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Effect of ethacrynic acid (EA) on the pharmacokinetics of Ex-RAD[®] (ON 01210.Na) in the isolated perfused rat liver model (IPRL)

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ABSTRACT

Ex-RAD[®] (ON 01210.Na), a novel benzyl styryl sulfone analog being developed as a promising new radioprotectant by Onconova Therapeutics Inc., has completed two Phase I clinical safety trials under an Investigational New Drug (IND) exemption. ON 01210.Na has demonstrated increased survival in cellular, tissue and animal radiation models. Its virtual lack of side effects at effective dosage makes ON 01210.Na an attractive candidate both as a prophylactic agent and as a therapeutic treatment for mitigation by enhancing cell survival and DNA repair mechanisms. Previous studies found that ON 01210.Na was extensively metabolized to a glutathione (GSH) conjugate *in vitro* and *in vivo* by glutathione-S-transferase (GST). The objective of this study was to determine the hepatobiliary disposition of Ex-RAD[®] in the isolated perfused rat liver (IPRL) model and to examine the effect of co-administration of ethacrynic acid (EA), a prototype GST inhibitor, on the pharmacokinetics of ON 01210.Na in the model. ON 01210.Na showed non-linear pharmacokinetics in dose escalation studies in the IPRL (target concentrations 10, 50, 100, 250 µg/mL) with clearance decreasing from 3.14 to 1.99 mL/min with increasing dose. The glutathione adduct metabolite of ON 01210.Na formed in liver was mainly excreted into the bile. Less than 1% of the parent drug was recovered from IPRL perfusate at lower doses (target concentrations < 100 µg/mL), but total recovery increased 10-fold at the highest dose tested (250 µg/mL). Perfusate recovery increased from 9.5% to 54% when EA (1mM) was co-administered with ON 01210.Na (250 µg/mL), and clearance was reduced by almost 5-fold. The results suggest that EA co-administration could significantly inhibit the conversion of ON 01210.Na to its glutathione conjugate in rats, resulting in decreased clearance, prolonged half-life and greater systemic exposure of ON 01210.Na. Accordingly, combination treatment with EA could potentially help achieve and sustain effective plasma levels of ON 01210.Na at a fraction of the dose by altering its metabolic profile, which in turn could open avenues for administering ON 01210.Na by extravascular routes thus enhancing patient compliance.

Key words: Ex-RAD[®], isolated perfused rat liver, glutathione-S-transferase inhibitor, metabolism, clearance

ON 01210.Na induces SLUG pathway to mitigate radiation-induced hematopoietic toxicity in mice

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Introduction: Unpredictable radiation exposure could occur due to either accident or terrorism related intentional incidents. Therefore, developing agents, which could block or minimize radiation toxicity after exposure is essential. Radiation at doses <8 Gy predominantly affects hematopoietic cells especially the bone marrow cells. Thus mitigators of radiation damages to the hematopoietic cells could be a life saving intervention. This study evaluates effectiveness of a new small molecule, ON 01210.Na, as a mitigator of radiation toxicity in hematopoietic cells.

Methods: Two doses of ON 01210.Na (500 mg/Kg) were administered by subcutaneous route to 6-8 weeks old male C3H/He mice (n=14) at 24h and 36h after 7.5 Gy of radiation from a ¹³⁷-Cs source. Vehicle group received equal volume of the vehicle solution. Survival was monitored for 30 days. Hematopoietic system was studied in these mice (n=5) after a sub-lethal dose of 5 Gy while keeping drug dosing the same. Complete peripheral blood count was performed along with bone marrow granulocyte-macrophage colony forming unit (GM-CFU), TUNEL assay, western blot, and Q-PCR analysis.

Results: Significant (p<0.003 compared to vehicle) survival advantage (80% with drug and 20% with vehicle) was observed after 7.5 Gy radiation. Peripheral white blood cell and platelet counts were higher in ON 01210.Na than vehicle treated mice. Increased GM-CFU along with reduced TUNEL positive cells in spleen and bone marrow were observed in drug treated mice. At the molecular level we observed downregulation of p53, Bax, p21, and upregulation of Mn-SOD, Bcl2, and NFκB in bone marrow cells.

Conclusions: In conclusion, ON 01210.Na administered at 24h and 36h post-irradiation could significantly mitigate the radiation toxicity leading to enhanced survival. Quantitative hematopoietic data evidently supports our survival results. Molecular pathway analysis indicates involvement of SLUG, a known hematopoietic stem cell survival factor, in ON 01210.Na mediated radiation mitigation. Furthermore, ON 01210.Na caused diminution of apoptotic and boosting of survival signals to reinforce mitigation effects.

**Prophylactic and therapeutic efficacy of a new formulation of
ON 01210.Na (Ex-RAD[®]) after total body radiation in mouse model**

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ON 01210 (Ex-RAD[®]), a novel small molecule kinase inhibitor, significantly protected mice against lethal gamma irradiation when administered subcutaneously (SC) 24 h and 15 min before irradiation at a dose of 500 mg/kg of body weight. The suspension formulation of Ex-RAD induced increases in GM-CFU colony and regeneration of gastrointestinal (GI) crypt cells post-irradiation, and prevented mice from radiation-induced cytopenia. We have investigated radioprotective efficacy (both prophylactic and therapeutic) with the new improved solution formulation in male C3H/HeN mice. Ex-RAD (500 mg/kg) improved survival by 60% compared to vehicle in mice irradiated to 8 Gy when administered SC 24 h and 15 min prior to irradiation. When mice were irradiated (8 Gy, 0.6 Gy/min) and treated with Ex-RAD (500 mg/kg) SC 4 and 24 h after total body irradiation (TBI), 50% improved survival was observed. Ex-RAD-treated irradiated animals maintained higher numbers of CFUs in bone marrow harvested from sublethally irradiated mice (6 Gy), indicating increased self renewing capacity of HSCs. Histopathology of sternal bone marrow indicated more regenerative microfoci for myeloid, erythroid and megacaryocytes and higher overall cellularity in Ex-RAD-treated mice compared to vehicle controls at days 7 and 14 after TBI (6 Gy).

Our results demonstrate that the new improved formulation of Ex-RAD shows efficacy given SC either before or after radiation. Ex-RAD treatment also protected hematopoietic tissue by preserving HSCs and HPCs.