

## **Apoptosis as a mechanism for low dose radiation- and amifostine-mediated chromosomal inversion responses**

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### **Abstract**

Low dose radiation and the chemical radioprotector amifostine have both been shown to protect cells from the immediate and delayed effects of radiation exposure. They display a number of distinct similarities including their ability to protect cells against radiation-induced DNA damage, radiation-induced cell death and metastases formation.

Amifostine, which protects cells from the toxic effects of ionizing radiation, has a broad range of activities including free radical scavenging, polyamine-like DNA binding, and induction of hypoxia and redox-regulated genes. Amifostine's ability to protect cells is often attributed to its free radical scavenging activity; however, amifostine can protect from radiation-induced *HPRT* mutations when administered after irradiation indicating that amifostine's anti-mutagenic properties are not solely due to antioxidant activity (Grdina et al., 2002). We previously demonstrated, using the pKZ1 mouse chromosomal assay, that low dose radiation can protect from radiation-induced chromosomal inversions also when delivered after high dose radiation exposure (Day et al., 2007). Subsequent studies of the effects of amifostine on the chromosomal inversion response have revealed further similarities with the effects of low dose radiation. We observed that both low dose radiation and amifostine induce a non-linear dose response for chromosomal inversions, can protect from endogenous inversions when administered singly and protect from radiation induced inversions when given in conjunction with high dose radiation (Hooker et al., 2009).

These results support the hypothesis that there are common mechanisms associated with both low dose radioprotective responses and amifostine-mediated cytoprotection. We hypothesised that the non-linear dose response for chromosomal inversions induced by amifostine is mediated via changes in apoptosis frequency. An induction of apoptosis by amifostine could remove inversion-containing cells thereby reducing the overall inversion frequency while an inhibition of apoptosis could account for an increase in chromosomal inversion frequency.

Amifostine was administered intraperitoneally to mice either alone or in conjunction with 250 mGy X-rays (the US EPA maximum dose for radiation emergency workers). Mice were euthanized at various timepoints (4 – 72 hours) and spleen tissues analysed for apoptosis using the TUNEL method.

In the absence of radiation, amifostine increased apoptosis in the spleen of mice 4 h following administration of 100 mg/kg and 400 mg/kg amifostine, doses at which a reduction in chromosomal inversions had previously been observed. Amifostine delivered at 1 mg/kg, which causes an increase in chromosomal inversions and 10 mg/kg, which has no effect on inversions, did not alter the apoptosis frequency.

Irradiation with 250 mGy X-rays induced apoptosis in mouse spleen at 7 h and administration of amifostine (400 mg/kg) 3 h following 250 mGy X-rays potentiated the radiation-induced apoptosis response. However 1 mg/kg amifostine, which previously protected against the chromosomal inversions induced by 250 mGy, did not show a potentiation of apoptosis.

Thus the hypothesis that amifostine-mediated induction of apoptosis is responsible for the reduction in chromosomal inversions is supported at high doses of amifostine alone and in combination with 250 mGy X-radiation. However, no significant changes in apoptosis frequency were found at lower doses of amifostine to support this hypothesis.

These observations of amifostine potentiating radiation-induced apoptosis are contrary to published reports in the literature which attribute amifostine's cytoprotection to its inhibition of apoptotic cell death. Normal cells are considered to have a greater apoptotic threshold following amifostine treatment as a result of the active import of the disulfide form of amifostine (WR-33278) into the cell providing greater defence against oxidative stress (List and Gerner, 2000). Our results indicate that this is not true in spleen and may indicate that different tissues have different apoptosis responses to amifostine administration. We are currently analysing bone marrow samples from the same mice, a tissue which has previously been reported to be protected from radiation-induced apoptosis by amifostine.

These results suggest that amifostine may not be able to protect all tissues from radiation-induced apoptosis, and may have implications for the clinical use of amifostine where it is used to ameliorate the cytotoxic effects of high dose radiotherapy.

### **References**

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