ニジマスに対する亜鉛の急性毒性

誌名	日本水産學會誌
ISSN	00215392
著者	児玉, 正昭 緒方, 武比古 山森, 邦夫
巻/号	48巻8号
掲載ページ	p. 1055-1058
発行年月	1982年8月

Bulletin of the Japanese Society of Scientific Fisheries 48(8), 1055–1058 (1982)

Acute Toxicity of Zinc to Rainbow Trout *Salmo gairdneri*

Masaaki KODAMA,* Takehiko OGATA,* and Kunio YAMAMORI*

(Received December 19, 1981)

Heavy metals such as zinc, copper, lead, cobalt and nickel hemolyzed erythrocytes of several kinds of animals. The hemolytic activity of each heavy metal was species-specific to test animals and zinc affected the erythrocytes of rainbow trout most profoundly. Microscopic observation showed that a part of the epithelium of the gills of rainbow trout was torn off in the later stage of zinc poisoning. In addition, heavy metals took away the elasticity from salmon eggs which we used as a model of cell membranes of the erythrocytes and the epithelial cells. There was a close relationship in the order of the efficacy of heavy metals between the degree of hemolysis and the loss in elasticity. Not only the hemolysis of erythrocytes but also the damage of the gills were probably due to the loss in elasticity of the cell membrane. Thus we concluded that zinc had a kind of cytotoxicity especially to rainbow trout.

Zinc in the environmental water is highly toxic to rainbow trout *Salmo gairdneri*. BURTON *et al.*[1] reported that tissue hypoxia was a major physiological change before death in zinc-treated trout. SKIDMORE[2] and SKIDMORE and TOVELL[3] indicated that the symptoms of fish in the acute intoxication to zinc involved the damage to the gill tissue and the subsequent severe arterial hypoxia. But they could not show whether the damage of the gills was caused by the direct action of zinc or it resulted from some physiological changes caused by the absorbed zinc. SELLERS *et al.*[4] reported that a sharp decrease in arterial pO_2 and pH of zinc-treated rainbow trout. On the other hand, we have found that hemolysis was one of the symptoms of zinc poisoning in rainbow trout.[5] Heavy metals including zinc harden the cell membrane of erythrocytes, which will weaken their elasticity.[6]

In this paper, we show that zinc has a kind of cytotoxicity that deprives the cell membrane of elasticity, and suggest that the hemolysis and the damage of the gills of zinc-treated rainbow trout were caused by the direct action of cytotoxicity of zinc.

Materials and Methods

Erythrocytes of Various Animals

Blood was collected from rainbow trout *S. gairdneri*, eel *Anguilla japonica*, rock fish *Sebastes schlegeli* by heart puncture or from the dorsal aorta. Rabbit blood was also collected from New Zealand albino rabbit. Sheep blood was purchased from Seikagaku Kogyo Co. The red blood cells were washed with saline and suspended in a veronal buffered saline, pH 7.4[7] to the concentration of 2% (v/v).

Hemolytic Activity of Heavy Metals

Two m*l* of the veronal buffered saline solutions containing each of $ZnSO_4$, $CuSO_4$, $PbCl_2$, $NiCl_2$ and $CoCl_2$ was mixed with an equal volume of the red blood cell suspensions. The final concentration of heavy metal ion was 40 ppm. These mixtures were incubated for 12 h at 4°C. After centrifugation at 3,000 rpm for 10 min, E_{541nm} of the supernatant was measured. Another aliquot of each red blood cell suspension was hemolyzed by distilled water as a control. The percentage of hemolysis was calculated based on E_{541nm} values of both sample and control.

Histological Examination of the Gills

Rainbow trout, weighing about 300 g, were treated with 40 ppm of Zn^{2+} as reported before.[5] The fish showed such typical symptoms of acute zinc poisoning as three successive responses characterized by surfacing of the fish, overturn, and immobilization of the gill opercula. The gills of the poisoned fish at these three stages were removed. Some pieces of each gill arc were fixed in 10% neutral formalin, embeded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin for light microscopic observation.

* School of Fisheries Sciences, Kitasato University, Sanriku, Iwate 022-01, Japan (児玉正昭・緒方武比古・山森邦夫: 北里大学水産学部).

Effect of Heavy Metals on the Elasticity of Cell Membrane

Eggs of salmon *Oncorhyncus keta* were immersed in veronal buffered salines containing heavy metals at room temperature for 2 h. The concentration of heavy metals varied from 1.25 to 40 ppm. Control eggs were also treated with the same buffer without heavy metals under the same condition. The elasticity of these eggs was measured by a slightly modified elastic load tester (Okada type, Chuoriken Co., Ltd.). The measurement was repeated ten times for each sample and a control. The elasticity was expressed as percentages of the force required to crush test eggs to that required to crush control ones.

Results

Hemolytic Activity of Heavy Metals

Fig. 1 shows the hemolytic activity of heavy metals on fish and mammalian erythrocytes. The erythrocytes of fish were more easily hemolyzed than those of mammals. Among the heavy metals tested, zinc was the most effective to erythrocytes of rainbow trout whereas it hemolyzed those of the other fishes slightly. These results showed that the hemolytic activity of heavy metals was species-specific. As for rainbow trout erythrocytes, the hemolytic activity of heavy metals was in the order, Zn^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+} and Co^{2+}.

Histological Examination of the Gills

Fig. 2 shows the changes of the gills affected by Zn^{2+}. The secondary lamellae were slightly curled over at the surfacing stage (Fig. 2-b) and then separated from the pillar cell system at the overturn stage (Fig. 2-c). Finally, the secondary lamellae were grossly curled over and the epithelium was further elevated (Fig. 2-d). These observations coincided well with those of SKIDMORE and TOVELL.[3] In addition, our results showed that the lifted epithelial cells were partially torn off, resulting in the exposure of the central blood space (Fig. 2-e).

Effect of Heavy Metals on the Elasticity of Cell Membrane

The changes in the elasticity of salmon eggs treated with heavy metals are shown in Fig. 3. The elasticity decreased when the eggs were treated with heavy metals and the degree of decrease varied depending on the kind of metals. Zn^{2+} was the most effective and over 80% of the elasticity of eggs was lost, even when treated with such a low concentration of 1.25 ppm. The degree of elasticity decrease by heavy metals was in the order, Zn^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+} and Co^{2+}, suggesting a close relationship with the hemolysis of rainbow trout erythrocytes.

Discussion

Heavy metals such as zinc, copper, lead, cobalt and nickel hemolyzed erythrocytes of several kinds of animals. The hemolytic activity of each heavy metal was species-specific to test animals and zinc affected the erythrocytes of rainbow trout most profoundly. BALL[8] observed the hemolytic action of silver, palladium and mercury to fish erythrocytes and described that the activity of these heavy metals varied with species of fish. Our results showed that the other heavy metals were also hemolytic to various erythrocytes and the activity was species-specific.

SKIDMORE and TOVELL[3] indicated the damage of epithelial cells of the gills in zinc-treated rainbow trout. We also examined the gills of zinc-treated rainbow trout and observed almost the same changes in the gill tissue as they reported.

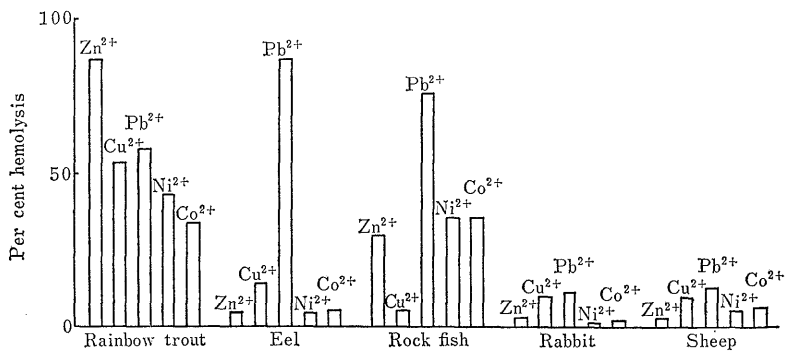

Fig. 1. Hemolytic activity of heavy metals.

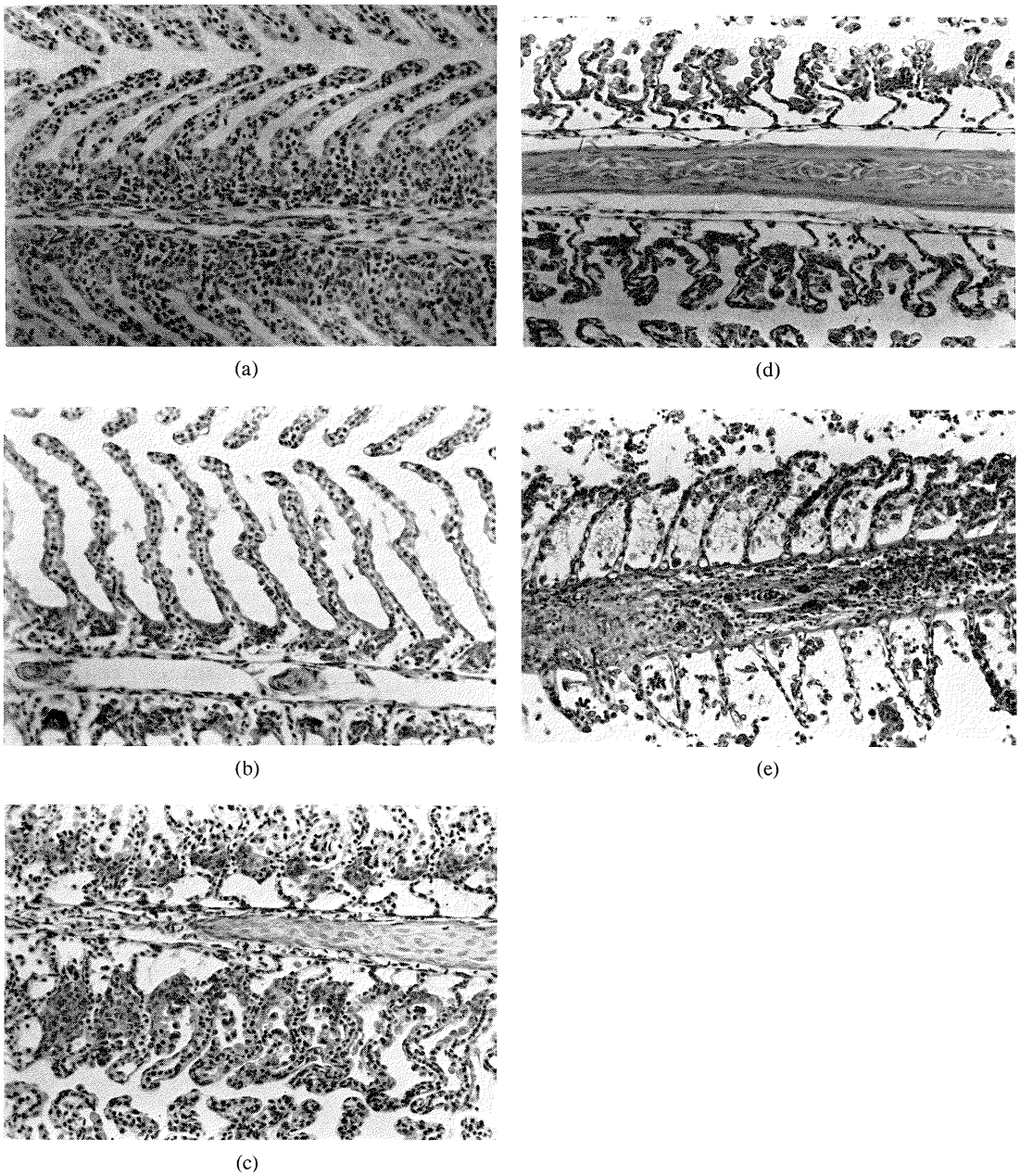

Fig. 2. Photomicrograph of a longitudinal section through a filament from a fish exposed to 40 ppm zinc. a) normal, b) surfacing stage, c) overturn stage, d) and e) opercula immorbilization stage.

But at the same time, we observed that a part of the lifted epithelium was torn off in the later stage of poisoning (Fig. 2-e). These results show that epithelial cells of the gills as well as erythrocytes became fragile when treated with zinc. In this connection, various heavy metals are known to harden the cell membrane of human red blood cells and to weaken its elasticity.[8] If the cell membrane was hardened and the elasticity was deprived, it would easily be broken by less physical force, and hemolysis or tissue damage would occur.

Because of the large cell size, availability and similar sensitivity to zinc toxicity,[9] we chose the eggs of salmon to measure the change in elasticity of cell membrane treated with heavy metals. As

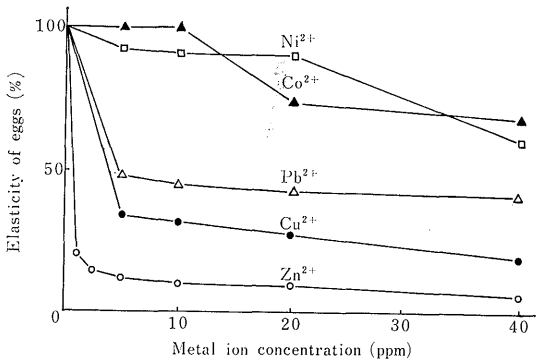

Fig. 3. The changes in elasticity of salmon eggs treated with heavy metals.

shown in Fig. 3, the elasticity of salmon eggs decreased when treated with heavy metals, of which zinc affected most markedly. Moreover, the tendency of decrease in elasticity of salmon eggs coincided well with that of hemolysis of rainbow trout erythrocytes. These results showed that heavy metals had a kind of cytotoxicity to harden the cell membrane and to weaken its elasticity. In connection to this, SELLERS *et al.*[4] described that asphyxia occurred even when rainbow trout was treated with zinc at a sublethal concentration of 1.43 ppm. Since they did not examine the gills, it was not clear whether the gills were damaged or not. Our results on the elasticity of eggs showed that over 80% of elasticity were lost when treated with zinc even at a low concentration of 1.25 ppm. BROCKS *et al.*[10] reported that a high concentration of zinc injected intravascularly to man caused pulmonary oedema and suggested that zinc was a kind of cellular toxin. Salmonid fish seem to be more sensitive to the cytotoxicity of zinc than other animals.

Thus we concluded that hemolysis and damage of the epithelial cells of zinc-treated rainbow trout were attributed to the cytotoxicity of this metal.

References

1) D. T. BURTON, A. H. JONES, and J. CAIRNS: *J. Fish. Res. Bd. Can.*, **29**, 1463–1466 (1972).
2) J. F. SKIDMORE: *J. Exp. Biol.*, **52**, 481–494 (1970).
3) J. F. SKIDOMRE and P. W. A. TOVELL: *Water Res.*, **6**, 217–230 (1972).
4) C. M. SELLERS, A. G. HEATH, and M. L. BASS: *Water Res.*, **9**, 401–408 (1975).
5) M. KODAMA, T. OGATA, and K. YAMAMORI: *Bull. Japan. Soc. Sci. Fish.*, **48**, 593 (1982).
6) Y. MISHIRO and Y. NAKAYAMA: in "Ketsueki Kagaku" (in Japanese) (ed. by K. KURODA, H. YOSHIKAWA, Y. NAKAO, and K. WAKISAKA) Asakura Shoten, Tokyo, 1963, pp. 25–26.
7) E. A. KABAT and M. M. MAYER: Experimental Immunochemistry, 2nd ed., C. C. THOMAS, 1961, pp. 133–240.
8) E. G. BALL: *Biol. Bull.*, **64**, 277–288 (1933).
9) G. J. FARMER, D. ASHFIELD, and H. S. SAMANT: *Environ. Pollut.*, **19**, 103–117 (1979).
10) A. BROCKS, H. REID, and G. GLAZER: *Br. Med. J.*, **28**, 1390–1391 (1977).