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Research Note

Dexamethasone treatment affects skin mucous cell density in *Gyrodactylus derjavini* infected *Salmo salar*

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Abstract

Atlantic salmon, *Salmo salar*, is normally rather refractive to infection with the ectoparasitic monogenean *Gyrodactylus derjavini* but dexamethasone treatment of the host increases the susceptibility. The causative mechanisms were elucidated in this work. Groups of Atlantic salmon were treated by intra-peritoneal dexamethasone injections and subsequently infected with *G. derjavini*. It was shown that both the infection level and the mucous cell density of caudal and pelvic fins were affected by the treatment. Significantly higher mucous cell densities were found on infected and treated fish whereas non-infected and treated fish showed no significant elevation of cell density. This suggests that mucous cell discharge elicited by infection is inhibited by the drug. The association with elevated parasite counts in these fish can be explained either by decreased anti-parasitic mucus action or by parasite predilection for intact mucous cells.

Atlantic salmon are naturally resistant to infection with the trout parasite *Gyrodactylus derjavini*. (Buchmann & Uldal, 1997; Bakke *et al.*, 1999, 2002). However, dexamethasone treatment interferes with this host specificity (Olafsdottir *et al.*, 2003). Other studies have shown that steroid treatments generally affect the susceptibility of salmonids to infections with various gyrodactylids (Lindenstrøm & Buchmann, 1998; Harris *et al.*, 2000). Host factors involved in this innate host response are less well defined but mucous cells have been suggested as a main player in such host–parasite associations (Buchmann & Bresciani, 1998; Sterud *et al.*, 1998). Molecular studies have demonstrated that interleukin 1-beta gene expression is induced in rainbow trout skin following *G. derjavini* infection (Lindenstrøm *et al.*, 2003). In addition, it is known that corticosteroids depress IL-1 gene expression in this host (Zou *et al.*, 2000). This pro-inflammatory cytokine has several effects in the host, including the mucous cell discharge (see Buchmann, 1999). The present work was initiated to elucidate if the corticosteroid dexamethasone, on this background, affects the mucous cell characteristics of salmon.

Hardened eggs of the Scottish Conon river strain of the Atlantic salmon *Salmo salar* were brought to the Bornholm salmon hatchery (Nexø, Bornholm, Denmark) and hatched and reared in pathogen free conditions (recirculated water) until fish reached a mean body weight of 3.2 g and a mean body length of 7.9 cm.

Fish were infected by a laboratory strain of the ectoparasitic monogenean *G. derjavini* originally isolated from the Danish trout farm Mosbjerg (Jutland, Western Denmark).

Municipal water was used in fish tanks. This has a pH at 7.43–7.46, temperature at 12°C, calcium carbonate

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390 mg l^{-1}, no nitrite and ammonia and a nitrate content below 20 mg l^{-1}.

Treated fish were injected intra-peritoneally (200 μg per fish) with the corticosteroid dexamethasone (Dexadresone®) corresponding to 0.1 ml per fish. Control fish were injected with a similar volume of phosphate buffered saline (PBS).

Infected fish were anaesthetized (MS 222 50 mg l^{-1}) and placed under the dissection microscope (sub-illumination) (7–40 × magnification). The total number of parasites were counted on each body part (Buchmann & Uldal, 1997).

Caudal and pelvic fins were excised from sub-samples of salmon on days 2 and 4 post-infection and subsequently fixed in phosphate buffered 4% formaldehyde. Following rinsing in PBS, the fins were stained for mucous cell detection in 1% Alcian Blue in 3% acetic acid (Wells & Cone, 1990). Fins were mounted on slides in Aquamount and four zones of 0.61 mm^2 from each fin were counted in the compound microscope (200× magnification).

Corticosteroid-treated fish (10) and PBS-injected control fish (10) were exposed by cohabitation to *G. derjavini* infection in a 128-litre fish tank containing 10 infected rainbow trout (infected with more than 700 parasites). Non-infected fish were kept in similar aquaria and treated likewise except for infection. Parasites spread from donor fish to salmon and the number of parasites on each fish was counted on days 2 and 4 post-infection. Fins were excised on day 2 and 4 post-infection and processed for mucous cell counting.

The mean number of parasites per fish or per fin and mucous cell densities in various groups were compared using the Student's *t*-test with a probability level of 0.05.

Parasites spread readily from infected donor fish to recipient salmon. Mucous cell counts were generally higher on pelvic fins compared to caudal fins and a general trend to a slight decrease from day 2 to day 4 in mucous cell density was observed in most groups which could be due to initial handling (table 1). Dexamethasone treatment of uninfected fish only caused a non-significant slight increase in mucous cell density (table 1). In contrast, the main difference between dexamethasone treated/infected fish and PBS treated/infected fish was the significantly increased mucous cell density in the former group (table 1). Dexamethasone treated fish also carried a significantly higher parasite burden after 4 days compared to untreated controls (table 1).

The present investigation confirmed previous studies showing that corticosteroid treatment of salmonids reduces the innate resistance of a host to infection with an inappropriate gyrodactylid (Harris *et al.*, 2000; Olafsdottir *et al.*, 2003). The underlying mechanisms have been incompletely described but this work suggests an association between a reduced mucous cell discharge and a reduced resistance to infection. Dexamethasone treatment alone does not increase the cell count significantly, but when subsequently stimulated by parasites treated fish exhibit a higher mucous cell number on both caudal and pelvic fins. Thus, it can be suggested that infection stimulates mucous cell production and immediate mucus discharge in untreated fish with an effective anti-parasitic response. Therefore the mucous cell count will not increase because cells are readily emptied in untreated fish. In contrast, when hosts are dexamethasone-treated the mucous cell discharge is inhibited whereby the total cell count increases significantly accompanied by a reduced response. This is more clearly shown in infected fish stimulated by skin parasites. Thus, the drug induced inhibition of mucous cell function could be one of more factors explaining the corticosteroid labile resistance of salmonids to gyrodactylid invasion. Interestingly, a similar association between increased infection level and increased mucous cell density was found in dexamethasone treated Scottish salmon infected by *G. salaris* whereas this was not the case in Swedish Lule salmon (Dalgaard *et al.*, 2003). In this Baltic strain of salmon, susceptibility and mucous cell density were only lightly affected by dexamethasone which emphasizes the link between mucous cells and susceptibility. The molecular basis of this event could be connected to cytokine production in the host. It was previously shown that the expression of IL-1 genes and production of mucous cells in rainbow trout skin are increased and subsequently discharged following *G. derjavini* infection (Lindenstrøm & Buchmann,

Table 1. Mean number of parasites per fish (SD) and mean number of mucous cells (SD) in 0.61 mm^2 zones of caudal and pelvic fins of PBS or dexamethasone-treated Conon salmon uninfected or infected with *Gyrodactylus derjavini*.

	Mean no. parasites per fish (SD)		Mucous cell density (SD)			
			Caudal fin		Pelvic fin	
Group	Day 2	Day 4	Day 2	Day 4	Day 2	Day 4
Uninfected						
PBS control	0	0	376.8 (166.6)	216.7 (62.8)	441.4 (186.2)	380.4 (124.3)
Dexamethasone treated	0	0	361.1 (159.1)	406.4 (211.0)	478.8 (160.5)	445.7 (207.0)
Infected						
PBS control	41.2 (19.1)	54.8 (29.0)	345.1 (143.9)	309.8 (139.3)	504.3 (238.7)	386.2 (121.6)
Dexamethasone treated	34.4 (14.0)	87.3* (43.9)	584.2 (244.3)	456.3* (146.3)	778.6* (226.2)	626.5* (149.2)

*Significantly different from control, $P < 0.05$.
Five fish in each sample.

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1998; Buchmann, 1999; Lindenstrøm *et al.*, 2003). It is noteworthy that expression of this cytokine is inhibited by dexamethasone (Zou *et al.*, 2000) and this could explain why mucous cells show decreased emptying in treated fish which subsequently can contribute to a reduced anti-parasitic response. It should be noted that mucous cell density may not be the only factor of importance. Thus, complement in salmon mucus was found in higher concentrations in salmon responding effectively to *G. salaris* infection compared to non-responding fish (Bakke *et al.*, 2000) and complement factors are known to have a devastating effect on gyrodactylids (Moore *et al.*, 1994; Buchmann, 1998; Harris *et al.*, 1998). Furthermore, other elements such as lectins (Buchmann, 2001), acute phase molecules (Bayne *et al.*, 2001) or leukocytes (Buchmann & Bresciani, 1999) which are affected by dexamethasone (Pagniello *et al.*, 2002) could be involved in these interactions. Finally, it cannot be excluded that non-immunological factors (still not elucidated) being sensitive to dexamethasone in the fish skin contribute to the parasite's preference for a certain host. In this context it is possible that parasite reproduction could be triggered by an increased number of undischarged mucous cells in the microhabitat.

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