

Ex-RAD[®] (ON 01210.Na)

PRESENTATIONS AT THE 56TH ANNUAL MEETING OF THE RADIATION RESEARCH SOCIETY

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- Maniar M, Kumar R, Moon B-H, Taft DR, Datta K (2010) Radioprotection and radiomitigation properties of Ex-RAD[™] upon oral administration. Radiation Soc. Annual Mtg: Abstract #MS701 (Oral presentation & Poster).
- Ghosh SP, Kulkarni S, Perkins MW, Gambles K, Hieber K, Maniar M, Seed TM, Kumar KS (2010) Recovery from radiation-induced hematopoietic and gastrointestinal sub-syndromes by Ex-RAD[™] in murine model. Radiation Soc Annual Mtg: Abstract #PS1.10.
- Kang AD, Cosenza SC, Reddy MVR, Reddy EP (2010) Radioprotection of human bone marrow by ON 01210.Na (Ex-RAD[™]) through AKT mediated signaling pathway. Radiation Soc. Annual Mtg: Abstract #258.
- Ren C, Tamhane M, Fegley GJ, Taft DR, Maniar M (2010) Metabolic disposition of Ex-RAD[™] (ON 01210.Na), a novel radioprotectant. Radiation Soc. Annual Mtg: Abstract #379.
- Ren C, Tamhane M, Taft DR, Maniar M (2010) Disposition of Ex-RAD[™] (ON 01210.Na), a new radioprotectant, in the isolated perfused rat liver model. Radiation Soc. Annual Mtg: Abstract #531.

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Poster Session: Radiation Protection – Protection / Mitigators / Treatment (PS1.56)
Mini-symposium MS7: Wednesday, September 29, 2010; 10:15AM-12:15PM (MS701)

Radioprotection and Radiomitigation Properties of Ex-RAD™ Upon Oral Administration

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Onconova Therapeutics has discovered a novel small molecule kinase inhibitor, Ex-RAD™, which has demonstrated significant protection against radiation damage at lethal doses of radiation *in vitro* and *in vivo*, with remarkable margin of safety. At present, Ex-RAD™ is being developed as a subcutaneous injectable product for first responders for prophylactic use. Onconova has completed several clinical trials with subcutaneous (SC) administration of drug. The oral route of administration of Ex-RAD™ would provide significant benefit of use in civilian population especially children and the elderly. Preclinical pharmacokinetics studies in rats, dogs, rabbits and monkeys reveal that Ex-RAD™ is well-absorbed, and has a relative bioavailability ranging from 47 to 91%. Here we report effectiveness of Ex-RAD™ upon oral administration in a mouse whole body irradiation (WBI) model.

Methods: Ex-RAD™ was administered SC or orally (PO) to 6-8 weeks old male C3H/Hen or female C57BL/6J mice (N=10) at 24h and 15 minutes prior to 7.5 Gy whole body irradiation (WBI). C3H/Hen mice (N=10) were also dosed 24h and 36h after WBI. Irradiation was done using a ¹³⁷Cs source. Survival was monitored for 30 days.

Results: We saw significant survival advantage with Ex-RAD™ administered pre- and post-radiation. Radioprotection results were similar with PO and SC administration of Ex-RAD™ at 500 mg/Kg. Significant radioprotection was also observed with lower oral dose (200 mg/Kg) of Ex-RAD™. Oral formulation (500 mg/Kg), when administered +24h and +36h after radiation, showed 90% survival compared to 50% in vehicle administered group.

Conclusions: Ex-RAD™ is equally effective when administered either orally or subcutaneously. Orally administered Ex-RAD™ offers the benefit of convenient dosing options to the civilian population for either prophylaxis or the treatment of Acute Radiation Syndrome.

Recovery from radiation-induced hematopoietic and gastrointestinal sub-syndromes by Ex-RAD™ in a murine model

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We tested the efficacy of a new chemical entity, ON 01210.Na (Ex-RAD™) in collaboration with Onconova Therapeutics to protect mice from radiation injury. Exposure to total body irradiation (TBI) results in hematopoietic and gastrointestinal (GI) sub-syndromes depending on the radiation dose. We reported that administration of Ex-RAD™ 24 h and 15 min before TBI protected mice from lethality with a dose reduction factor of 1.16. Ex-RAD™ accelerated recovery of peripheral blood elements in sublethally irradiated mice. In this study, we report amelioration of radiation-induced bone marrow suppression, protection of GI crypt cells, and inhibition of p53-mediated apoptosis in spleen.

C3H/HeN mice were injected subcutaneously with various doses of Ex-RAD™ 24 h and 15 min prior to radiation (7 Gy @ 0.6 Gy/min). Bone marrow cells were isolated on 4, 8, and 14 days post-radiation and granulocyte macrophage colony forming units (GM-CFUs) were found to be maintained at higher levels in drug-treated relative to vehicle-treated controls (*p<0.01). Crypt cell analysis was performed to assess GI injury in irradiated mice (exposed to 13 and 14 Gy). Numbers of crypts regenerated per circumference in the jejunum of mice treated with drug were significantly higher compared to vehicle alone on 12 h and 3.5 days post-radiation (*p<0.001). Expression of phosphorylated p53 was down-regulated in spleen from mice irradiated and treated with Ex-RAD™.

These data suggest that the mechanisms of radiation protection by Ex-RAD™ involve protection from hematopoietic and gastrointestinal sub-syndromes and down-regulation of phosphorylated p53.

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Session: Experimental therapeutics and translational research

**Radioprotection of human bone marrow by ON 01210.Na (Ex-RAD™)
through AKT-mediated signaling pathway**

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Background: Limited availability of non-toxic radioprotective agents prompted us to identify a small molecule compound ON01210.Na (Ex-RAD) from our small molecule chemical library using a high throughput cell based assay screening system. If successfully evaluated, this radioprotective agent could be used by military personnel and civilians during a nuclear-radiological disaster or radiation therapy. ON01210.Na is a novel benzyl styryl sulfone that protects mice from ionizing radiation through the prevention of radiation-induced DNA damage and inhibition of apoptosis. The purpose of the current studies was to evaluate the radioprotective efficacy of the drug in human bone marrow cells and identify the mechanism of protection.

Methods: Human bone marrows (hBM) cells were grown in methylcellulose semi-solid medium and colony forming units (CFU) determined after treatment with various doses of ON01210.Na and ionizing radiation. Toxicity of the drug was determined following 2 and 24 hr of incubation in the presence of 0 to 50uM of the drug. Efficacy of various concentrations of the drug was tested after exposure of cells to 2-4 Gy and was compared with amifostine for its dose reduction factor (DRF) and toxicity. An antibody array was used to identify the signaling pathway responsible for radioprotection.

Results and Discussion: Treatment with varying doses of ON01210.Na for 2 and 24 hr did not inhibit the growth and differentiation of normal hBM. The drug radioprotected normal hBM cells irradiated with 2, 3, and 4 Gy and had a DRF of 1.6. This DRF is comparable to FDA-approved, amifostine. Incubation of lysates from vehicle or drug treated HFL-1 cells to the Phospho-MAPK Array comprising of 21 different anti-kinase antibodies and 7 different controls printed in duplicate showed that ON01210.Na activates the phosphorylation of AKT and GSK3 α/β following irradiation. Activation of AKT and inhibition of GSK3 β has been directly correlated with cytoprotection from ionizing radiation. Thus it was concluded that ON01210.Na is a safe and non-toxic alternative radioprotective drug with a novel mechanism of action by activating the AKT signaling survival pathway in the presence of radiation.

Session: Experimental therapeutics and translational research

**Metabolic disposition of Ex-RAD™ (ON 01210.Na),
a novel radioprotectant**

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ABSTRACT

Ex-RAD™ (ON 01210.Na), sodium salt of a benzyl styrylsulfone, is being developed by Onconova Therapeutics as a novel radiation protection agent. Ex-RAD™ has completed two Phase I clinical safety trials under an Investigational New Drug (IND) exemption. Initial development, in collaboration with the Armed Forces Radiobiology Research Institute (AFRRI), is focused on its use as a prophylactic agent. We have characterized the metabolic disposition of this drug in various species as part of the regulatory submission process for approval of this drug under the “Animal Rule,” which applies to drugs that are developed without human efficacy studies. We investigated the metabolic fate of Ex-RAD™ in *in vitro* and *in vivo* systems. A major metabolite, glutathione conjugate (1210-GSH), was identified *in vitro* using hepatocytes from various species. In mice dosed subcutaneously with ¹⁴C-labelled ON 01210.Na, five additional metabolites were identified by HPLC/RAD and mass spectrometry, tentatively assigned as ON 01210-cysteine (1210-Cys), ON 01210-(N)-acetylcysteine (1210-NAC), ON 01210-thiol (1210-SH), ON 01210-unsaturated thiol (1210-uSH) and ON 01210-methylsulfide (1210-SCH₃). The formation of these metabolites potentially involves two pathways. Initially, ON 01210.Na undergoes Michael addition with glutathione, catalyzed by glutathione S-transferase. Subsequently, 1210-GSH is further metabolized to 1210-Cys via the mercapturic acid pathway; this key metabolite thus forms thiols and methyl sulfides through the cysteine s-conjugate β-lyase pathway. An LC-MS/MS method was optimized to monitor the levels of all six metabolites and ON 01210. The same set of metabolites was also monitored in the plasma of rats following oral and IV administration of Ex-RAD™ at various doses. All of these metabolites except 1210-SH were detected in rat plasma. ON 01210.Na follows nonlinear pharmacokinetics, especially at higher doses. The results indicate that the metabolic pathway of Ex-RAD™ is conserved from mouse to rat, and the same metabolic disposition pattern appears irrespective of the route of administration. These results and ongoing studies will help support the regulatory approval for this novel radioprotectant.

Key words: Ex-RAD™, radiation, glutathione transferase, metabolites

Session: Experimental therapeutics and translational research

Disposition of Ex-RAD™ (ON 01210.Na), a new radioprotectant, in the isolated perfused rat liver model

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ABSTRACT

Ex-RAD™ (ON 01210.Na) is a promising new radioprotective treatment being developed by Onconova Therapeutics, Inc., in collaboration with the Armed Forces Radiobiology Research Institute (AFRRI) for prophylactic or therapeutic use. The compound has been shown to be non-toxic and effective in increasing survival in cellular, tissue and animal radiation models by enhancing cell survival and DNA repair mechanisms. Ex-RAD™ is currently in Phase I clinical development, and is targeted for use by emergency first responders. Ex-RAD™ demonstrates rapid clearance from plasma in rats following IV dosing. A major metabolite, glutathione conjugate (1210-GSH), was identified *in vitro* using hepatocytes. In this study, the disposition of Ex-RAD™ was evaluated in the isolated perfused rat liver (IPRL) model. The aim of the research was to assess the dose-linearity of Ex-RAD™ disposition in the IPRL, and to evaluate the rate of formation of 1210-GSH in the model. Perfusion experiments (n=3/group) were performed at 4 doses (0.8, 4, 8, 20 mg), targeting a range of perfusate levels between 10 and 250 µg/ml. Perfusate binding was measured by ultrafiltration. Ex-RAD™ was assayed in perfusate by HPLC and in bile using LC/MS/MS. Non-compartmental analysis was used to determine pharmacokinetic parameters. The protein binding results demonstrated high binding to perfusate proteins (88-95%) over the doses studied. Ex-RAD™ displayed nonlinear disposition in the IPRL. The increase in AUC was disproportional at higher doses and consequently, the clearance decreased almost 2-fold at dose 20 mg. The half-life of ON 01210.Na was on an average 14 minutes at lower doses and increased to ~40 minutes at the high dose of 20 mg. The biliary clearance was found to be very low (0.01- 0.019 mL/min) and a very high abundance of the metabolite 1210-GSH was detected in the bile, which indicated a rapid degradation of Ex-RAD™. The results presented here demonstrate that IPRL model is a useful tool in studying the role of the liver in the kinetics and metabolism of Ex-RAD™. A better understanding of how Ex-RAD™ is taken up, metabolized and excreted by the liver will help to predict *in vivo* kinetics of the drug under various routes of drug administration.

Key words: Ex-RAD™, isolated perfused rat liver, glutathione