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原著論文

An Infectious Maker in Intestine and Kidney of Japanese Flounder Challenged with *Edwardsiella tarda*

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ABSTRACT

We examined the heat shock protein (hsp) 60 expression level by western blot analysis in the Japanese flounder tissues on the edwardsiellosis induced with *Edwardsiella tarda*. In the intestine, when fish were infected with this pathogen, the hsp 60 expression level showed significantly ($p<0.05$) lower than non-infected fish (control). In kidney, the expression levels in the infected fishes were significantly ($p<0.05$) higher than the control after infection. The hsp 60 synthesis are different depended on the tissue and the measurement of the level might be shown on a biomarker of infectious stress.

KEY WORDS: heat shock protein, *Edwardsiella tarda*, Japanese flounder

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INTRODUCTION

Japanese flounder, *Paralichthys olivaceus,* is one of the commercially important cultured marine fish in Japan, and the edwardsiellosis caused by infection with *Edwardsiella tarda* has been brought about serious damage in a fish farm. The infected fishes with this pathogen show abscess in the liver and kidney, swelling of abdomen with accumulation of ascites and hernia of the intestine[1].

Heat shock proteins (hsps) are induced by exposure organisms to a variety of stimuli such as a heat, a drug, an infection and an inflammation, and the protein structure and function are well conserved in all organisms from bacteria to humans. The functional roles of the hsp 60 and hsp 70 families are as well characterized, transport and assembly of functional protein structures. Dietz[2] reported that hsps were increased in several tissues of marine toleost fish by raising the temperature. In the present study, we analyzed the relationship between hsp 60 expression in the tissues and the edwardsiellosis of Japanese flounder.

MATERIALS and METHODS

Japanese flounder weighting 117±10 g were acclimatized to our laboratory conditions before use. Fish were separated into two groups, and reared for 1 week at 22 ℃ in 200 *l* tanks until the challenge. The infection group of fish was injected intraperitoneally with 1.0×10^6 CFU/fish of *E. tarda* EF-1 strain, the other was injected the same volume of sterile saline and subsequently quantity analysis.

The removed intestine and kidney of fish on day 11 after the infection were added 9-folds of 10 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride, then the samples were sonicated and centrifuged at 100,000 ×*g* for 30 min at 4 ℃. Proteins in the supernatants were separated by 10 % SDS-PAGE (25 μg protein/lane). The protein bands on gels were transferred to a polyvinylidene difluoride membrane, and then detected by anti-human hsp 60 mAbs (Stress Gen). Arbitrary unit (A.U.) were calculated using a NIH-image software

(National Institute Of Health, Bethesa, Md.). Statistical analysis was calculated using Student's *t*- test.

RESULTS and DISCUSSION

The infected Japanese flounder with *E. tarda* had the accumulation of milky or bloody ascites, hepatic abscess, swollen kidney and hernia of the intestine. The relative weight (%) of the intestine was decreased after infection, but this tendency was not significantly compared with the control. On the other hand, that of the kidney were increased significantly ($p<0.05$) after infection to compared with control (Fig. 1).

Fig. 2 showed the result of samples from the western blot analysis of the intestine and kidney. In the intestine, when fish were infected with this pathogen, the expression level of hsp 60 decreased into one-sixth being, and being significantly ($p<0.05$) lower than the control. It was suggested that the surfaces of mucosal tissues such as intestine, respiratory and urogenital tracts develop a special immune defense system: M cells exist in intestinal epithelium behave like taking antigens for efficient endocytosis and transcytosis[3]. The antigens transported by M cells are processed and presented by macrophages, and the macrophages activate γ , δ -T cells in intraepithelial pocket[4]. The activation of mucosal immune difference is thought to be caused by hsps recognized by γ , δ -T cells[5,6]. In our experiments, the hsp 60 expression level intestine of the control fish was higher than in the kidney, and seem to regulate mucosal immune defense system. Thus, hsp 60 seems that hsp regulates the T cells response against this disease as important function in the activation of a mucosal immune system in fish. However the infected fish decreased the hsp 60 expression level of intestine. It appears that chiefly two cases, i.e. the cells of intestine were broken or mucosal immune system was damaged by edwardsiellosis.

Hsp 60 expression level in the kidney increased about five times and after infection the amount being were significantly ($p<0.05$) higher than the control. Forsyth *et al.* [7] reported that Coho salmon, *Oncorhynchus kisutch*, with a bacterial kidney disease had a significantly higher hsp 70 expression level than the control fish. The abnormal expression of stress proteins has been widely observed in a number of the infected

conditions including oxidant injury, ischemia and inflammation[8]. Miyazaki and Kaige[9] reported that the kidney in some cases produced abscesses involving nephrons by bacterial invasions to the hematopoietic tissue, when Japanese flounder were infected with edwardsiellosis. These events that the inflammation connected with the activation of immune systems would be raise bacterial infection stress in kidney. Hsp 60 families are molecular chaperones that support several steps during synthesis, transportation, and degradation of proteins[10,11]. The hsp 60 in kidney may have protected the organ from an oxidative stress caused by bacterial infection. Thus, the measurement of hsp 60 expression level is an index of the structural integrity in infectious tissues. Habich *et al.*[12] discussed that the important role of hsp 60 epitopes in regulating self and inflammatory responses. The hsp 60 induction may behave an important event to infection with edwardsiellasis.

It is important point out that following the infection with edwardsiellosis, the induction styles of hsp 60 was different in intestine associated with a mucosal immune system and the kidney associated with hemopioessis when the fish were infected with edwardsiellosis. In the intestine, the hsp 60 expression level decreased with edwardsiellosis, which suggests the damages of the mucosal immune system, whereas the hsp 60 expression level in the kidney increased. Thus, it seems that the expression of hsp 60 are different on account of the tissue character and the measurement of a hsp 60 expression level is shown an biomaker of infectious stress.

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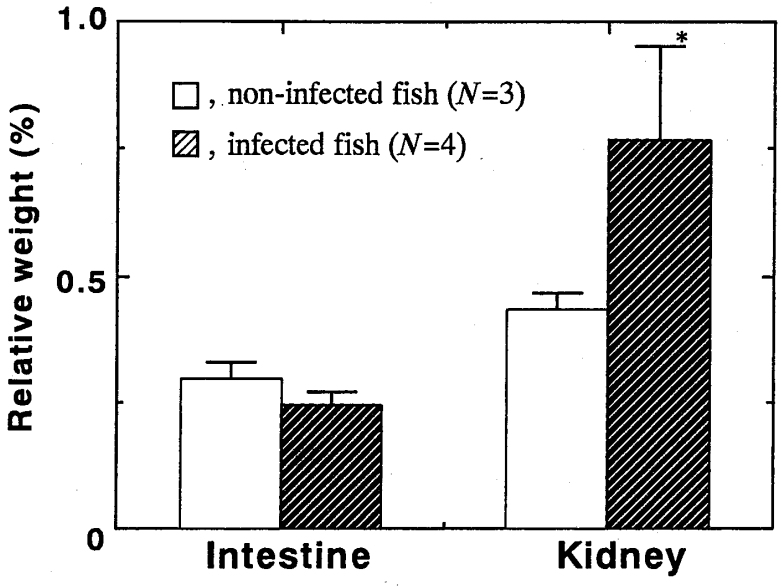

Fig. 1. The intestine and kidney expressed as % of body weight in Japanese flounder on day 11 after infection.

Fish were infected intraperitoneally with 10^6 CFU/fish of *E. tarda*. *Significantly difference from the control with Student's *t*-test ($p<0.05$).

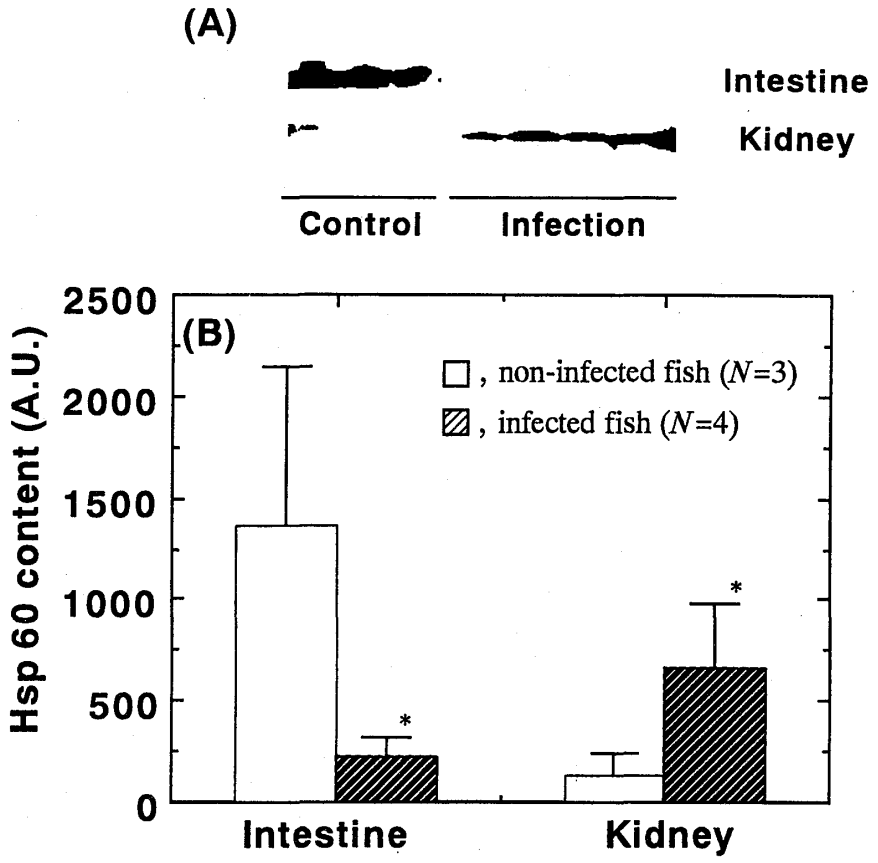

Fig. 2. Expression of heat shock protein 60 (hsp 60)in the intestine and kidney of Japanese flounder on day 11 after infection.

(A) Western blot analysis of hsp 60 from the infected and non-infected fish. (B) Quantitation of relative expression of hsp60. *Significantly difference from the control with *t*-test ($p<0.05$).