Is superoxide dismutase a physiological radioprotector?[1]

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Summary. Prolonged treatment with relatively low doses of ionizing radiation did not induce synthesis of superoxide dismutase, either in *Drosophila melanogaster* or in mice liver.

The ubiquitous presence of superoxide dismutase (SOD) in all aerobic organisms[3] points to the importance of the biological function(s) performed by this enzyme. From among different biological roles attributed to this protein, resulting from its superoxide dismutase activity, the protective role with respect to ionizing radiation has been suggested and demonstrated in several instances. Addition of SOD protected irradiated calf myoblasts[4] and *Acholeplasma laidlawii* cells[5]. When injected into animals, it reduced lethality in X-irradiated mice[6,7]. However, in all these systems SOD was a factor introduced artificially. It is not clear whether the constitutive cellular SOD acts as a physiological radioprotector, too. If so, one could expect an adaptative synthesis of this enzyme in response to treatment with ionizing radiation, as is the case with other factors, especially oxygen[8]. To approach this question, we studied SOD activity after a prolonged treatment with moderately low doses of ionizing radiation in 2 objects: *Drosophila melanogaster* and *Mus musculus.*

Material and methods. Culturing and homogenization of *Drosophila,* as well as estimation and expressing of SOD activity, is described in the accompanying paper. Half of the *Drosophila* (wild strain) culture bottles were subjected to daily irradiations from a ^{60}Co γ-source (7 × 400 rad, dose-rate of 16.7 rad/sec, room temperature) throughout the developmental stages from eggs until pupae. 2–3 days after emergence, SOD activity was estimated in homogenates of control and irradiated flies.

Female Swiss mice, weighing 20–25 g, fed with standard LSM diet, were subjected to 6 irradiations from a ^{60}Co source (48.6 rad each, dose-rate of 9.7 rad/sec) over a fortnight. After 7, 16 and 18 days from the beginning of the treatment, the animals were killed by cervical dislocation. Livers and spleens were taken, perfused with buffered saline in order to remove as much blood as possible and homogenized in the same buffer as that used for *Drosophila.* Hemoglobin was determined as described elsewhere[9].

Results and discussion. SOD activity did not differ in homogenates of control and irradiated flies (table 1). Data for SOD activity in mouse organs are given in table 2. These results demonstrate clearly that no increase in SOD activity was induced in *Drosophila* or in the relatively radioresistant mammalian organ, mouse liver. On the other hand, some elevation of this activity was noted in a radiosensitive organ, mouse spleen ($p > 0.05$ in all cases). However, it is not possible to ascribe this phenomenon to the radiation-induced SOD synthesis in the spleen. It is still plausible that radiation can bring about an increased destruction of erythrocytes and a deposition of erythrocyte SOD mainly in the spleen. The contamination of spleen homogenates with hemoglobin did not differ significantly between control and irradiated animals (table 3), but hemoglobin may be more rapidly decomposed in the spleen than SOD, which is more resistant to the action of proteolytic enzymes[10]. Therefore, our results do not prove a radiation-induced SOD synthesis. Nevertheless, they do not exclude this possibility. In similar experiments with induction of SOD by oxygen, an increase[8] in the enzyme activity, or no changes[11] and even a decrease[12], were found, depending on the experimental design. Further experimentation is needed to elucidate this question.

Table 1. SOD activity in homogenates of control and irradiated *Drosophila melanogaster* (units/g protein)

Flies	C+M	M
Control	786±175	168±44
Irradiated	770±155	158±10

Mean±SD from 6 experiments. C: cytosol enzyme, M: mitochondrial enzyme.

Table 2. SOD activity in organs of control and irradiated mice (units/g protein)

Days after beginning of treatment	Control C+M	Control M	Irradiated C+M	Irradiated M
Liver				
7	3732±959	237±107	2565± 297	191±33
14	3743±256	227± 6	3497± 960	208±24
16	3543±429	313±130	3403±1084	199±32
Spleen				
7	374±175	81± 10	819± 56	105±18
14	651±169	65± 39	1047± 90	68±13
16	713±130	91± 26	950± 93	106±37

Mean±SD from triplicate determinations on 5–10 animals in each group. C: cytosol enzyme, M: mitochondrial enzyme.

Table 3. Contamination of mice organ homogenates with hemoglobin (hemoglobin as per cent of homogenate proteins, mean ±SD)

Time	Control	Irradiated mice
Liver		
7 days	3.7±0.67	3.6±0.19
14 days	2.2±0.05	2.7±0.48
16 days	3.3±0.04	3.7±0.25
Spleen		
7 days	20.8±6.45	25.4±1.20
14 days	12.0±2.31	14.8±1.50
16 days	18.7±3.28	16.2±2.69

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