Prevention of Low Dose Radiation-Induced Genomic Instability with Clinically Relevant Non-Protein Thiols and Vitamin E. J.S. Murley[1] , Y. Kataoka[1] , W.F. Morgan[2] , and D.J. Grdina[1]. *The University of Chicago, Chicago, IL[1], The University of Maryland Medical School, Baltimore, MD[2]*

Induced or delayed radioprotection is a novel phenomenon that shares many similarities with the low dose radiation-induced radiobiological phenomenon referred to as the adaptive response. Induced or delayed radioprotection is defined as an enhancement in the radiation resistance of cells at long times following their exposure to non-protein thiols (NPT) such as WR1065, the free thiol form of amifostine. This effect is the result of the induction of a cascade of intracellular molecular events leading to an elevation in the levels of endogenous anti-oxidant molecules. The precise mechanism of action involves the activation of nuclear transcription factor κB (NFκB) as a result of the SH-mediated alteration of the redox state of cysteine residues on its p50 and p65 subunits. Following NFκB activation only a limited number of genes having NFκB binding motifs are activated (Kataoka et al., 2002), with the most responsive gene being manganese superoxide dismutase (*MnSOD*) (Guo et al., 2003). MnSOD is an inducible anti-oxidant enzyme localized in the mitochondria that protects against oxidative damage caused by reactive oxygen species generated not only by normal aerobic metabolism but also by ionizing radiation (Murley et al., 2004; Murley et al., 2006). We have in our study tested the hypothesis that human RKO-derived cells stably transfected with a green fluorescent protein (GFP) and designated RKO36 when exposed to both low or high doses of the NPT WR1065 will experience a subsequent elevation in intracellular levels of MnSOD, and if challenged at that time with a 2 or 4Gy dose of radiation will exhibit an enhanced radiation resistance similar to that observed in cells expressing the classical radiation-induced adaptive response. RKO36 cells were grown to confluence and were first evaluated as a function of their GFP fluorescence state with regards to γH2AX formation following a dose of 4 Gy. Both GFP+ and GFP- cells responded similarly. RKO36 cells were then exposed for thirty min to either a 40 μM or 4 mM dose of WR1065. Aliquots of cells were sampled at time intervals from 4 to 32 h post WR1065 exposure to determine the relative levels of intracellular anti-oxidant proteins MnSOD, catalase, and glutathione peroxidase (GPx) by Western blot analysis. Both GPx protein levels and enzymatic activity remained level

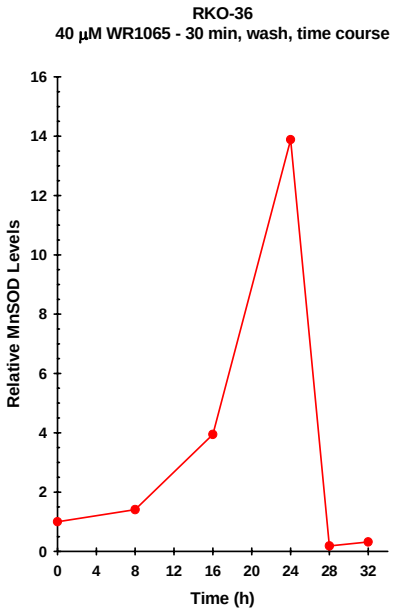

Figure 1

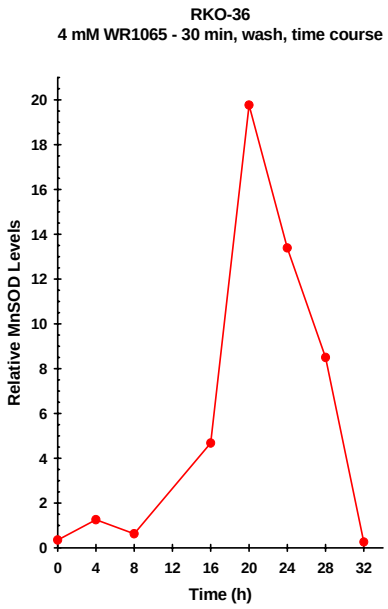

Figure 2

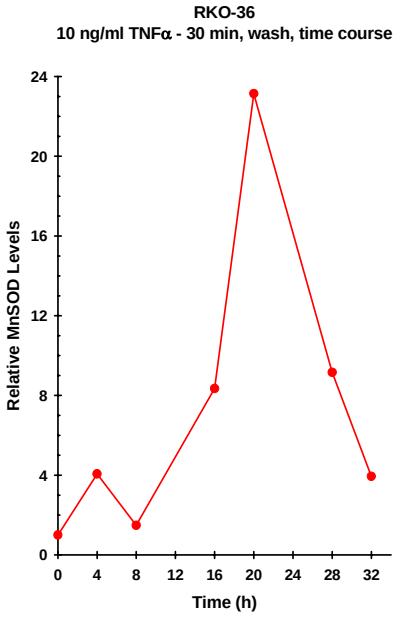

Figure 3

over this period of time. However, MnSOD levels increased as a function of time, regardless of the dose of WR1065 used, reaching 15- to 20-fold elevated levels 24 h after drug treatment and then falling to background levels by 32 h (see Fig. 1 and 2). Tumor necrosis factor-alpha (TNF-α), a known inducer of MnSOD, was used as a control for comparative purposes and was observed to induce a similar kinetic response as observed for WR1065 (see Fig. 3). Irradiation studies were performed under two separate conditions to test both the classical immediate radiation response of cells to WR1065 exposure as well as the induced or delayed radiation response. RKO36 cells were exposed to either the non-cytoprotective dose of 40 µM WR1065 or to the standard cytoprotective dose of 4 mM 30 min prior to irradiation with 2 Gy. Following irradiation cells were washed free of WR1065, counted, and plated to determine surviving fractions. Parallel experiments were performed on cells not exposed to WR1065 to assess the relative protective effects of these two doses of WR1065. The results are shown in Fig. 4. Only the dose of 4 mM was found to be cytoprotective under these conditions. In contrast, if RKO36 cells were exposed to either dose of WR1065 for 30 min and then washed free and incubated for an additional 24 h to allow for the maximum elevation in intracellular MnSOD levels to occur before exposure to 2 Gy of radiation, cellular resistance was significantly enhanced, as evidenced by about a 40% increase in the respective surviving fractions (see Fig. 5). Cells expressing elevated levels of MnSOD 24 h following exposure to10 ng/ml TNFα responded in a similar fashion (see Fig. 6). The ability to induce a delayed enhanced radiation resistance in cells following exposure to NPT appears to parallel the enhanced radiation resistance induced in

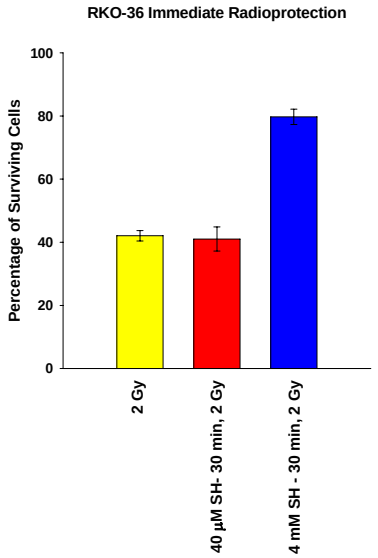

Figure 4

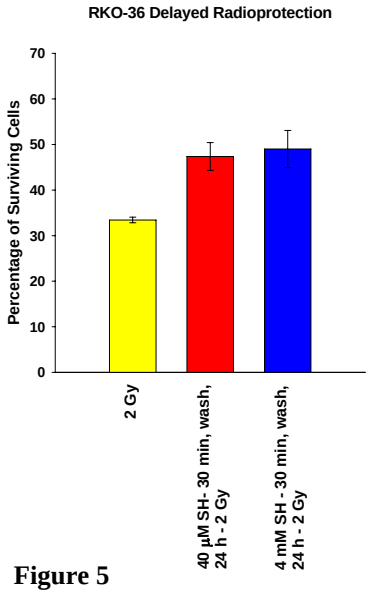

Figure 5

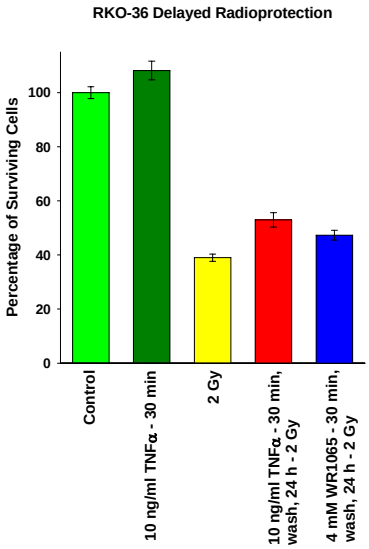

Figure 6

certain cells following an exposure to a low dose of radiation, eg, the adaptive response. These data support not only our hypothesis that endogenous radioprotective molecules such as MnSOD can be induced by very low doses of NPT leading to enhanced cellular radioresistance, but that NPT may be useful probes with which to better study and characterize molecular processes underlying the low radiation dose induced adaptive response.

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Murley JS et al. (2004) Radiat Res 162:536-546.
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