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Xenopus laevis embryos: biochemical evaluations in simulated microgravity condition

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Abstract

Exposure to space-flight environment, notably microgravity and radiations, can induce changes in living systems. Life in space increases the amount of stress hormones, insulin resistance, altered musculoskeletal system structure and function, inflammation and mitochondrial function with increased oxidative stress (Biolo et al., 2003; Zhang et al., 2007; Schatten et al., 2001). Only few animal systems are suitable for space experiments which allow to investigate the countermeasure for microgravity and radiation damages. One of the best model is represented by Xenopus laevis (X.l.) (Ikuzawa and Asashima, 2008) for the reasons indicated below.
Xenopus laevis embryos are easily produced by in vitro fertilisation, develop at room temperature in fresh water and do not need to be fed during the first few days of development. The two layers of cells constituting the embryo skin are directly exposed to the external environment without the stratum corneum, which functions as a barrier in adult animals. Moreover, Xenopus embryo has already well differentiated and functional organs. Finally Xenopus embryos are usually utilized to assay the toxicity of many molecules using the so called "FETAX" test. As they represent a very sensitive system, they could be considered also a biosensor for space deleterious effects.
The development of X.l. was investigated in microgravity utilizing a Random Positioning Machine (RPM), that is essentially a 3-axis clinostat.
Embryos were placed on RPM 8 hrs after in vitro fertilization and kept in microgravity conditions till day 3 of development; another set of embryos was maintained in microgravity conditions from day 3 to day 6, while a third group was maintained from day 1 till day 6 of development. Embryos developed in the Clinostat showed the same viability compared to the control ones even if their size was lower and they were less pigmented, in particular the ones exposed for 6 days. The antioxidant defenses of the embryos studied by the content of glutathione, by the activity of enzymes involved in its metabolism and in reactive oxygen species detoxification showed an up regulation.

Introduction

Exposure to microgravity and radiations can induce changes in living systems. Life in space is both associated with undesired and unexpected effects on physiological processes, such as increased stress hormones incretion, insulin resistance, anaemia, altered musculoskeletal system structure and function and altered inflammatory response and mitochondrial function with increased oxidative stress. Particularly in unmanned missions, only few animal systems are suitable for space experiments allowing to investigate the countermeasure for microgravity and radiation damages.
Oxidative stress is a more pronounced pro-oxidant state that results from a serious imbalance between oxidation and antioxidation; it is caused by the excessive production of Reactive Oxygen and Nitrogen Species (ROS and RNS, respectively) and free radicals or from the weakening of the antioxidant defense system. A number of defense mechanisms have evolved to protect the non-radical molecules from radical attack by ROS, thus limiting the damage that they can produce.
One example of the production of ROS is depicted in the following scheme (**Fig. 1**).
In **Figure 2** the origin of cellular ROS, the damage they produce and the enzymatic systems which counteract directions are schematically reported.
Xenopus laevis (*X.l.*) is a vertebrate. Their embryos are easily produced in large number by in vitro fertilisation. They can be kept in plastic Petri dishes, developed at room

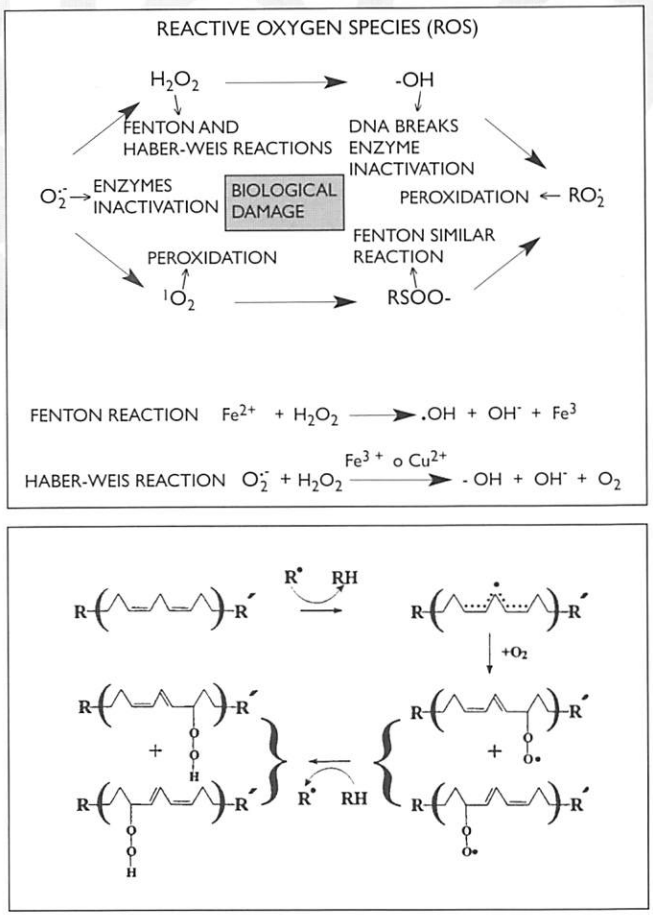


Fig. 1 - *one mechanism for ROS production.*

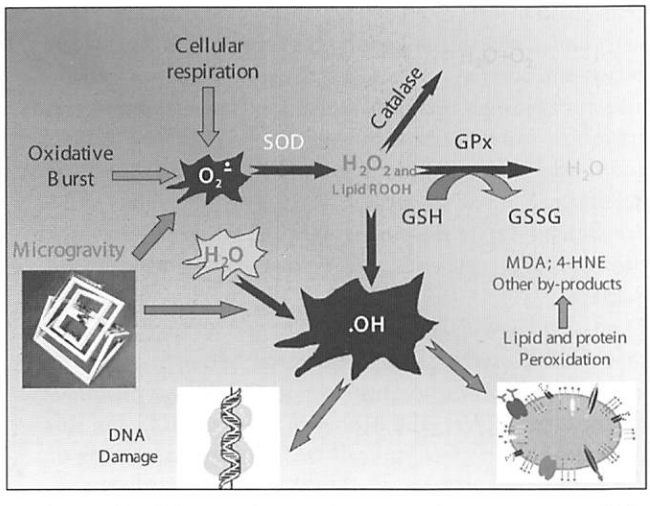


Fig. 2 - *ROS cellular production, damage and counteractions. SOD: superoxide dismutase; GPx: Glutathione peroxidase; GSH: reduced glutathione; GSSG: oxidized glutathione; MDA: malondialdehyde; 4-HNE: 4-hydroxynonenal.*

temperature in fresh water and do not need to be fed during the first days of development. The two layers of cells constituting the embryo skin are directly exposed to the external environment without the presence of the stratum corneum, which acts as a barrier in adult animals.

Xenopus embryos are widely used to assay the toxicity of molecules using the so called "FETAX" test (see materials and methods) and could be considered a biosensor also for space generated deleterious effects. Worth of note, a few day old *Xenopus* embryo already has well differentiated and functional organs.

Materials and Methods

Simulation of microgravity was performed utilizing a random positioning machine (RPM) shown in **Figure 3.**

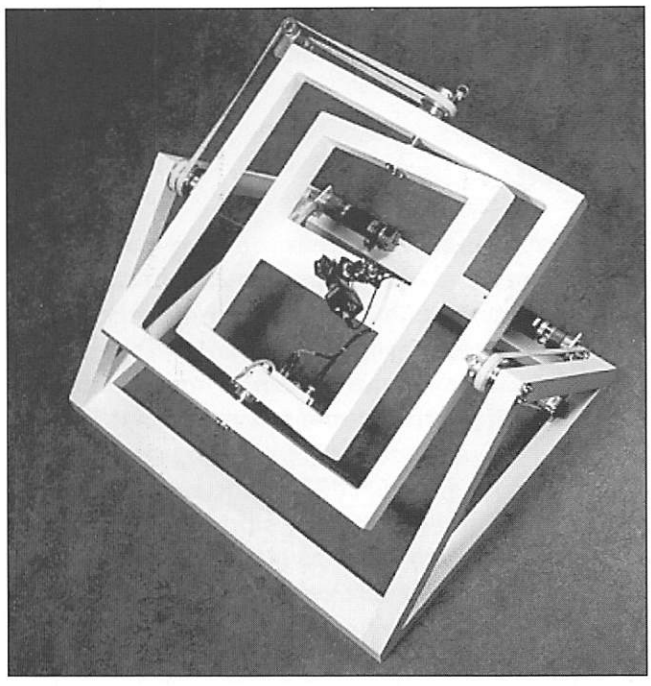


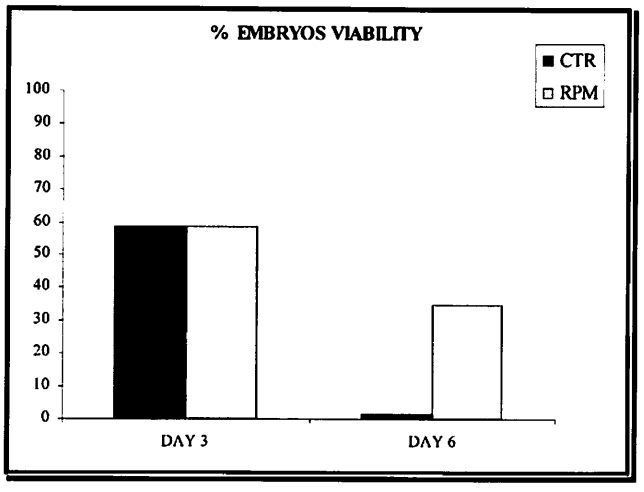
Fig. 3 - *RPM machine. The RPM is essentially a 3-axis clinostat.*

Embryos of *X.l.* were obtained by in vitro fertilisation (Rizzo *et al.*, 1994) and maintained in a thermostatic chamber at 23 ± 0.5 °C. The embryos used in the experiment were divided into three groups: **Group A:** 8 hrs after *in vitro* fertilization the embryos were placed on the RPM till day 3 of development; **Group B:** 8 hrs after *in vitro* fertilization the embryos were maintained in a thermostatic chamber at 23 ± 0.5 °C till day 3 and then placed on the RPM till day 6 of development; **Group C:** 8 hrs after *in vitro* fertilization embryos were placed on the RPM from day 1 till day 6 of development. For every group, control embryos were grown in the same conditions of RPM ones but placed on the fixed base of RPM.
Alive, dead or malformed embryos were counted at the end of the experiments.
Heat Shock Proteins (HSP) were separated by SDS–PAGE and analysed by Western blotting, utilising specific monoclonal antibodies (Calbiochem, USA). Specific proteins were detected by the ECL method (Amersham, UK), followed by densitometric scanning to quantify the bands (Camag, Switzerland).

Total glutathione (GSH) content, catalase, GSH reductase, and GSH peroxidase activities were determined as previously described (Griffith, 1985; Aebi, 1984; Pinto *et al.*, 1984; Prohaska and Ganther, 1976).

Results and Conclusion

The viability of *Xenopus* embryos after exposure to microgravity is depicted in **Figure 4**.



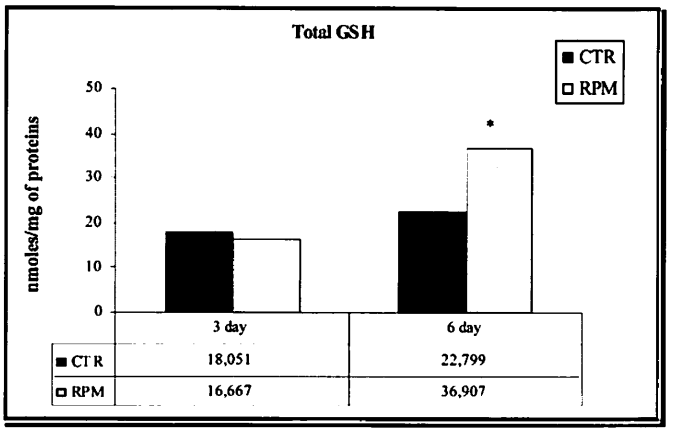
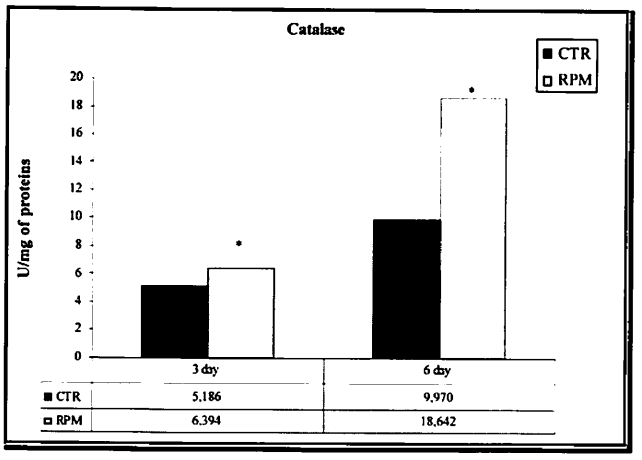
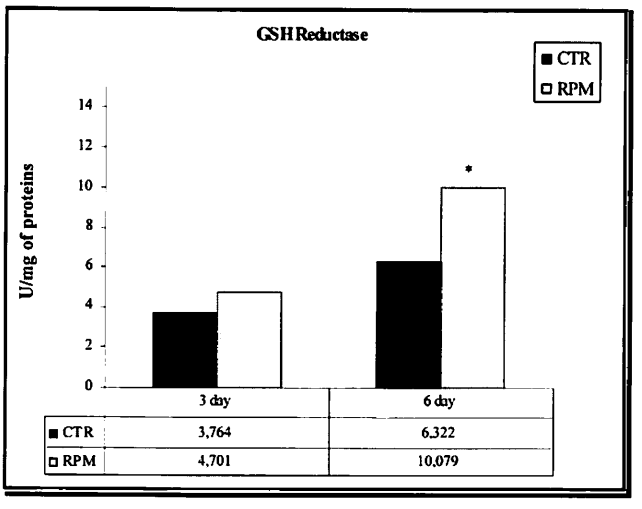


Fig. 4 - *viability (%) of control (CTR) and microgravity (RPM) X.l. embryos at different endpoints.*

Total GSH content in *Xenopus* embryos developed in microgravity conditions increased significantly only in six days old embryos while no differences were found for three days old embryos between treated and controlled. The same results, i.e. increase at six days and no significant variation for three days old embryos, were shown when GSH reductase activity was measured.
On the contrary, significant variation in the group grown in microgravity conditions was shown at three and six days for what catalase and GSH peroxidase activity is concerned.
Results are shown in **Figure 5**. In **Figure 6** the activation of HSP 60 and HSP 70 is shown.
In conclusion, microgravity did not cause an increase of mortality compared with controls but we observed some





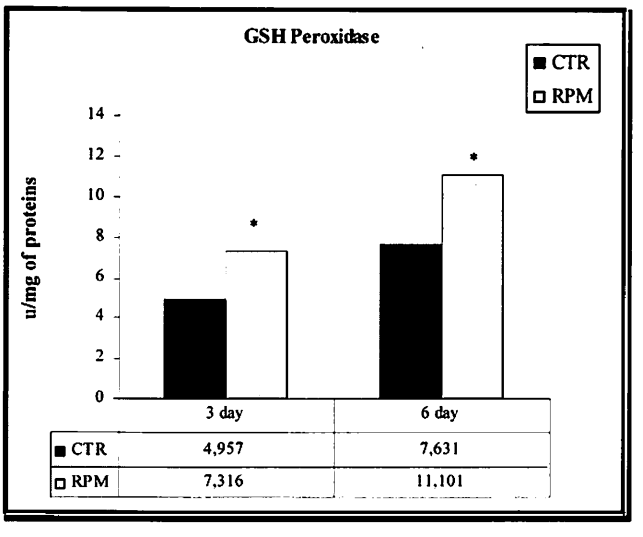


Fig. 5 - *total GSH content, catalase, GSH reductase and GSH peroxidase activities results for control (CTR) and microgravity (RPM) X.l. embryos at different endpoints. *= statistically significant difference.*

malformation more evident at 6 days (data not shown).
The exposure of embryos to microgravity for 3 days caused an activation of HSP-60 and HSP-70; longer periods of incubation did not cause the same effect. When the embryos were grown in simulated microgravity there is a

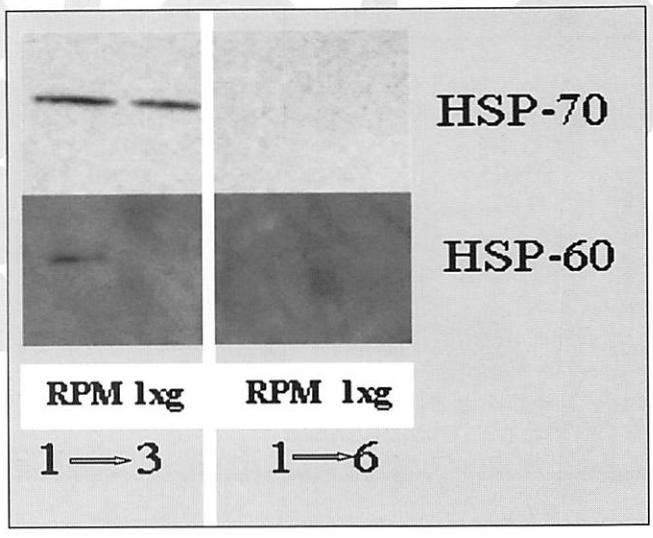


Fig. 6 - *western blot analysis for HSP-60 and HSP-70 expression in control (1xg) and microgravity (RPM) X.l. embryos at different endpoints (from day 1 to day 3; and from day 1 to day 6).*

decrease of DNA content after 6 days of incubation (data not shown). It is possible to observe that the embryos up regulated their antioxidant system, based on GSH utilization. In fact all the enzyme involved in GSH metabolism are increased after 3 days of exposure to microgravity, reaching the higher values of the end of the experiment; at that time also the total GSH content was increased.

References

Aebi H. 1984. Catalase in vitro. Meth. Enzymol; 105:121-126.

Biolo G., Heer M., Narici M., Strollo F. 2003 Jan. Microgravity as a model of ageing. Curr Opin Clin Nutr. Metab. Care., 6(1):31-40.

Griffith O.W. 1985. Glutathione and glutathione disulphide. In: Bergmeyer HU editor. Methods of Enzymatic Analysis.New Yprk: Academic Press; vol VIII: 521-529.

Ikuzawa M., Asashima M. 2008 Aug. Global expression of simulated microgravity-responsive genes in *Xenopus* liver cells. *Zoolog Sci.*, 25(8):828-37.

Pinto C., Mata A., Lopez Barea J. 1984. Reversible inactivation of Saccharomyces Cerevisiae glutathione reductase under reducing conditions. Arch Biochem Biophys, 228:1-12.

Prohaska J.R., Ganther H.E. 1976. Selenium and glutathione peroxidase in developing rat. J Neurochem, 27:1379-1387.

Rizzo A.M., Gornati R., Galli C., Bernardini G., Berra B. 1994. Cholesterol, triacylglycerols and phospholipids during *Xenopus* embryo development. Cell Bio Int, 18:1085-1090.

Schatten H., Lewis M.L., Chakrabarti A. 2001 Aug-Nov. Spaceflight and clinorotation cause cytoskeleton and mitochondria changes and increases in apoptosis in cultured cells. *Acta Astronaut.*, 49(3-10): 399-418.

Zhang P., Chen X., Fan M. 2007. Signaling mechanisms involved in disuse muscle atrophy. *Med. Hypotheses.*, 69(2):310-21.