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Effects of Consecutive Blood Collecting Stressors on the Plasma Glucose Level and Chemiluminescent Response of Peripheral Blood Phagocytes in Cultured Sea Bass, *Lateolabrax japonicus*

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The stress of consecutive blood collectings resulted in evident elevation of plasma glucose level and significant lowering of chemiluminescent response of peripheral blood phagocytes in sea bass (*Lateolabrax japonicus*). Fish responded to the consecutive stressors in cumulative manners. The plasma glucose level in response to consecutive stressors depended on the stressor intervals. When the plasma glucose level of individual fish was compared with the chemiluminescent response, statistically significant ($P<0.05$) negative correlations existed.

Key words: Sea bass, Stress, Glucose, Blood phagocytes, CL response

Various types of stressors have been shown to elicit a primary endocrine response of fish including elevation of plasma cortisol and catecholamine levels (Mazeaud *et al.*, 1977; Donaldson, 1981; Barton & Iwama, 1991), and these hormonal responses induce a series of secondary responses including elevation of blood glucose (Mazeaud *et al.*, 1977; Barton *et al.*, 1986; Biron & Benfey, 1994) and lactic acid (Soivio & Oikari, 1976; Swift, 1983; Barton *et al.*, 1986; Reubush & Heath, 1997), increase of glycogen metabolism (Mazeaud *et al.*, 1977; Schwalme & MacKay, 1991; Vijayan & Moon, 1992), and disturbance of osmotic regulation (Barton *et al.*, 1986; Reubush & Heath, 1997). It has also been postulated that stress increases the susceptibility of fish to infectious agents *via* stress-mediated immunosuppression (Snieszko, 1974; Ellis, 1981; Angelidis *et al.*, 1987; Maule *et al.*, 1989).

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The stress response of fish is closely related to the severity and duration of the stressor (Barton *et al.*, 1980; Pickering *et al.*, 1982), and may be cumulative (Barton *et al.*, 1986; Mesa, 1994). However, the cumulative effect of consecutive stressor on the peripheral blood phagocytes activity in fish is poorly understood. Resistance and susceptibility to infectious disease-causing agents depend to a large degree on non-specific immune responses, and phagocytes play a major role in the defensive activities. Therefore, the objective of this study was to evaluate the effects of consecutive blood-collecting stressors on the plasma glucose level and chemiluminescent (CL) response of peripheral blood phagocytes in cultured sea bass, *Lateolabrax japonicus*.

Materials and Methods

Fish

Three individuals of sea bass, *Lateolabrax*

japonicus, weighing 350-430 g were obtained from a local sea bass farm, and were acclimated to a 300-l fiberglass tank supplied with running seawater (salinity 33) for 3 weeks prior to the experiment. The fish were not fed throughout the experimental periods. Each fish could be distinguished by the difference in body weight.

Experimental procedure

Each sea bass was subjected to six consecutive blood collecting stressors at 0, 2, 6, 24, 26 and 30 h. Fish were anaesthetized with MS222, and 0.5 ml of blood was taken from the caudal vessel using heparinized syringes, then the fish were restored by returning to the culture tank until the next following blood collections. The collected blood at each time was used to analyze blood glucose and CL response. The blood collected at 0 h in each sea bass served as pre-stress baseline values of glucose and CL response.

Glucose

Plasma glucose was measured using a glucose oxidase/peroxidase enzymatic assay based upon the method of Werner *et al.* (1970).

Chemiluminescence (CL) assay

Each collected blood (0.3 ml) was immediately placed on a 34/51% Percoll (Sigma) density gradient and centrifuged at 400 g for 30 min at 4°C. The interphase was collected and the cells were washed twice at 400 g for 5 min in Hank's balanced salt solution (HBSS, Sigma) containing heparin and antibiotics. The cell viability was examined with trypan blue exclusion and was evaluated to be greater than 98%. The leucocytes including neutrophils and monocytes were adjusted to 5×10^5 cells/ml HBSS.

Zymosan (Sigma) was mixed with the serum of sea bass and incubated at 30°C for 30 min. The opsonized zymosan was separated by centrifugation, washed three times and suspended in HBSS.

The ROIs (reactive oxygen intermediates) produced by stimulated phagocytes was quantified using an automatic photoluminometer (Bio-Orbit 1251, Sweden). Each test cuvette contained 0.7 ml luminol (Sigma) made according to the method of Scott and Klesius (1981), 0.5 ml cell suspension, and 0.3 ml opsonized zymosan, which was added just prior to measurement. The measurements were made for 100 min with triplicates, and the light emission was recorded as mV.

Statistical analysis

All data were subjected to ANOVA using Statistix 3.1 (Analytical Software, St. Paul, MN, USA). When a significant effect was observed, a least significant difference (LSD) test was used to compare means. The criterion for statistical difference was $P<0.05$.

Results

Plasma glucose

The stress of consecutive blood collecting resulted in evident elevation of plasma glucose levels in each sea bass (Fig. 1). Plasma glucose levels were sharply increased at 2 h and 6 h by

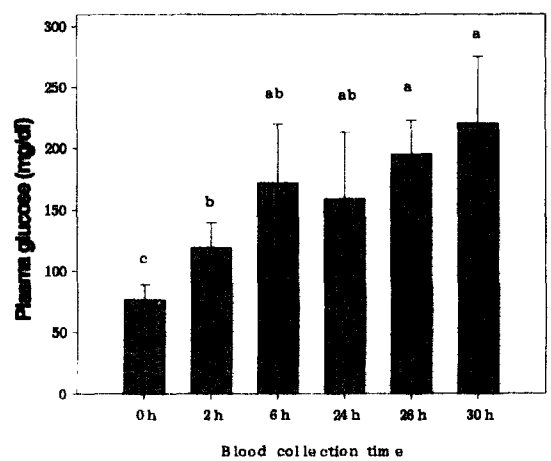

Fig. 1. Changes in plasma glucose level in the sea bass subjected to consecutive blood collecting stressors. Values are mean±S.E.M. and different letters indicate statistical significance at $P<0.05$.

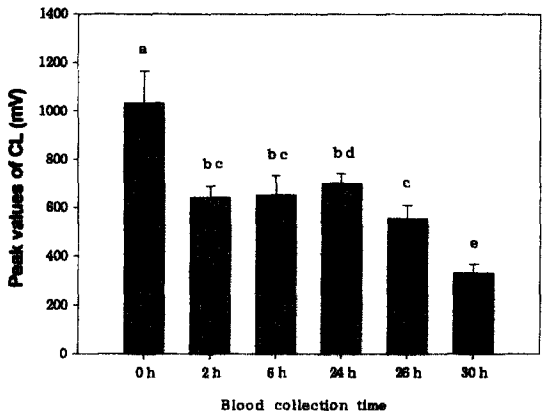

Fig. 2. Changes in chemiluminescent response of peripheral blood phagocytes in the sea bass subjected to consecutive blood collecting stressors. Values are mean±S.E.M. and different letters indicate statistical significance at $P<0.05$.

short-term consecutive blood collecting, then dropped slightly at 24 h by relatively long-term of stabilization between blood collectings, but remained significantly elevated above pre-stress level. The rise in plasma glucose levels at 26 h and 30 h was more gradual than at 2 h and 6 h.

Chemiluminescent (CL) response

Although there were some differences in the pattern of CL responses according to individual sea bass, it was evident that the stress of consecutive blood collectings resulted in significant lowering of chemiluminescent response of blood phagocytes (Fig. 2).

When the plasma glucose level of individual fish was compared with the CL response, significantly ($P<0.05$) negative correlations existed.

Discussion

The results from the present study clearly indicated that sea bass responded to consecutive stressors in cumulative manner both in plasma glucose concentration and CL response of peripheral blood phagocytes. Elevated blood glucose concentration is commonly used as an indicator of secondary stress response to an acute stress in fish (Barton & Iwama, 1991). Elevations in plasma glucose in response to stress presumably reflect the metabolic response of the fish (Schreck, 1981; Leach & Taylor, 1982; Andersen *et al.*, 1991; Vijayan & Moon, 1992). Therefore, the cumulative increase in plasma glucose in the present experiment indicated an increasing demand for energy in response to the repeated stressors. The slight decrease of plasma glucose at 24 h by relatively longer stabilization period in contrast to the sharp increases by short-term blood collections suggests that the plasma glucose level in response to consecutive stressors depends upon the stressor intervals. Pickering *et al.* (1982), also, reported that the time taken for complete stabilization of blood glucose concentrations was influenced by the time course and severity of the stress itself and, in general, stabilization following an acute stress occurred within 1-4 days.

Fish, like other vertebrates, respond to infectious pathogens in specific and nonspecific ways. Stress may influence the ability of fish to resist pathogens invasions by affecting the specific and nonspecific immune system (Kaattari & Tripp, 1987; Tripp *et al.*, 1987; Maule *et al.*, 1989; Narnaware *et al.*, 1994). The nonspecific defences are the first pathogen encounters, and it has been suggested that they are very important in the resistance of fish to infectious agents (Blazer, 1991). Granulocytes and macrophages possess a phagocytic activity which is the initial step in the immune response in fish, and is the major line of defence for all foreign materials, including pathogenic agents (Olivier *et al.*, 1986). Therefore, the reduction of phagocytic activity may be related to the increase of disease susceptibility. In the present study, the CL response of peripheral blood phagocytes of sea bass was clearly reduced by blood collecting stressors. Thompson *et al.* (1993) demonstrated that Atlantic salmon stressed by confinement had reduced respiratory burst and bactericidal activities of head kidney leukocytes. Angelidis *et al.* (1987) also reported that rainbow

trout showed a lower CL response of head kidney phagocytes and a higher susceptibility to *Aeromonas salmonicida* by handling and anoxic stressors. The mechanism of stress-mediated suppression of phagocytic activity in fish is not fully understood, but appears to be mediated by the endocrine system (Bayne & Levy, 1991). Angelidis *et al.* (1987) assumed that the decrease in the CL response in stressed fish might be based on the corticosteroid effects. In the present study, the significantly negative correlation between plasma glucose concentration and CL response of blood phagocytes in response to consecutive stressor suggests that endocrine hormones in fish influence simultaneously on both physiological and immunological responses.

In conclusion, the consecutive blood collecting stressors had deleterious effects on sea bass health by consuming energy sources for adjusting energy budgets and by impairing ability of phagocytes.

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연속 채혈 스트레스가 양식 농어의 혈장 Glucose 농도 및 말초 혈액내 식세포의 Chemiluminescent 반응에 미치는 영향

김기홍 · 황윤정 · 조재범 · 안경진 · 권세련

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양식 농어에 있어서 연속 채혈 스트레스는 혈장내 glucose 농도의 뚜렷한 증가 및 말초 혈액내 식세포의 chemiluminescent 반응을 유의적으로 감소시켰다. 실험어는 연속적인 스트레스에 대해 축적적인 반응을 나타내었으며, 혈장내 glucose의 농도는 스트레스 부과 시간간격에 의존하는 것으로 나타났다. 혈장내 glucose의 농도와 chemiluminescent 반응간에는 통계적으로 유의적인 ($P<0.05$) 음의 상관관계를 나타내었다.

Key words: Sea bass, Stress, Glucose, Blood phagocytes, CL response