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Cadmium-Induced Scale Deformation in Carp *(Cyprinus carpio)*

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Various morphological patterns including ridges are formed in fish scales. Scale patterns provide information about not only the growth rate of fish, but also about the time fish have been in a particular environment, such as those with pesticides or having a stressful water temperature or salinity (Johal and Dua, 1994; Hubbs, 1959; Ikeda and Ozaki, 1978). Studies using fish scales have many advantages. Scales can be taken repeatedly from the same fish with little harm, the composition of the removed scales is chemically stable for a long time, and new scales rapidly regenerate after the original scales are removed. These characters of scales make the scales highly suitable for studying the effect of water pollution on fish.

Cadmium is well known as a cause of severe health problems in man. In fish, cadmium has been reported to cause bone distortion and to decrease its calcium concentration (Koyama and Itazawa, 1977a,b; Muramoto, 1981a,b). The composition of fish scales is similar to that of bone, which is mainly composed of a calcium phosphate mineral (hydroxyapatite) embedded in a collagenous protein matrix (Yamada and Watabe, 1979). Therefore, we attempted to determine whether cadmium ingestion by fish caused any morphological disorders in their scales or not. Further, we employed a method of scale regeneration because the influence of cadmium on the scale formation was thought to be seen most efficiently in the process of regeneration.

MATERIALS AND METHODS

Fifteen immature carp, *Cyprinus carpio*, with an average body weight of 57.2±6.3 g (mean±SD) were used. The fish were separated into three groups of five fish and each group was maintained in a 50 L aerated tank with a charcoal filter at a temperature of 25°C. Prior to the experiment, all fish were acclimated under these conditions for 30 days. The fish were fed with freeze-dried pellets at a rate of 1% body weight per day. The control group, the low-dose group and the high-dose group were given pellets containing 0, 250, and 2500 μg/g of cadmium in the form of cadmium chloride ($CdCl_2.2½H_2O$), respectively, according to Koyama and Itazawa (1977a). These levels have been shown to cause tissue alterations but are below the lethal dose for fish. Half of the aquarium water was replaced with fresh water every other day. The concentration of cadmium in the tanks was kept at less than 0.1 μg/ml. At the beginning of this study, a scale at a fixed position, behind the head and above the lateral line on the left side of the fish body, was surgically removed for the scale regeneration study. After 15 days of cadmium treatment, fish were taken from each tank, and a regenerating scale was taken at the same place,

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and scales at the same place on the right side were used to observe morphological patterns of the original scales.

Scales were washed with distilled water, dried, mounted on stubs, and sputtered with platinum to a thickness of 300Å. The morphology on a growth region, the basal edge of the scale, was observed with a scanning electron microscope (SEM; Hitachi, S-4000) at accelerating current of 15 kV .

The regenerating scales were washed with distilled water and their diameters, the distance between the cranial and caudal edges, were measured under the light microscope with a micrometer eyepiece. The regeneration ratio (%) was obtained by dividing the diameter of the regenerating scale by the diameter of the original scale. Results were expressed as means±SD. Statistical comparisons for the differences between control group and cadmium-treated groups were tested by unpaired t-test. To examine the accumulation of calcium in the scale, synchrotron radiation-excited X-ray fluorescence (SR-XRF) imaging (Iida and Gohshi, 1991) was employed. SR-XRF imaging was made on beam line 4A of the Institute of Materials Structure Science, High Energy Accelerator Research Organization, Japan. Measurements were performed on the regenerating scale from one of the five fish in each group used for the above morphological measurements. The specimens were first dried completely before being attached to a Mylar membrane backed by a plastic frame. The specimens were kept in a vacuum of 10^{-2} torr. The energy of the incident X-rays was 18 keV, which were monochromatized by a Si(111) double crystal monochromator. The angle between the incident beam and the sample surface was 45°. The incident X-ray intensity during the measurements was monitored by an ionization chamber and was used to normalize the fluorescent X-ray intensities. Since the variation in the incident intensity during the whole measurement was less than 5%, the data shown below are those before the normalization. The fluorescence X-rays were detected by a Si(Li) solid state detector. Two-dimensional analyses were carried out by mounting scale samples on an X-Z stage under the following conditions: beam size=300 μm x 300 μm, step size=150 μm/step, counting time=5 sec/pixel. For mapping the distribution of calcium, the peak areas of the Kα and Kβ lines were used.

The blood was collected from the caudal vein with a heparinized (1%) plastic syringe. To examine the plasma calcium level, the plasma was immediately separated by centrifugation at 3000 rpm for 10 min, and the calcium concentrations were analyzed with a clinical chemistry analyzer (Shimadzu, CL-7100). The calcium concentration was measured by the cresolphthalein complexone method (HA-Test Kits, Wako Pure Chemical Industries, Ltd, Osaka, Japan). Results were expressed as means&D. Statistical comparisons for the differences between control group and cadmium-treated groups were tested by unpaired t-test.

RESULTS AND DISCUSSION

During the acclimation, before administering cadmium, the ridges that formed were parallel and evenly spaced in all the groups. After 15 days of cadmium administration, the scales of some individuals in the low cadmium-treated group showed distortion and loss of ridges at the basal edge of the scales (Figure 1-A). In the high cadmium-treatment group, scales were remarkably deformed; the ridges at the basal edge disappeared in all individuals (Figure 1-B, C). On the other hand, in the control, the ridges that formed were parallel and evenly spaced (Figure 1-D).

Fifteen days after the original scales were removed, the regeneration ratios of regenerating scales were determined (Figure 2). In the cadmium-treated groups, a

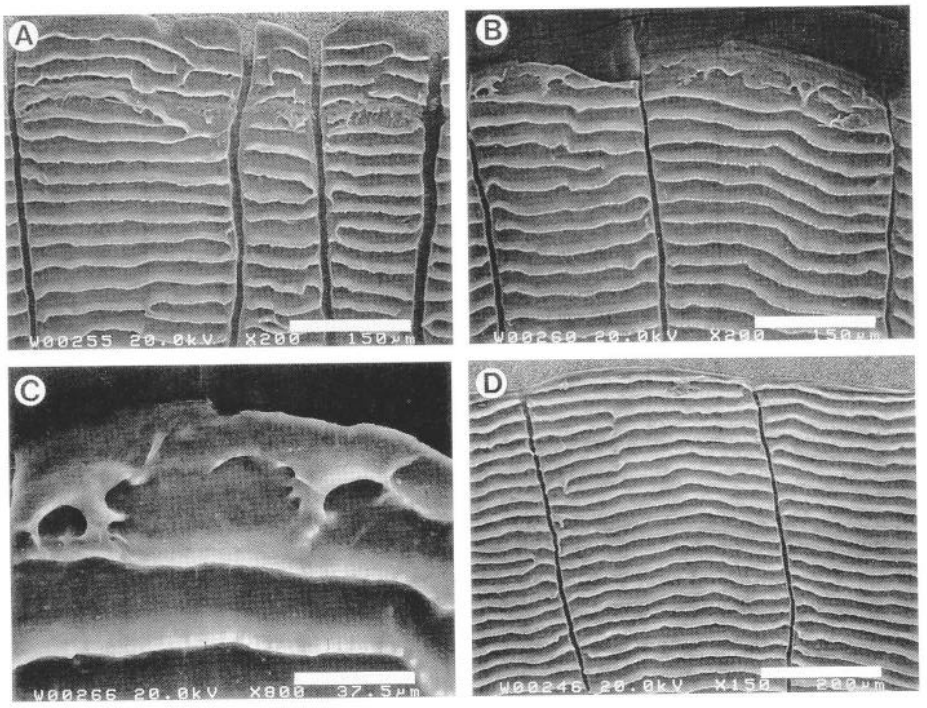

Figure 1. Effect of 15-day cadmium exposure on scales of carp. A) Scale from a low cadmium-treated fish. Distortion and loss of ridges at a basal edge of the scale was observed. B, C) Scale from a high cadmium-treated fish. Deformation of scales was remarkable, disappearance of ridges at the basal edge can be observed. The denticles disappeared on the ridge. D) Scale from a control fish. The ridges were parallel and evenly spaced at the basal edge of scales. Scale bar=150 μm (A, B), 37.5 μm (C), 200 μm(D).

significant decrease in the regeneration ratios was found and the decrease was found to depend on the cadmium concentration. Representative maps of calcium in the regenerating scales are shown for all groups in Figure 3. A comparison of the control and the cadmium-treated groups revealed that the accumulation of calcium in the whole area of the regenerating scales decreases with increasing cadmium concentration.

The blood plasma calcium concentrations after 15 days significantly decreased in the cadmium-treated groups (Figure 4).

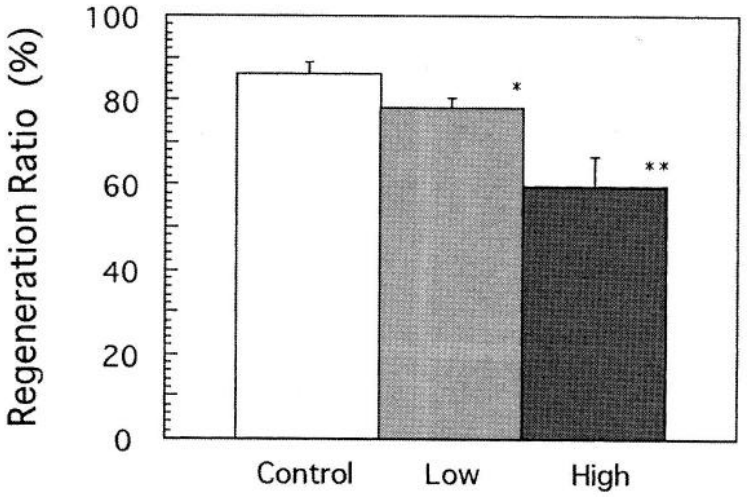

Figure 2. Regeneration ratios of regenerating scales in the control, the low cadmium-treated group, and the high cadmium-treated groups on the 15th day. Results are expressed as the mean±SD. Asterisks indicate significant difference compared to the control value (*: $P<0.01$, **: $P<0.001$).

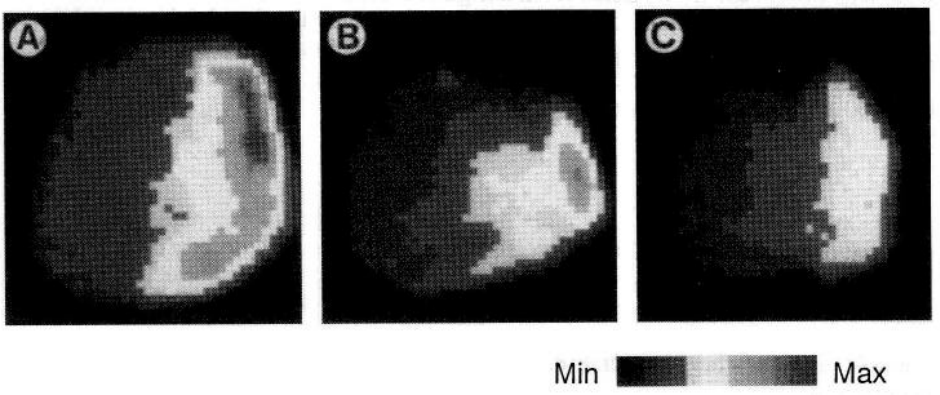

Figure 3. Distributions of calcium in regenerating scales in the control (A), the low cadmium-treated group (B), and the high cadmium-treated groups (C). The maximum intensities of calcium in the control, the low cadmium-treated, and the high cadmium-treated groups were 2289, 1856, and 1317, respectively. The left side is the basal edge of the regenerating scales. The fields of view are 6mm x 6mm.

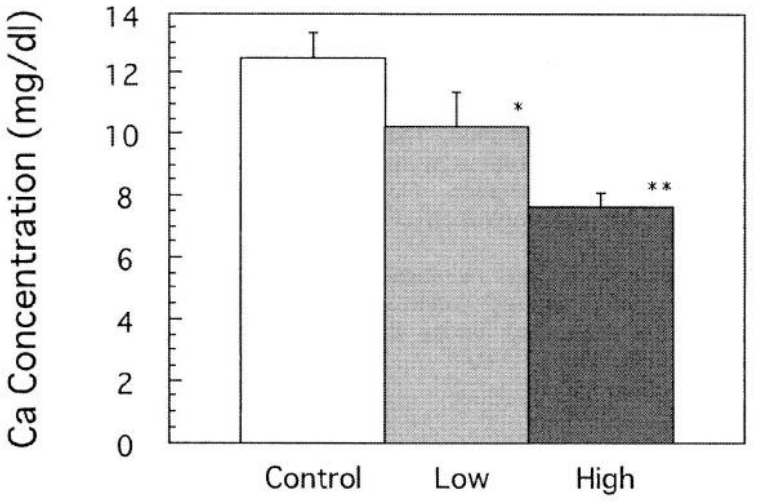

Figure 4. Blood plasma calcium levels in the carp after 15 days with oral administration of cadmium. Results are expressed as the mean±SD. Asterisks indicate significant difference compared to the control value (*: $P<0.01$, **: $P<0.001$).

Deformations at the basal edge of scales were observed in the cadmium-treatment groups. Similar morphological disorders on ridges at the growth region have been reported to be caused by environmental stresses, such as high water temperatures, high salinities, and pesticides (Hubbs, 1959; Ikeda and Ozaki, 1978; Johal and Dua, 1994). It is interesting that the morphology of fish scales appears to be altered by cadmium. However, it remains to be confirmed that the observed disorder was specifically due to cadmium.

In the scale regeneration experiments, a significantly smaller size of the regenerating scales was confirmed in the cadmium-treated groups. The SR-XRF imaging indicated that the amounts of calcium in the regenerating scales were reduced. In view of the changing distribution patterns of calcium with time (Yoshitomi et al., 1997), the patterns in the cadmium-treated groups can be attributed to a delay of the calcium intake in the regenerating scales. Using a ^{45}calcium tracer, Sauer (1987) found that cadmium exposure had a similar effect on scale regeneration in the mummichog, *Fundulus heteroclitus.* Sauer and Watabe (1988) reported that inhibition of the ATPase responsible for calcium transport in scleroblasts may cause the observed decrease in calcium intake in scales due to cadmium exposure. In addition, Ichii and Mugiya (1983) reported that the accumulation of calcium into the scales occurs not directly from the ambient water but via the blood. In our experiments, the blood plasma calcium level decreased significantly as the dosage increased. It also seems that the deformation of scales may have resulted from a deficiency of blood plasma calcium. Ultrastructural investigations have shown many morphological similarities between the cells associated with scale formation

and bone-producing cells (Onozato and Watabe, 1979). Cadmium causes a decrease in calcium concentration of bone and a distortion weakness of the vertebral column in carp (Koyama and Itazawa 1977a,b; Muramoto 1981a,b). Koyama and Itazawa (1977b) suggested that weakness of the vertebral column is due to a decrease in the blood plasma calcium concentration. The disorders of ridges on the scales of carp under oral administration of cadmium may be caused by the same metabolism that occurred in the scale regeneration. Our results clearly showed that cadmium contamination had a morphological effect in the form of scale deformations.

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