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Relationships between secondary lamellar structure and dorsal aortic oxygen tension in Salmo gairdneri with gills damaged by zinc

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Rainbow trout (*Salmo gairdneri*, Richardson) were exposed in fresh water to zinc (1 mg/l) for 17–20 hours. The O_2-tension in dorsal aortic blood (Pa_{O_2}) and external water (PI_{O_2}) was measured and the sedondary lamellae of the gills were analysed morphometrically. Zinc exposure increased the PI_{O_2} - Pa_{O_2} difference significantly. The PI_{O_2} - Pa_{O_2} difference was positively correlated with the increase in the fractional volume of blood channels to which the diffusion distance from water was over 15 μm. The results are discussed in the light of the hypothesis that gill O_2-transfer is dependent on the intralamellar shunting of blood.

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1. Introduction

Low concentrations of dissolved oxygen increased the toxicity of zinc to rainbow trout (Lloyd 1960), indicating some disturbance in the gas exchange of the fish. During the exposure to lethal concentrations of zinc, fish died of tissue hypoxia (Skidmore 1970; Burton et al. 1972). Sublethal concentrations of zinc decreased dorsal aortic P_{O_2} significantly (Sellers et al. 1975).

Disturbances in gas exchange of the fish seem to be due to the gill damage induced by zinc (Lloyd 1960; Skidmore & Tovell 1972; Matthiessen & Brafield 1973: Tuurala & Soivio 1982). The main effect of zinc on the gills is the detachment of the secondary lamellar epithelium from the underlying pillar cell system. This increases the diffusion distance from water to blood and also the fusion of the adjacent secondary lamellae, decreasing the gill area (Skidmore & Tovell 1972). Tuurala & Soivio (1982) using morphometrical methods analysed the structural and circulatory changes in rainbow trout gill secondary lamellae exposed to sublethal concentrations of zinc. During 96 h exposure to 1.25 mg/l of zinc the fusion of the secondary lamellae decreased the free gas exchange surface to less than 40 % of that in the control fish. The lymphatic space under the epithelium increased by 147 % owing to the detachment of the epithelium. Both changes evidently greatly impaired the O_2-transfer capacity of the gills.

In recent studies (Pärt et al. 1983; Tuurala et al. 1983) we have proposed a mechanism for the regulation of gill gas transfer capacity by the intralamellar shunting of blood through the basal channels of the secondary lamellae in the gills. While the diffusion distance to most of the blood spaces of the secondary lamellae is so short that P_{O_2} of blood flowing through them should reach the same value as in the external water, the distance from water to the basal channels is so long that blood flowing through them does not reach full saturation. Consequently the difference between PI_{O_2} and Pa_{O_2} should depend on the fractional blood flow through channels to which the O_2-transfer is limited by diffusion.

In this study, zinc was used as a tool in order to increase the diffusion distance from water to the blood spaces of the secondary lamellae, especially in the basal parts of the lamellae. Dorsal aortic oxygen tension of the fish was measured and the gills were analysed using morphometric methods in order to see how the structural changes correlated with the increase in the PI_{O_2} - Pa_{O_2} difference in the same fish.

2. Material and methods

The experiments were carried out at Laukaa Fish Culture Research Station in May - June 1982. The fish used were 3-year old rainbow trout (*Salmo gairdneri*, Richardson) (weight, mean and *SE*, 535 ± 31 g; length 34.5 ± 0.6 cm). Throughout

the experiments local lake water was used with a flow of 2-4 l/min/kg fish. The water quality was as follows: pH 6.9, specific conductivity at 20°C 4.9-5.2 mS/m, total hardness 0.16 mmol $CaCO_3$/l. The oxygen saturation of the water was over 90 %.

Before the experiments the fish were anaesthetised with MS-222 (0.1 g/l) and cannulated to the dorsal aorta according to the method of Soivio et al. (1975). After the operation fish were allowed to recover and acclimatise to the experimental temperature (8.5-10.2°C) for two weeks. The fish were fed with pelleted Ewos trout food daily until 48 h before the experiments.

The fish were enclosed in individual restrainers 24 h before the experiments were begun in order to avoid any visual disturbance (Soivio et al. 1975). The fish were divided into two groups. The first group (n= 6) served as control fish. The other group (n = 7) was exposed to zinc in a continuous flow-through system. Zinc was administred as $ZnSO_4$-solution with a peristaltic pump (Ismatic mp-ge) so that the final calculated concentration of Zn^{2+} was 1 mg/l.

Just prior to, and after, the 17-20 h exposure the P_{O_2} of inspired water (PI_{O_2}) was measured with a Radiometer BMS-3 Mk2 system at the experimental temperature. Immediately after these determinations 0.2 ml blood samples were drawn through the dorsal aortic cannula in a lml unheparinised syringe. The blood P_{O_2} (Pa_{O_2}) was then measured with the same system. The blood samples were not taken from struggling fish. The same sampling and measuring procedure was carried out with the control fish.

After the final P_{O_2} measurements in both groups, the fish were quickly "poured" out from the restrainers and stunned with a blow on the head within 10 sec from the first disturbance. The blood flow in the first left gill arch was stopped simultaneously both dorsally and ventrally with tongue forceps. The gill arch was cut outside the forceps and immersed in 2.5 % glutaraldehyde (in 0.1 M phosphate buffer, pH 7.2) for at least 20 min at + 4°C. After this initial fixation the forceps were removed and fixation continued for at least 24 h. The time interval from stunning the fish to the immersion of the gill sample was less than 15 seconds. After this, the middle parts of the gill filaments were selected, postfixed in 1 % osmium tetroxide solution (in 0.1 M phosphate buffer, pH 7.2), dehydrated in ethanol and embedded in Epon 812 (Luft 1961). Semithin (1μm) sections of the gill samples were cut parallel to the long axis of the gill filaments and perpendicular to the surface of the secondary lamellae with a Sorvall MT 2B ultramicrotome. The sections were mounted on glass slides and stained with 1% toluidine blue in 1 % borax solution.

The morphometric analyses were carried out at a magnification of 1410 x with a Wild M 20 microscope fitted with a projection head containing a 36-point Mertz grid (Mertz 1967). Points falling on the profiles of the following components of the secondary lamellar area (cf Hughes & Perry 1976; Tuurala & Soivio 1982; Tuurala et al. 1983) were counted: tissue from the epithelium covering the secondary lamellae, the non-tissue (lymphatic) component of the epithelium, the pillar cells and their basement membrane, space between the secondary lamellae ('water'), blood plasma and erythrocytes in the blood channels of the secondary lamellae. Points lying on the blood were divided into two groups: 1) points lying on blood in channels to which the diffusion distance from the free surface of the secondary lamellae was less than 15 μm. Intersections between the lines of the test lattice and the outer surface of the secondary lamellae were also counted. The points and intersections were counted for 20-35 fields of the test lattice in each section.

The results of point and intersection countings made it possible to evaluate the proportional volumes of different components in the secondary lamellar area as well as the surface/volume rations of the secondary lamellae.

For statistical comparison of means, Student's t-test was used for P_{O_2} values and the Mann-Whitney U-test for morphometric data.

3. Results

The difference between oxygen tensions in inspired water (PI_{O_2}, 20.4 ± 0.27 kPa) and dorsal aortic blood (Pa_{O_2}) in the control group was 5.33 ± 0.67 kPa and in the experimental group before exposure to zinc 5.43 ± 0.87 kPa. During the zinc exposure PI_{O_2} - Pa_{O_2} difference increased significantly to 12.91 ± 1.36 kPa ($p< 0.001$). The variation of PI_{O_2} - Pa_{O_2} difference in zinc exposed fish was great, the lowest value being 8.63 kPa and the highest one 17.59 kPa.

The reason for the increase in PI_{O_2} - Pa_{O_2} difference in zinc exposed fish was evidently the structural changes in the secondary lamellae of the gills.

In this study the effects of zinc were essentially the same as in Tuurala & Soivio (1982) in spite of the shorter exposure time. However, no significant vasoconstriction was found in this study, the fractional volume of blood in the secondary lamellar area being 16.1 ± 2.2 % in control fish and 12.7 ± 1.9 % in zinc exposed fish. Neither did the morphometrically determined haematocrit value of the blood in the secondary lamellae change, being 0.666 ± 0.053 in control fish and 0.667 ± 0.025 in zinc exposed fish. The fusion of the secondary lamellae decreased the free surface of the lamellae. This was reflected in the surface to volume ratio of the lamellae which decreased from 1.07 ± 0.05 to 0.55 ± 0.08 ($p< 0.001$). The detachment of the epithelium from the pillar cell system increased the lymphatic space from 6.0 ± 1.1 % to 20.5 ± 4.5 % ($p< 0.001$) of the volume of the secondary lamellae, thus increasing the diffusion distance from water to the blood spaces of the secondary lamellae greatly. During the zinc exposure the proportional volume of blood channels to which the diffusion distance from water was over 15 μm increased from 16.7 ± 2.8 % to 55.8 ± 6.2 % ($p< 0.001$) of the total volume of the blood spaces in the secondary lamellae.

A significant and positive correlation (r = 0.921***, Fig 1.) was found between the fractional volume of the blood channels to which the diffusion distance was over 15 μm and the PI_{O_2} - Pa_{O_2} difference.

4. Discussion

The morphometric measurements of the structure of the secondary lamellae have proved to be useful when analysing the changes in rainbow trout gill O_2-transfer (Pärt et al. 1982; Tuurala et al. 1983). It can be argued tha the use of fixed material does not give correct information of the situation in living fish when studying the structural and circulatory factors affecting the gas

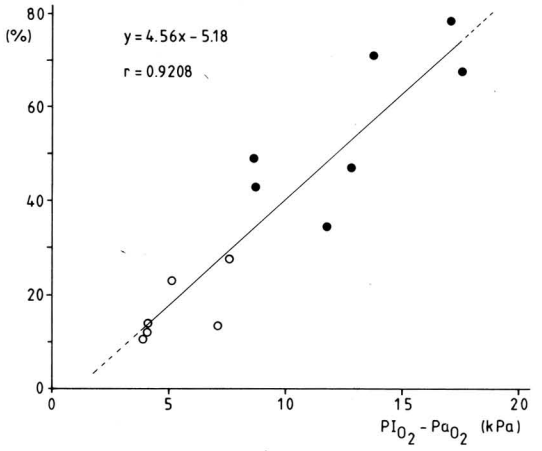

Fig. 1. The relationship between the fraction (% volume) of blood spaces to which the diffusion distance is over 15 μm (%) and the PI_{O_2} - Pa_{O_2} difference (kPa). The correlation between the parameters was significant ($p < 0.001$). Open circles = control fish; filled circles = exposed fish. $n = 13$.

exchange in the gills. However, the results of morphometric and physiological measurements agree quite well with each other (Pärt et al. 1982; this study). Also the results of morphometric measurements in different studies agree with each other provided that the fixation procedure is the same (Soivio & Hughes 1978; Hughes et al. 1978). In this study the morphometric measurements gave essentially the same picture of zinc induced changes in secondary lamellar structure as in Tuurala & Soivio's (1982) study.

The increase in PI_{O_2} - Pa_{O_2} difference in zinc exposed fish agree with the results of Sellers et al. (1975) who exposed rainbow trout to 1.43 mg/l zinc for 24 hours in harder water than in this study. The PI_{O_2} - Pa_{O_2} difference, at least in some of the zinc exposed fish in this study, was so great that they evidently suffered from tissue hypoxia. However, no circulatory changes typical to the gills of hypoxic fish (Soivio & Tuurala 1981) were observed in this study.

The most promiment structural change induced by zinc was the fusion of the adjacent secondary lamellae and the detachment of the lamellar epithelium. These two factors together increased the number and fractional volume of the blood channels to which the diffusion distance from water is long, especially in the basal parts of the secondary lamellae. The mean harmonic distance from water to the majority of the blood channels in rainbow trout secondary lamellae varies between 2.3 - 2.9 μm (Soivio & Tuurala 1981). The diffusion distance to the basal channels of the secondary lamellae is 13 - 18 μm (Tuurala et al. 1983). The oxygenation time of the erythrocytes in isolated carp and eel secondary lamellae is about 1 second, this corresponding to a diffusion distance of 5.3 μm according to Hills et al. (1982). As the blood transit time in the secondary lamellar circulation has been estimated to be 1-3 seconds in different species of fish with undamaged gills (Hughes 1966; Farrell 1980; Hughes et al. 1981; Randall & Daxboeck 1982), it should be sufficient for a complete oxygenation of the erythrocytes, especially in rainbow trout gills, where the diffusion distance is less than 5.3 μm. Consequently, the O_2-transfer to the respiratory blod spaces of the normal secondary lamellae is not limited by diffusion as also proposed by Randall & Daxboeck (1982). Daxboeck et al. (1982) observed that O_2-transfer in artificially blood-perfused spontaneously ventilating rainbow trout was limited by perfusion, not by diffusion. Thus, Pa_{O_2} should be nearly equal to PI_{O_2} since the gills function as a counter current gas exchanger. However, Pa_{O_2} does not reach PI_{O_2} in this or other studies (Holeton & Randall 1967; Nikinmaa 1981) performed with undamaged gills.

One explanation for the P_{O_2} gradient between external water and blood in the dorsal aorta could be the shunting of blood via channels to which the diffusion distance from water is so long and the surface available for O_2-diffusion so small that O_2-transfer to them is restricted (Tuurala et al. 1983). As intrafilamental shunting of blood from the afferent to the efferent filamental artery in rainbow trout gills seems to be improbable (Morgan & Tovell 1973; Cameron 1974; Laurent & Dunel 1976; Vogel et al. 1976): the only possibility is intralamellar shunting of blood. In recent studies Pärt et al. (1983) and Tuurala et al. (1983) have shown that the basal channels of the secondary lamellae can function as such shunt vessels. Due to the long diffusion distance and unfavourable gill outer surface / blood volume ratio these channels were considered to be non-respiratory.

Against this background the fractional volume of blood channels to which the diffusion distance from water was more than 15 μm was estimated separately. The positive correlation between the fractional volume of these channels and PI_{O_2} - Pa_{O_2} difference (Fig. 1) serves as additional proof for the hypothesis that shunting of blood through channels to which the diffusion distance from water is long (basal channels in undamaged gills) can decrease the dorsal aortic oxygen tension (Pa_{O_2}).

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