A Novel Radiomitigating Medical Countermeasure against Acute High Dose Ionizing Radiation Exposure using a Designer Cerium Oxide Nanozyme and P7C3

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Purpose: Exposure to acute levels of ionizing radiation (IR) causes cellular harm and eventually leads to multi-organ dysfunction and acute radiation syndrome. Currently, there is no effective countermeasure. We discovered that acute IR-induced bone toxicity in rats can be protected after pretreatment with either cerium oxide nanoparticles (CeONPs) engineered with increased (↑)Ce^{3+} *vs.* Ce^{4+} surface sites, or the aminopropyl carbazole, P7C3. ↑Ce^{3+}-CeONPs also protected nontargeted organs and key blood cells. A question is whether there is a synergistic effect. We explored whether this innovative strategy abrogates damage when given 24 and 48 h post-IR *in vitro*.

Methods: Human bone marrow-derived mesenchymal stromal cells (hBMSCs) and macrophages (RAW 264.7) were subjected to ± a single 7 Gy dose and supplemented with ↑Ce^{3+}-CeONPs, P7C3, or a combo, 24 or 48 h post-IR. Metabolic activity and morphology were examined. Osteogenesis was evaluated *via* alkaline phosphatase (ALP) and alizarin red staining, DNA damage, a human bone metabolism, and TGF-β pathway phosphorylation array (±↑Ce^{3+}-CeONPs or P7C3) were investigated. Adipogenic differentiation was assessed. Multinucleated giant cell (MNGC) formation and macrophage proinflammatory (*IL-1β* and *IL-6*) and osteoclast expression (*RANKL* and *CTSK*) were assessed using qRT-PCR. Data were compared (Mann-Whitney U, GraphPad Prism, v8.0). *p* values <0.05 were considered significant.

Results: ↑Ce^{3+}-CeONPs, P7C3 or their combo exhibited no non-IR toxicity. After IR, ↑Ce^{3+}-CeONPs, P7C3 or their combo showed a minimal mitigating effect to metabolic activity. However, hBMSCs+IR with ↑Ce^{3+}-CeONPs or in combo, significantly reduced DNA damage (p<0.0001 all groups (AG)). Similarly, ↑Ce^{3+}-CeONPs, P7C3 (both >4-fold), and the combo (>6-fold) resulted in increased ALP formation and mineralization compared to control, indicating enhanced osteogenesis (p<0.0001 AG). hBMSCs+P7C3 or the combo reduced IR-induced adipogenesis (>90%, p<0.0001AG). IR increased MNGC formation, *IL-1β*, *IL-6*, *RANKL*, and *CTSK* expression while ↑Ce^{3+}-CeONPs, P7C3, or the combo significantly decreased MNGC and expression >2-fold despite IR (p<0.0001 AG). GO enrichment showed P7C3 upregulated T cell activity while ↑Ce^{3+}-CeONPs augmented angiogenesis. Both therapeutics utilized TGFβ signaling but *via* differing proteins.

Conclusions: ↑Ce^{3+}-CeONPs and P7C3 could serve as novel multifunctional radiomitigators. Further *in vivo* analyses are warranted.