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Influence of space flight conditions on phenotypes and function of nephritic immune cells of swordtail fish (*Xiphophorus helleri*)

K. Piepenbreier, J. Renn *, R. Fischer, R. Goerlich

Department of Molecular Biotechnology, RWTH Aachen, Worringer Weg 1, D-52074 Aachen, Germany

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Abstract

During space fights, animals and astronauts have to live and act under unusual environmental conditions characterised by reduced gravity. Due to interactions with several physiological systems, the immune system is sensitive to endogenous and exogenous influences. The present study provides the first data of parameters of the defence system, namely differential haemogram and spontaneous cell proliferation activity of nephritic tissue as well as phagocytosis activity of isolated nephritic phagocytes, in teleost fish after 9 days (STS-89) and 16 days (STS-90) of space flight. The artificial aquatic ecosystem C.E.B.A.S. (Closed Equilibrated Biological Aquatic System) was the habitat of swordtail fish (*Xiphophorus helleri*) during space flights and for ground controls. No statistically significant differences were observed between fish after space flights and ground controls either after 9 or 16 days. Additionally, all values of the space flight experiments remained within the physiological normal area. However, in comparison to the values of fish that were kept in aquaria as ground controls, the environmental conditions of some C.E.B.A.S. ground experiments showed a decrease of monocytes and lymphocytes as well as inhibition of the activity of phagocytosis and spontaneous cell proliferation. Swordtails from C.E.B.A.S. ground experiments showed typical symptoms of a stress reaction, namely a decrease of monocytes and lymphocytes and an inhibition of phagocytosis activity. These results indicate that short-term space flights of 9 and 16 days have no effects on the immune system of the swordtails, whereas specific environmental conditions such as those found in the C.E.B.A.S. module during the experiments have the potential to influence defence parameters.

Keywords: Swordtail fish; Space flight; Immune system; Differential haemogram; Phagocytosis; Cell proliferation; Monocytes; Lymphocytes

1. Introduction

When developing an aquatic life support system like the Closed Equilibrated Biological Aquatic System (C.E.B.A.S.) (see Blüm et al., 1994, 1995; Blüm, 2000; Slenzka, 1998, 1999) that is able to regenerate water and oxygen and produce fish as food for astronauts, it is necessary to make sure that space flight conditions do not endanger the health of vertebrates that are part of the ecosystem. To analyse the effects of altered environmental conditions on the health status of vertebrates, parameters of the immune system can give valuable information.

In teleost fish, like the swordtail (*Xiphophorus helleri*), the kidney is the main haemato- and immunopoietic organ (Goerlich and Hamers, 1994; Hamers et al., 1997). In general, determination of blood parameters is an important tool for diagnoses of fish diseases, as they reflect alterations of physiological status according to endogenous and exogenous influences (Lehmann and Stürenberg, 1981; Goerlich and Hamers, 1994, 1996; Schütt et al., 1997; Köllner et al., 2002). Alterations of

* Corresponding author. Tel.: +49 241 8026603; fax: +49 241 8022148.
E-mail addresses: k.piepenbreier@gmx.de (K. Piepenbreier), joerg.renn@rwth-aachen.de (J. Renn), fischer@ime.fraunhofer.de (R. Fischer), goerlich@molbiotech.rwth-aachen.de (R. Goerlich).

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the cell proliferation activity of leucocytes can reflect influences on the cellular defence system (Hamers and Goerlich, 1995a) and can give general information about the activation status of the defence system (Köllner et al., 2002).

The ability of phagocytes to internalise, kill and digest invading microorganisms is a crucial protective mechanism of the non-specific cellular defence system of fish (Secombes, 1996). Therefore, the determination of the nephritic phagocytotic activity is suitable to evaluate the health status of fish (Falk et al., 1990; Lamas and Ellis, 1994; Hamers and Goerlich, 1995b). Stress reactions caused by non-physiological environmental conditions can influence the phagocytosis activity of nephritic phagocytes (Bly and Clem, 1992; Cooper and Parinello, 1996; Ortuno et al., 2001; Boleza et al., 2001; Köllner et al., 2002).

Furthermore, the differential haemogram, i.e., the percentage of monocytes and lymphocytes of leucocytes in nephritic tissue, can indicate changes of the health status of teleost fish (Goerlich and Hamers, 1994). The effectiveness of the immune system is correlated with the ability of the haematopoietic organs to produce defence cells (Ellis, 1981), because an adequate percentage of leucocyte types in nephritic tissue is essential for the function of the defence system. External factors are able to influence the cellular composition of the teleost kidney (Hamers et al., 1997).

Both phagocytes and monocytes internalise, kill and digest invading microorganisms (Ainsworth, 1992; Secombes and Fletcher, 1992; Steinhagen and Jendrysek, 1994). Additionally, they are important accessory cells as they present antigens on their cell surface and secrete soluble mediators involved in lymphocyte activation (Secombes, 1996).

Lymphocytes are key cells for the functions of the specific immune system. B-lymphocytes produce specific antibodies (Kaattari and Piganelli, 1996). T-lymphocytes are able to kill virus infected cells (Somamoto et al., 2000) and produce co-stimulatory molecules that are necessary for other immune functions (Miller et al., 1994; Cain et al., 2002).

To determine the health status of swordtails after 9 days (STS-89) and 16 days (STS-90) of space flight conditions, we analysed the status of their immune system using the differential haemogram of nephritic tissue, phagocytotic activity of isolated nephritic phagocytes, and the spontaneous cell proliferation activity of nephritic tissue. This study provides the first data of immunological parameters of teleost fish after short-term space flights.

2. Materials and methods

2.1. Experiments and animals

Four healthy and sexual mature female swordtail fish (*Xiphophorus helleri*, strain HELLERI 3) were aboard of the STS-89 (89FE) and STS-90 (90FE) space shuttle missions for 9 and 16 days, respectively. Due to reproductive biological studies that were performed simultaneously by another research group and were not object of this work, the selected animals were pregnant. Induction of pregnancy took place short before or short after the shuttle liftoff with ovarial stored sperm (Paris and Blüm, 2000). Selected fish had similar weights (for details see Table 1).

A compartment of the artificial aquatic ecosystem C.E.B.A.S. (Blüm et al., 1994, 1995, 2000; Slenzka, 1998, 1999) with a volume of ≈2 l was used as the habitat of the swordtails during the space flights. In parallel, C.E.B.A.S. ground control experiments were performed (89GE, 90GE). In preparation of both space flight experiments, two C.E.B.A.S. test runs (called payload

Table 1
Weight of the *Xiphophorus helleri* used in the C.E.B.A.S. experiments

	F1 start/end	F2 start/end	F3 start/end	F4 start/end
PVT1[a]	12.9/11.0			
PVT2[a]	12.9/10.3			
89GE[b]	2.40/2.44	2.69/2.89	2.98/2.48	3.00/dead
89FE[c]	4.08/3.82	2.21/1.81	2.39/2.00	2.29/1.97
PVT3[d]	14.8/3.7 (only one surviving fish)			
PVT4[d]	11.9/10.3			
90GE[e]	1.48/1.43	1.59/1.64	1.22/dead	1.71/dead
90FE[f]	2.02/1.96	1.71/1.41	1.72/1.78	1.56/1.31

The weight of the swordtail fish at the start and the end of the C.E.B.A.S. experiments is given in grams. For the payload verification test (PVT) 1–4 only the total weight of all four fish used in the C.E.B.A.S. module is given. F1–F4: fish in the C.E.B.A.S. experiments.

[a] PVT of the STS-89 mission.

[b] Ground control of STS-89 mission.

[c] Flight experiment of STS-89 mission.

[d] PVT of STS-90 mission.

[e] Ground control of STS-90 mission.

[f] Flight experiment of STS-90 mission; Data by Prof. Dr. V. Blüm, Ruhr-University Bochum, Faculty of Biology, C.E.B.A.S. Center of Excellence, FRG.