Difference in the Mechanism of Radioprotection between Carbon Particles and Bacterial Endotoxin

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Radioprotection/Bacterial Endotoxin/Carbon Particles

Difference between carbon particles and bacterial endotoxin in their modes of radioprotection was examined. Radioprotection by bacterial endotoxin was carried out without corresponding enhancement of the haemopoietic stem cell survival, whereas the increased survival of the carbon treated animals was in good accordance with the enhancement of the survival of the haemopoietic stem cells in the treated animals. Radioprotection by bacterial endotoxin, therefore, seems to be due to the enhanced induction of the differentiation of the stem cells to recruit functioning blood cells.

Survival of sublethally irradiated animals depends mainly on the recovery of haemopoietic function. Restoration of such haemopoietic cells can be greatly enhanced by the administration to animals of particulate substance such as carbon particles or bacterial endotoxin\(^1\). The former has been shown to blockade reticuloendothelial system (RES), which as a result gives a favourable environment for the recovery of haemopoietic stem cells\(^2\), whereas the latter is known to simulate RES and enhance the immune response\(^3\).

Present work deals with the attempt to distinguish the mechanism of radioprotection by carbon particles from that by endotoxin, by comparing the effective dose for the radioprotection with that for the enhancement of the survival of haemopoietic stem cells in the irradiated animals treated with either with carbon particles or with bacterial endotoxin.

Male mice of DDY-strain, 8–10 weeks old were injected i.v. with Pelikan india ink (C 11/1431 a, Günther-Wagner, Germany) or with lipopolysaccharide B from \textit{E. coli} 0 111: B\(_4\) (Difco, U. S. A.) 24 hrs before the irradiation of the animals with various doses of X-rays as reported previously\(^4\). Haemopoietic stem cells were assayed according to the method of Till and McCulloch\(^5\). The mice were irradiated with appropriated doses of X-rays and the endogenous spleen colonies were counted 9 days later.

Table 1 shows the radioprotective effect of carbon particles and of bacterial endotoxin given separately or simultaneously. Substantial radioprotection was observed in
the mice injected with not less than 1 mg carbon particles. Endotoxin showed strong radioprotective effect with the injection of more than 0.01 μg. Simultaneous injection of carbon particles and endotoxin did not enhance the survival of the irradiated animals any further, or rather decreased the survival. Thus, the administration of bacterial endotoxin had an antagonistic effect on the carbon-induced radioprotection.

A significant increase in the survival of the endogenous spleen colony-forming unit (CFU) was observed when no less than 1 mg carbon particles were given, the dose corresponding with the dose required for the effective radioprotection (Table 2). In contrast, treatment of mice with less than 0.1 μg endotoxin scarcely showed favourable effect on the survival of CFU. Administration of ca. 1 μg was necessary to obtain substantial increase in the number of surviving CFU. Thus, the minimum dose required for the radioprotection of the irradiated mice was much less than that necessary for the enhancement of the survival of CFU.

Although the survival and recovery of haemopoietic stem cells is mainly responsible for the survival of sublethally irradiated animals, the actual survival would ulti-
mately depend on the restoration of differentiated functioning blood cells by the critical
time after the irradiation. Bacterial endotoxin is known to stimulate reticuloendothelial
system and release granulocytes from the bone marrow to peripheral blood's. It is
also reported that endotoxin triggers the proliferation, and probably differentiation as
well, of haemopoietic stem cells'. Accordingly, our interpretation of the present
results is that the radioprotective effect of a small amount of bacterial endotoxin is
mainly due to the stimulation of the induction of the differentiation of haemopoietic
stem cells into functioning cells, which as a consequence recruits the damaged peripheral
blood cells, rather than enhancing the survival of the stem cells, whereas increased
survival of the stem cells is responsible for the carbon-induced radioprotection.

Table 2.

| Doses of X-rays | 420 R No. of colonies | 420 R Ratio | 480 R No. of colonies | 480 R Ratio | 540 R No. of colonies | 540 R Ratio |
|-----------------|-----------------------|-------------|-----------------------|-------------|-----------------------|-------------|
| Control (Normal) | 6.7 1 | 2.7 1 | 0.7 1 |
| Saline alone    | 6.6 1.0 | 3.8 1.4 | 0.8 1.2 |
| Carbon*         | 4.0 0.6 | 4.1 1.5 | N. T. |
| 0.5 mg          | 10.0 1.5 | 2.5 0.9 | 0.6 0.9 |
| 1.0 mg          | 25.6 3.8 | 19.3 7.1 | 17.5 25.0 |
| 5.0 mg          | numerous | numerous | 30.0 42.9 |
| Endotoxin**     | 6.0 0.9 | 3.2 1.2 | N. T. |
| 0.005 µg        | 7.4 1.1 | 4.3 1.6 | 0.6 0.9 |
| 0.05 µg         | 14.1 2.1 | 3.0 1.1 | 0.8 1.2 |
| 0.1 µg          | 12.7 1.9 | 11.5 4.2 | 1.6 2.3 |
| 1.0 µg          | numerous | numerous | numerous |

Carbon particles* or endotoxin** were given i.v. 24 hrs before irradiation, and spleen
colonies were counted 9 days later. Average of 3 separate experiments. N. T.: not tested.

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