Radioprotective effects of ginsan and amifostine (WR-2721) against γ-ray-induction of micronuclei in mice bone marrow erythroblasts

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It is common knowledge that the use of combinations of agents is a promising approach for maximizing radioprotection with minimal adverse effects.

So it has been known that amifostine has powerful protective action against irradiation. However, its toxicity has limited its application in medicine or in hazardous radiation environments.

It was shown that some biological compounds, including cytokines, have at least an additive effect when administered with amifostine. It was previously reported that ginsan immunomodulator elevates the endogenous production of radioprotective cytokines. The aim of the present study was to assess the effects of combinations of ginsan, amifostine and γ-rays on induction of micronuclei in the mouse erythropoietic system, by determining the frequency of micronucleated polychromatic (MNPCEs) in the bone marrow.

The frequency of micronucleated polychromatic erythrocytes (MNPCEs) was assessed in the bone marrow of adult male C57Bl/6 treated with ginsan and amifostine (WR-2721, S-2-/3-aminopropylamino/ethyl phosphorothioic acid), at a dose of 200 mg/kg body weight, and exposed to 1.5 Gy of γ-rays (39cGy/min) from a ^{60}Co Tele therapy source (Theratron, Atomic Energy Agency, Canada). This dose of amifostine represents 2/5 of the maximum tolerated dose (500mg/kg) for wich is about one-thirds of its LD_{50} dose and for ginsan - 1/5 the maximum tolerated dose. Ginsan or amifostine were injected intraperitoneally alone, or 30 min prior, or 15 min after to γ-ray-exposure. The number of MNPCEs was determined at 24 h after the γ-irradiation. For the estimation of the cell cycle perturbation during erythropoiesis in bone marrow the PCE/NCE ratio was determined. Statistical significance among different treatments was determined by one-way ANOVA and Newman-Keuls multiple comparison test (Graph PAD Instar Software).

Radiation exhibited about a 30-fold increase in the frequency of MNPCE and resulted in a significant decrease in the PCE/NCE ratio compared to the concurrent control groups ($p<0.001$). Dose 1.5Gy resulted also in the induction of PCEs bearing of more than one micronucleus ($p<0.001$).

Ginsan showed the stronger radioprotective effect compare with amifostine application. The percentage of reduction in the frequency of micronucleated polychromatic erythrocytes following ginsan administration both prior to γ-irradiation and after was 50% ($p<0.01$). It was shown that means and variances for frequencies of MNPCE and PCE/NCE ratio between the ginsan + irradiation and irradiation + ginsan groups were not significantly different ($p>0.05$). Treatment with ginsan significantly reduced the occurrence of more than one MN both in the ginsan + irradiation and irradiation + ginsan groups compared to the PBS + irradiation group ($p<0.01$). This indicates that ginsan protected against the multiple breaks induced by gamma-rays with their significant reduction.
Administration of ginsan prior to and after irradiation did not decrease the PCE/NCE ratio significantly compared to those for irradiated mice given only the control solutions ($p>0.05$).

Among mice treated with amifostine both prior and after to exposure to γ-rays, the decrease of the total frequency of MNPCE and also MNPCE with multiple micronuclei was not significant ($p>0.05$). Means and variances for MNPCE were not differed significantly between these groups. In contrast, the PCE/NCE ratio increased significantly compared to the those in control irradiated groups when amifostine was injected 30 min prior to irradiation ($p<0.01$) and it was not significantly higher in irradiation + amifostine group ($p>0.05$).

The combination of ginsan and amifostine given before γ-ray-exposure resulted in the most radioprotective effect as compared to the respective single-drug treatment of mice and to the same combination given 15 min after irradiation. The percentage of reduction in the frequency of micronucleated polychromatic erythrocytes was almost 70% and the level of the MNPCE declined significantly compared to those for the concurrent PBS + irradiation mice ($p<0.001$). But the difference was not significant compare with the only ginsan administration ($p>0.05$). The frequency of multiple MNPCE was also reduced drastically and was not differed significantly from those in mice given only the control solutions. The PCE/NCE ratio was increased but did not significantly compared to those for irradiated mice ($p>0.05$).

The treatment schedule of amifostine 30 min before and ginsan 15 min after irradiation (amifostine + irradiation + ginsan) showed also good radioprotection (58% reduction). Both the frequency of total MNPCE ($p<0.01$) and multiple MNPCE ($p<0.001$) has declined significantly when compared to those in the irradiation treatment mice and not significantly compared with the only ginsan administration group. PCE/NCE ratio was

increased not significantly compared to those for irradiated mice ($p>0.05$).

The treatment by combination ginsan + amifostine given after irradiation showed also radioprotective effect (reduction 38.4%). But it was less compare to the others used schedules: ginsan + irradiation – 49.5%; irradiation + ginsan – 50%; ginsan + amifostine + irradiation – 69.9%; amifostine + irradiation + ginsan – 58.2%. PCE/NCE ratio was less compared to the PBS + irradiation group, but difference was not significant ($p>0.05$). The frequency of multiple MNPCE was higher compare to those following combination ginsan + amifostine injected before irradiation or amifostine + irradiation + ginsan treatment. The difference was not significant ($p>0.05$).

To summarize, the present results demonstrate that:

1 – 30 min pre- and 15 min post-treatment by ginsan at a dose 200 mg/kg showed the stronger radioprotective effect compare with amifostine (200 mg/kg) application;

2 - ginsan administered simultaneously with amifostine before irradiation or after irradiation or following schedule amifostine (30 min) + irradiation + Ginsan (15 min) has tendency to enhance the radioprotective efficacy of amifostine;

3 - the most effective radioprotection against γ-ray-induction of micronuclei in the normal erythroblasts was obtained in the case of application of the combination of ginsan and amifostine prior to exposure of mice to ionizing radiation.