemical characteristics (Haas and Schmitt 1982a, b; Haas and van de Roemer 1998), but our data show that *T. szidati* cercariae respond more sensitively to the typical penetration stimulus linolenic acid. This seems to be an adaptation to

invade duck hosts whose skin surface has much lower fatty acid content than human skin. And in fact, the penetration behavior of *T. szidati* cercariae is much more stimulated by human skin surface lipids than by duck skin surface lipids (Haas and van de Roemer 1998). The penetration of the skin is supported by the enzymatic activity of acetabular gland contents. The secretion of the glands is stimulated in the two species by different host cues, in *S. mansoni* by fatty acids, glycosylceramides, and phospholipids (Haas et al. 1997) and in *T. szidati* by fatty acids, ceramides, and cholesterol (Haas et al., unpublished results). It is not clear whether this different secretion mode contributes to the faster entry of *T. szidati* cercariae. However, also the gland secretions themselves differ between the two species. The main penetration enzyme of *S. mansoni* is thought to be a serine peptidase, elastase (Salter et al. 2000). But in *Trichobilharzia* cercariae, no elastase activity was found (Kasny et al. 2007). The same holds true for cercariae of *S. japonicum* (Dvorák et al. 2008), a species which enters the skin of mammals very fast (Pan et al. 1954) and migrates faster through the human skin than *S. mansoni* (Ruppel et al. 2004; Wang et al. 2005). It was suggested that, in *S. japonicum*, as well as in the bird schistosomes *T. szidati* and *Trichobilharzia regenti*, cathepsin B-like cysteine peptidases may act as the main penetration enzymes (Salter et al. 2002; Mikes et al. 2005; Kasny et al. 2007; Dvorák et al. 2008). It is well possible that this specific enzymatic equipment of *T. szidati* enables the cercariae to penetrate the skin faster than *S. mansoni*. Likewise, after penetration and transformation to schistosomula, *T. szidati* shows other behavioral characteristics than *S. mansoni*. The schistosomula respond much more sensitively to the directing cues within serum (arginine, arginine-containing peptides, and glucose; Grabe and Haas 2004). In case of these cues already being used as attractants during the penetration process, they may also contribute to the faster entry of the skin by *T. szidati*. The faster invasion of human skin by *T. szidati* may be a consequence of the adaptation of these parasites to invade ducks as hosts. Duck foot-skin has a thicker keratinized surface layer which is probably more difficult to disrupt than the thinner human skin surface. Nevertheless, *T. szidati* cercariae are able to penetrate this barrier relatively fast. Therefore, it is not surprising that they invade the human skin faster than *S. mansoni* for which the penetration of human skin is natural.

It is noteworthy that 60% of the *S. mansoni* cercariae had their tails still attached when the body disappeared in the entry pores. This is in contrast to the assumptions of many authors that schistosome cercariae lose their tails at the onset of penetration. However, Stirewalt and Hackey (1956) also observed a delayed tail loss (DTL) when *S. mansoni* cercariae penetrated the skins of various animals. Furthermore, Whitfield et al. (2003) found a DTL in *S.*

mansoni cercariae entering excised human skin samples and they suggested that DTL is a specific adaptation of *S. mansoni* to evade the host's immune system.

During our initial infection series, many irradiated *S. mansoni* cercariae must have penetrated the skin, but we observed no signs of immediate cercarial dermatitis. This suggests that, in *S. mansoni* infections, similarly as in infections with bird schistosomes (reviewed by Horák et al. 2002), the immediate skin response occurs only after sensitization by repeated cercarial exposure (in our case, after 19 days and eight infection series). This sensitization seems to disappear in the course of time, as the senior author developed no immediate cercarial dermatitis although he had three histories of acute *S. mansoni* schistosomiasis. There is also no cross-reaction between infections with cercariae of *T. szidati* and *S. mansoni* as both authors responded with intensive cercarial dermatitis to infections with *T. szidati* but not to the infections with *S. mansoni*. We observed a delayed cercarial dermatitis 17–22 days after infection with irradiated cercariae. This must be a response to dying schistosomula which had not left the skin. That irradiated cercariae can persist for more than 10 days in the skin has been shown in animal experiments (reviewed by Coulson 1997; Riengrojpitak et al. 1998).

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