CXCL12 EXPRESSION IN HEMATOPOIETIC TISSUES OF MICE EXPOSED TO SUBLETHAL DOSE OF IONIZING RADIATION IN THE PRESENCE OF iNOS INHIBITOR.

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We study the production of CXCL12, a stem cell homing chemokine, in spleen and bone marrow of mice exposed at LD50% of γ-radiation, w/wo a iNOS blocker, aminoguanidine, to test if inflammatory nitric oxide is involved in necrotic processes of stem cell death after ionizing radiation exposure. Groups of 10 male 6-week old C57Bl/6j mice were killed at specific time points after a 8Gy dose irradiation (^{60}Co source; 4,22kGy/h dose rate) and spleen and bone marrow samples were immersed and stored in TriZOL® for total mRNA extraction. RT-PCR assays were performed to determine the production of CXCL12 as compared to murine β-actin at days 2th, 5 th, 7 th, 9 th and 15 th days after radiation in a semiquantitative way. PCR was performed after cDNA synthesis using Oligo-dT primers and specific primers for CXCL12 and β-actin. Artificial optical density was determined in silver-stained PAGE resolved specific amplification products of CXCL12, using amplification of murine β-actin as standard, and measurements obtained by the Image J freeware. CXCL12 production in spleen samples reached its maximum at 5 th day after radiation exposure in animals not treated with aminoguanidine, but this peak was extended to at 7 th day in treated animals. Non-treated animals presented a decrease of CXCL12 expression up to 15 th day of experiment, and aminoguanidine treated animals showed sustained increase of expression levels between 9 th and 15 th days. In bone marrow samples, the main difference among the two different experimental groups was a maintenance of CXCL12 mRNA expression between 7 th and 9 th days, persisting until the end of the experiment. Our data demonstrates that the effect of aminoguanidine appears to sustain the CXCL12 mRNA synthesis in hematopoetic tissues of irradiated mice, providing some evidences that the axis iNOS –NO – inflammation must be involved in stem cell death, aside to the direct radiation effect, suggesting their use associated to the CXCL12 chemokine to enhance the post-transplantation grafting of hematopoietic tissue and/or treatment of accidentaly exposed individuals to sublethal doses of ionizing radiation.
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