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Study of enhanced radio-resistance induced by hibernation

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Summary. — During hibernation/torpor, mammals show an enhanced resistance to radiation. This effect, whose mechanisms are still unclear, could have important applications in radiotherapy and for space travels. In recent years a procedure to induce a state mimicking torpor (synthetic torpor) was described for non-hibernators (rats), renewing the interest in possible human applications. The multi-disciplinary HIBRAD experiment was conceived to test the possible radio-resistance induced by synthetic torpor and investigate its molecular mechanisms, focusing on the early response. Analysis of tissue damage and gene expression indicate that synthetic torpor reduces radiation damage, possibly by the activation of specific gene pathways.

1. – Introduction

Torpor is a peculiar metabolic state used by some mammals to save energy in case of scarce food or low ambient temperature, that can last up to many months during hibernation. During torpor mammals undergo a reduction of the metabolic rate, entailing a decrease of the body temperature that depends on the environmental temperature [1,2].

One trait of the torpor is an enhanced radio-resistance (see [3] for a detailed discussion). Experiments on squirrels, hamsters and mice irradiated with lethal doses have shown higher surviving fractions and survival times in animals irradiated during torpor, compared to the ones irradiated at normal body temperature. However the mechanisms beyond this radioprotective effect of torpor are still unknown and may be related to the possible cell hypoxia, the halt of cell replication, or the activation of more effective DNA repair mechanisms.

The possibility to induce a torpor state in non-hibernators would have broad implications on the development of new clinical practices and strategies for space exploration.

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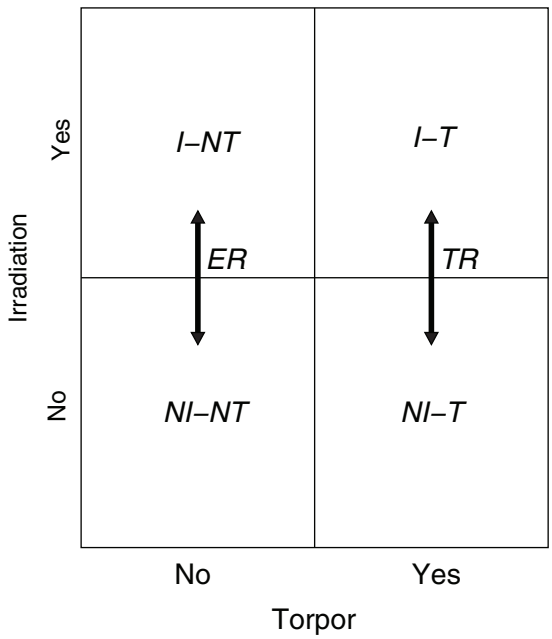

Fig. 1. – Schematic representation of the four experimental groups, as described in the text. The arrows indicate the groups that are compared to identify the genes that are up- or down-regulated as an effect of radiation.

The Raphe Pallidus is a region of the brain with a central fuction in the body temperature regulation. A method to induce a state resembling torpor in non-hibernators through the pharmacological inhibition of the neurons in the region of the Raphe Pallidus was recenlty developed [4]. However, whether the enhanced radio-resistance characterizing the hibernation is also present in this state of "synthetic torpor" is still an open question.

The HIBRAD experiment aims at the investigation of the molecular mechanisms at the basis of the possible enhanced radio-resistance induced by synthetic torpor, tested on a non-hibernator animal model such as the rat. The cellular response to radiation is obtained by comparing irradiated tissues to non-irradiated controls by means of gene expression analysis. In order to identify the radioprotective mechanisms that come into play, the response to radiation for animals in torpor is compared to the one of animals in the euthermic state.

2. – Methods

This section presents a concise overview of the experimental method, more details can be found in [5].

The HIBRAD experiment is designed to investigate the early cell response (few hours after the irradiation) when the first repair mechanisms are activated. Four experimental groups, each one composed by 8 animals (rats), are defined depending whether the animals were irradiated (I) or not (NI) and whether synthetic torpor was induced (T) or not (NT), as schematically represented in fig. 1.

Experiments were conducted on male Sprague-Dawley rats weighing 250–300 g. Af-

ter one week of adaptation, they underwent surgery under general anaesthesia for the implantation of a thermistor probe to monitor the internal body temperature and a microinjection guide cannula implanted within the brainstem region of the Raphe Pallidus.

After the recovery period of one week at standard laboratory conditions, synthetic torpor was induced to the T group through the microinjection of the GABA–A agonist muscimol (1 mmol) within the Raphe Pallidus and keeping them at ambient temperature $T_a = 15\,^{\circ}\mathrm{C}$. Animals enter in synthetic torpor shortly after the first injection and their body temperature decreases, reaching approximately $T_b = 20\,^{\circ}\mathrm{C}$ in 3–6 hrs. All animals in the T group are kept in such conditions until the end of the experiment. The same treatment was applied to animals of the NT group, except that artificial cerebrospinal fluid was injected instead of muscimol.

A thermostatic transportable cage, with sound attenuated and homogeneously radiotransparent walls, was developed to allow the transportation of the animals from the housing site to the irradiation site at constant $T_a = 15\,^{\circ}\mathrm{C}$.

The irradiation setup was calibrated using a ionization chamber inside a plexiglass phantom resembling a typical rat thickness. Animals were irradiated 4 hrs after the first injection with a target average dose of 3 Gy total body at the dose rate of 23 cGy/min, using an X-ray tube operated at 180 kV at the S. Orsola Hospital in Bologna. The source produces a uniform field in the whole region where the animal is located inside the thermostatic cage. Verification of the dose is obtained by means of radiochromic films placed below the animal, showing deviations of the order of 20% from the target dose in the upper and lower part of the animal, as an effect of the X-ray absorption in the animal tissue (irradiated from above).

Four hours after the irradiation, animals were given a lethal dose of anaesthetic for organs collection. The analysis of the gene expression of the different organs is then obtained through RNA sequencing, provided by a specialized firm (Mentotech S.R.L.).

3. – Results

The peculiar cell response to radiation that is activated during torpor can be identified by comparing the gene regulation of animals irradiated during synthetic torpor (I-T) with respect to controls irradiated in euthermic state (I-NT). However, this picture is too simplistic and cannot be directly applied: inducing synthetic torpor in animals will completely change their overall metabolic state, in which several genes can show up- or down-regulation even without the external irradiation, as would be observed in the comparison of the gene expression of non-irradiated animals in synthetic torpor (NI-T) with respect to controls non-irradiated in euthermic state (NI-NT). For this reason, in order to extract the different response to radiation in euthermia and in synthetic torpor, it is necessary to extract the response to radiation in both metabolic states, this means comparing the gene expression of group I-NT with the control NI-NT ("euthermic response", ER) and the one of group I-T with the control NI-T ("torpor response", TR).

Possibly as an effect of the overall reduction of methabolic functions, we observed just a small number of genes that are up-regulated in TR. Moreover, almost 90% of the down-regulated genes in TR are down-regulated also in ER, so they represent a general response of the cell to radiation, not a peculiar response induced by synthetic torpor.

Preliminary results regarding the gene expression analysis of liver of 3 animals are obtained by focusing on the mechanisms that are activated by the cell only during synthetic torpor, therefore we identify genes that are up-regulated in TR but not in ER.

Within this set, multiple gene ontologies were represented, and of particular interest were the following pathways:

- genome activation;
- anti-inflammation;
- activation of fat acid metabolism;
- DNA stabilization and repair [6].

The specific response induced by radiation during torpor support the hypothesis that the cells, during torpor, switch to a different phenotype that may provide a better response to cell damage.

4. – Summary

HIBRAD is a proof of concept experiment for the investigation of the enhanced radio-resistance induced by hibernation, that successfully proved the feasibility of the technique for future experiments.

Preliminary results regarding the gene expression in the liver of animals irradiated during synthetic torpor indicate that an enhanced radio-resistance could be the result of different repair mechanisms, that occur during this peculiar methabolic state.

A complete gene expression analysis, that will also consider different tissues, is ongoing and will be the subject of a future paper.

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