EFFECTS OF TETRACHLOROGUAIACOL (TeCG) ON THE OSMOREGULATION OF ADULT COHO SALMON (Oncorhynchus kisutch)

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1. Introduction

Fish are sensitive to many water-borne toxicants and alterations in fish physiology have been considered as potential diagnostic tools in environmental risk assessment. In spite of their great physiological importance, fish gills are delicate structures, vulnerable to all kinds of environmental influences[1]. Any damage to the gills will have immediate effects on ion homeostasis and will evoke compensatory osmoregulatory responses.

Tetrachloroguaicol (TeCG) is a xenobiotic released in water as a result of pulping processes. As the pulp and paper industry is a major industry in British Columbia, the impact of the paper effluent is of considerable interest. Every year, hundreds of thousands of smolts migrate into the pacific ocean from freshwater rivers which are also the receiving body of various pulp and paper effluent discharges. In order to provide some information to answer questions as to whether fish migration will be affected by the local pulp and paper industry, the toxic action of tetrachloroguaiacol (TeCG) was investigated, determining its toxic effect on osmoregulation of adult coho salmon (Oncorhynchus kisutch) in freshwater as well as during seawater challenge.

2. Materials and methods

Fish used in this experiment were adult coho salmon (Oncorhynchus kisutch) with a mean weight of 25.35 ± 0.65g. Once the experiment started, the fish being tested were held in 70-L plastic garbage cans sitting in three large square tanks full of continuously running water. There were three toxicant treatment groups and two control groups. Two TeCG treated groups were set up for freshwater tests, i.e. 1) freshwater group where fish were exposed to 100 $\mu g l^{-1}$ TeCG (final concentration) for three days with the exposure solution partially changed (20-L per day) to ensure adequate toxicant loading. The dosed fish were then kept in clean freshwater during the eight-day depuration period; 2) Freshwater group where fish

were exposed to 100 $\mu g \cdot l^{-1}$ TeCG for continuously 11 days with the exposure solution partially changed (20-L per day). In the seawater challenge groups, the fish followed the same dosing process as in the freshwater group but were transferred directly to ~30‰ seawater after toxicant exposure termination. Fish in the control group were simply held in toxicant free freshwater, still with 20-L water changed every day in the first three days of the experiment, and then were transferred to ~30‰ saltwater for the seawater challenge test, which lasted for 8 days. Fish in all groups were put in airstone equipped plastic garbage cans with either freshwater (except in the continuous exposure group) or seawater overflowing at the turnover rate of 2 hours during depuration or seawater challenge periods. There were 30 fish in each group and every garbage-can contained twelve fish with the density being approximately 8 g $fish \cdot l^{-1}$ water. The experiment lasted for three weeks and the mean water temperature was 12.0 ± 1.2 °C.

Before exposure started, six control fish were sampled and on each successive sampling day six fish were also sampled both in the control and the toxicant treated groups. The sampling days were as follows: exposure-day-3 (d3) in all groups; depuration-day-1, 3, 8 (dd1, dd3, dd8) in seawater challenge groups and all freshwater groups with the exception in the continuous exposure group where fish were sampled at exposure-day-4, 6 and 11. Fish sampling was achieved by terminal anaesthetization with MS222 (200 $mg \cdot l^{-1}$). Anaesthetization prior to sampling has been demonstrated to have no effect on plasma ion concentrations[2]. Blood was sampled through the caudal ventral vein using heparinized 100-µL syringe and collected with 60-µL microhematocrit tubes. At the same time duplicate 20-µL blood samples were also taken into 10-mL small test tubes with Drabkin's solution for later haemoglobin measurement. The microhematocrit tubes were then centrifuged at 11,500 rpm for 3 min in a Damon IEC MB microhematocrit centrifuge and haematocrit (Hct) was measured in quadruplicate. Fish were then weighed and dissected. From each fish approximately 0.1g gill and 0.3g kidney tissues were taken and maintained in eppendorff tubes with 500-µL SEI (0.3M sucrose, 0.02M EDTA, 0.1M Imidazole) solution for the Na^+/K^+ activated ATPase assay[3]. The remaining fish carcasses along with the tissues and the plasma were stored at -80°C for later analysis.

Blood haemoglobin concentrations were determined by analyzing the hemolyzed blood and Drabkin's solution mixture, at 540nm using a Shimadzu UV-160 visible recording spectrophotometer. Plasma sodium concentrations were measured on a Perkin-Elmer model 2380 atomic absorption spectrophotometer (aa). The plasma held in microhematocrit tubes under -80°C prior to ion level determination were thawed and diluted to within the linear range of the machine's detection. Gill and kidney Na^+/K^+ ATPase activity assays were conducted according to the simplified preparation for adenosine triphosphatase determination procedure described by Zaugg[3].

All data are expressed as mean ± standard error. Statistical differences between treatment and control were determined using parametric one way ANOVA followed by a Dunnett's test for freshwater groups, and student t-test for seawater groups. Under certain conditions, the non-parametric equivalent was used as appropriate. A probability level of 0.05 was chosen as the limit of statistical significance.

3. Results

After a 3-day sublethal exposure to 100$\mu g \cdot l^{-1}$ TeCG, the experimental fish showed no change in either haematocrit (%) or haemoglobin concentration ($g \cdot dl^{-1}$) over the whole test duration (Fig. 1a & b). In the freshwater groups, plasma sodium concentration ($mmol.l^{-1}$) was fairly stable until 4 days after the beginning of toxicant exposure, i.e. depuration-day-1 and exposure-day-4 in partial and continuous exposure groups respectively, when a significant decrease ($p<0.05$) was seen (Fig. 1a). Then, plasma [Na^+] climbed again from depuration day-3 toward the end of the experiment during which time no statistical difference was recorded. In the seawater challenge groups, plasma [Na^+] in fish exposed to TeCG was not significantly higher ($p<0.05$) than that in control group fish until 8 days after being transferred to toxicant free seawater (depuration day-8) (Fig. 1b). The muscle moisture content (%) was significantly elevated ($P<0.05$) on depuration day-1 for partially dosed fish and exposure day-4 for continuously dosed fish (Fig. 1a), but on depuration day-3 only those fish still exposed to TeCG had a significantly higher muscle moisture content compared with the controls. The differences between treated and control animals in muscle moisture content were not maintained during the rest of the freshwater test. After the fish were transferred to seawater no significant difference was observed between control and treated animals (Fig. 1b). Gill Na^+/K^+-ATPase activities ($\mu mol\ Pi \cdot mg\ Protein^{-1} \cdot hr^{-1}$) in these smolts were significantly upregulated ($p<0.05$) immediately after the three day exposure to 100 $\mu g \cdot l^{-1}$ TeCG, the enzyme activity, however, gradually returned to normal in both freshwater (Fig. 1a) and seawater (Fig. 1b) groups, showing no difference from the control fish during the remaining eight days of the experiment. The kidney Na^+/K^+-ATPase activity in the exposed fish, on the other hand, was not influenced by the sublethal exposure to TeCG except on depuration day-3 and 8 in the seawater group and depuration day-8 in the freshwater group, when the enzyme was significantly inhibited ($p<0.05$) (Fig. 1a & b).

4. Discussion

Osmoregulatory dysfunction was seen in TeCG treated coho salmon according to the changes in plasma Na^+ concentrations together with the corresponding changes in the muscle moisture contents (Fig. 1a & b). In freshwater the reduction in plasma [Na^+] is associated with an increased muscle water content. That is, the dilution is both in blood and muscle. The reasons responsible for either the plasma ion loss or gain shown respectively in the freshwater group of fish or seawater challenged fish might be due to the change in the permeability of the gill epithelium and/or the impairment of active ion transport systems. Increase in membrane permeability, enhancing water influx and ion efflux in hypoosmotic water and vice versa in hyperosmotic water, could cause the decreased sodium level in plasma 4 days after the initial dosing in freshwater group fish and the significantly higher plasma [Na^+] in TeCG treated fish 8 days after seawater transfer. It appears that a wide range of organic xenobiotics are capable of affecting fish osmoregulatory function by disturbing active ion transport[5]. The inhibition of gill Na^+/K^+ ATPase has been shown in different fish

Fig. 1a

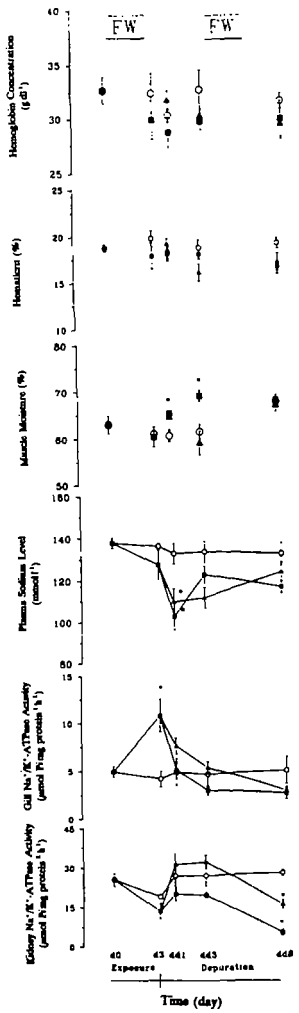

Fig. 1b

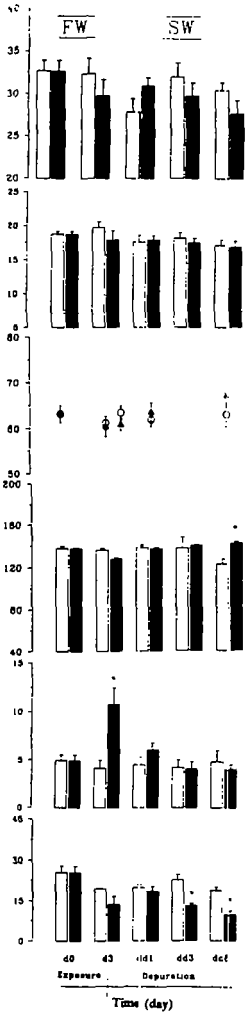

Figure 1. Effects of sublethal exposure (100 μg TeCG l^{-1}) in freshwater (FW) on coho salmon (Oncorhynchus kisutch) haemoglobin concentration ($g dl^{-1}$), haematocrit (%), plasma sodium level ($mmol l^{-1}$), muscle moisture content (%), gill/kidney Na^+/K^+-ATPase activity ($\mu mol\ Pi \cdot mg\ Protein^{-1} \cdot hr^{-1}$) during freshwater depuration (1a) and seawater adaptation (1b). The asterisk indicates a significant difference from control group ($p<0.05$). Values are expressed as mean ± standard error (n=6). (○ & □ : control; ▲ & ▮ : 3-day-exposure; ■ : continuous exposure)

exposed to a variety of organic chemicals, e.g. it was reported that the organochlorines, DDT, dicofol, and endosulfan inhibited rainbow trout gill Na^+/K^+ activated ATPase by 60 to 100% in vitro at concentrations between 10^{-5} and 10^{-4} M[4]. It was found that adult rainbow trout (Oncorhynchus mykiss) exposed to 200 $\mu g \cdot l^{-1}$ TeCG for three days showed an inhibition in the gill Na^+/K^+-ATPase activity (unpublished data). In this study, a different species was used and the toxicant level was lower (100 $\mu g \cdot l^{-1}$), which may be the reason why an upregulation in the gill Na^+/K^+-ATPase was observed (Fig. 1a). This increase in Na^+/K^+-ATPase activity was clearly due to the toxicant rather than a seawater challenge as the enzyme was affected with the same pattern in both freshwater and seawater. The upregulation seen in the gill Na^+/K^+-ATPase activity could also be described as a form of hormesis, a term referring to an overcompensation to some inhibitory challenge[6].

The kidney also plays a very important role in osmoregulation and it could be a point of chemical toxic action, although this has not received much attention. According to the results of this study, kidney Na^+/K^+-ATPase was inhibited in the TeCG treated fish, although the response was delayed for 3 and 8 days, respectively, after the termination of toxicant dosing in the seawater and freshwater groups. The inhibition of the kidney Na^+/K^+-ATPase was similar in freshwater and seawater fish, indicating the reduction was a direct effect of the toxicant on the ATPase rather than any secondary response to osmotic and ionic changes. Adult rainbow trout (Oncorhynchus mykiss) pre-exposed to the same chemical with a similar regime, but at a higher concentration (200 $\mu g \cdot l^{-1}$), showed no significant change in the kidney Na^+/K^+-ATPase activity even after an 8 day depuration period (unpublished data). Thus, it would seem that coho kidney Na^+/K^+-ATPase is more vulnerable than that of trout.

Plasma $[Na^+]$ levels of toxicant exposed fish were elevated above that of control only after eight days in clean seawater. This increase in plasma $[Na^+]$ was associated with no change in gill Na^+/K^+-ATPase activity and a fall in kidney Na^+/K^+-ATPase activity. A decrease in kidney Na^+/K^+-ATPase would presumably impair Na^+ reabsorption which would cause a fall in sodium levels in the body, rather than the observed increase. In freshwater, fish increases in gill Na^+/K^+-ATPase, which might be expected to cause an elevation of plasma $[Na^+]$, was followed in fact by the opposite, a drop in plasma sodium a day later. It would appear that the changes in plasma sodium are not brought about by changes in Na^+/K^+-ATPase in the body and, therefore, are due to increases in the passive flux of water and/or sodium. Thus, it would seem that the toxicant effects the permeability of the gills to sodium and water. We do not know if changes in sodium efflux or water influx were more important. There were no changes, however, in haematocrit, haemoglobin concentration (Fig. 1a & b) or mean red cell volume, so there was little or no hemodilution, indicating that sodium loss was perhaps more important. The changes in Na^+/K^+-ATPase are also caused by the toxicant, as discussed earlier, the effect, however, may be to ameliorate the changes in plasma sodium caused by changes in sodium flux across the gills as the result of the action of the toxicant on the gill epithelium.

It is still not clear, if or, how the sodium pump and membrane permeability is affected by these hydrophobic xenobiotics. TeCG is a hydrophobic chemical, with log K_{ow} (octanol/water partition coefficient) being 4.41. One hypothesis is that TeCG may change the bilayer

structure of the gill epithelial phospholipid membrane due to their high lipid solubility, which may cause the change in epithelial osmotic and ionic permeability as well as the activity of Na^+/K^+-ATPase bound to the membrane of chloride cells. However, there is no clear evidence of the actual mechanism of action of these chemicals on the ion transport pump.

In conclusion, TeCG can adversely effect the osmoregulation of adult coho salmon smolts in freshwater and during seawater adaptation. The rationale behind this experiment was to see if fish subjected to organic chemicals in the pulp mill effluents will successfully make the transition from freshwater to seawater. It is noticeable that the osmoregulatory function in toxicant exposed coho salmon tended to recover in freshwater regardless of the dosing regime, i.e. whether fish were only exposed for 3 days or continuously. The effect of TeCG exposure on fish seawater adaptability was observed only after several days in seawater. The impact of these toxicants, therefore, could be minimized if the exposed fish are left for several days to recover in the freshwater stream before they head for the ocean. In this regard, location of pulp mill plants to either freshwater or seawater sites, but away from the estuary might reduce their adverse impact on the local fishery as well as the aquatic ecosystems in general.

5. References

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