

**CARDIOVASCULAR FUNCTION IN *SALMO SALAR* WITH
INFECTIOUS SALMON ANAEMIA (ISA):
PUTATIVE ROLE OF NITRIC OXIDE**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

In higher vertebrates, particularly in mammals, a depressed myocardial contraction during sepsis or exposure to bacterial endotoxin (EDTX) has been documented (Parrillo, 1993). Recent studies have shown that alterations in transduction pathways in cardiac myocytes during sepsis or after exposure to cytokines involve NO (Kelly et al., 1996). Under these conditions there is often an induction of a calcium-independent NO synthase (iNOS or NOS2), whose role could be important in the determination of myocardial dysfunction. One cardiac action of NO can be via the Starling mechanism, as shown in the isolated ejecting guinea pig heart, in which basal intracardiac production of NO increased preload-induced rises in cardiac output (Prendergast et al., 1997). Our aim was to investigate in ISA salmon the potential role of NO-transduction pathway in the determination of cardiac dysfunction and to provide some information on the time course of heart dysfunction during the infection. We have studied the chronology of cardiac impairment from the time of the virus administration to the time of the typical pathological features at the peak of the disease.

Salmon hearts (13 from control group and 30 from infected group) were isolated from specimens of *Salmo salar* (weighing 79,41 ± 1,73 g; mean value ± S.E.M.) and connected to the perfusion apparatus. Experiments were carried out at

10±2°C in November 1999. The saline composition was (in mmol/l): NaCl 110; KCl 4,8; CaCl₂ ·2H₂O 1,5; NaHCO₃ 15; NaH₂PO₄·H₂O 2,5; MgSO₄·7H₂O 1,25; glucose·H₂O 5,55; pH was adjusted to 7,5-7,65 by adding NaOH (1N). The saline was equilibrated with 95% O₂ and 5% CO₂. In all experiments the diastolic afterload pressure was set at 2.94 kPa (30 cm H₂O) and the input pressure was regulated to obtain a cardiac output of about 20 ml/min/kg (wet body weight). The statistical significance of differences was assessed on parameter changes using the paired Student's t-test (p<0.05). Percent changes were evaluated as mean ± S.E. of percent changes obtained from individual experiments.

In the control group the Starling response showed that: 1) by increasing preload, a 3 fold significant increase of cardiac output (CO) and stroke volume (SV) (an index of contractility) occurred; 2) CO and SV were less sensitive to afterload challenges. Heart rate (HR) was independent from both preload and afterload, so that the increases of CO reflected those of SV. These results were in agreement with the Starling response described in salmonids (Graham and Farrell, 1989). The synthetic catecholamine, isoproterenol (from 10⁻⁸ to 10⁻⁶ M), classically induced an increase of HR (not significant) without a correspondent increase of CO and peak pressure. L-NMMA (10⁻⁵ M) induced a significant increase of CO and SV, indicating that in control fish a basal nitrenergic tone is present and it exerts a mild negative inotropism. L-arginine (10⁻⁷ M and 10⁻⁶ M), induced a significant decrease of CO and SV at both concentrations. The infected fish were used starting from the 7th until the 17th day from the beginning of the infection and were divided in the following three groups: I group (8th, 9th, 10th days), II group (11th, 12th, 13th days), III group (14th, 15th, 16th, 17th days). The ISA cardiac preparations were less sensitive to Starling response, this being particularly dramatic in the III group (Fig.1).

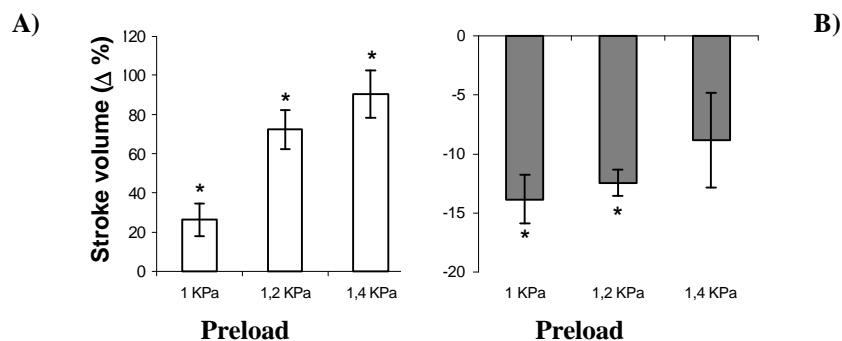


Figure 1. Starling response in control (A) and ISA-infected fish (third period) (B). N=3-4; *= p<0.05.

Under adrenergic stimulation (isoproterenol), no differences were observed between control and ISA fish. However in the first and second period of the infection there was an effect on HR more evident than in other periods. This may indicate that during the infection the adrenergic stimulation becomes more sensitive in comparison with the control animals. In contrast to the control fish, the inhibition of NOS by L-NMMA induced the decrease in both CO and SV in all 3 periods of infection (Fig.2). The ISA infected hearts were not sensitive to exposure to L-arginine, which, only at higher concentrations (10^{-5} M), exerted a mild but not significant reduction of CO and SV.

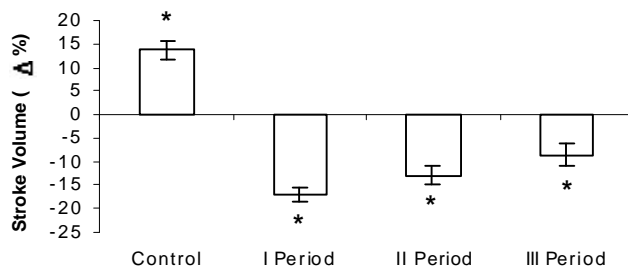


Figure 2. Effects of L-NMMA (10^{-5} M) in control and infected fish. N= 4; *= p<0.05.

The data are consistent with the hypothesis that cardiac dysfunction via impairment of the NO-signalling pathway is an early and significant problem in ISA-infected salmon, probably further aggravated in the adrenaline-activated, stressed fish.

References

- Graham M.S., A.P. Farrell 1989 The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. *Physiol. Zool.* 62(1): 38-61.
- Kelly R., J.L. Balligand, T.W. Smith 1996 Nitric oxide and cardiac function. *Circul. Res.* 79: 363-380.
- Parrillo J. 1993 Pathogenic mechanisms of septic shock. *N. Engl. J. Med.* 328: 1471-1477.
- Prendergast B.D., B.M. V.F. Sci Sagach, A.M. Shah 1997 Basal release of nitric oxide augments the Frank-Starling response in the isolated heart. *Circulation* 96: 1320-1329.

