Additive Radioprotection by the Combination of DNA Binding Antioxidants and Aminothiol Radical Scavengers

Jai Smith[1,2], Andrea Smith[1], Colin Skene[3], Jonathan M. White[3], Pavel N. Lobachevsky[1] and Roger F. Martin[1,2,3]

[1] *Peter MacCallum Cancer Centre, Melbourne, Australia.*
[2] *University of Melbourne, The Sir Peter MacCallum Department of Oncology, Melbourne, Australia.*
[3] *School of Chemistry, University of Melbourne and Bio 21 Institute, Melbourne, Australia*

Despite improvements in the targeting of radiation for radiotherapy some normal tissue is unavoidably irradiated. During head and neck radiotherapy the irradiated normal tissue often includes the radiosensitive oral mucosa that can result in serious dose limiting side effects such as oral mucositis.

The need for pharmacological radioprotection of normal tissue during radiotherapy has long been recognised by radiation biologists. The aminothiol radical scavenger amifostine (and its parent WR1065) was the first clinically approved radioprotector for this purpose. M2PB is a much newer radioprotector that protects via a unique mechanism. M2PB binds in the minor groove of DNA facilitating electron transfer from the ligand to the DNA molecule. As a result, M2PB acts as a reducing agent of transient radiation induced oxidising species formed on the DNA.

Additive radioprotection has been observed *in vitro* using the clonogenic survival endpoint; WR1065 alone gave a DMF of 2.5, M2PB gave a DMF of 2.8 and a combination of the two drugs resulted in a DMF of 4.4. Similar results have also been obtained for WR1065 with 2PH and Methylproamine (two related analogues of M2PB). *In vivo* radioprotection using the Withers micro colony survival assay has also been reported for M2PB (DMF=1.21), amifostine (DMF=1.13) and the drugs in combination (DMF=1.49).

In addition, additive radioprotection by M2PB (70 mg/kg SC) and amifostine (175 mg/kg IP) in the mouse oral mucosa ulceration endpoint will be reported. A 3mm by 3mm area on the dorsal surface of the mouse tongue was irradiated with 25 KeV X-rays (20 Gy) either with or without the drug treatment. The appearance and severity of radiation induced ulcers was quantified as the endpoint for this assay. The combination of the two drugs given systemically is not well tolerated, necessitating a reduction of the doses compared to experiments when either drug is used alone. Accordingly, pre-treatment with amifostine or M2PB alone did not result in a significant reduction in the formation of ulcers. However, a clear reduction in the severity of the ulcers was observed when the drugs were used in combination. The mice treated with the combination had a 50% reduction in ulcer formation and an 80% reduction in the formation of severe ulcers compared to the radiation only control. This result demonstrates additive radioprotection by the combination of DNA binding antioxidants and aminothiol radical scavengers in a clinically relevant endpoint.