

An Agonist of Toll-Like Receptor 5 Has Radioprotective Activity in Mouse and Primate Models

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Poster by Stéphanie Häuselmann

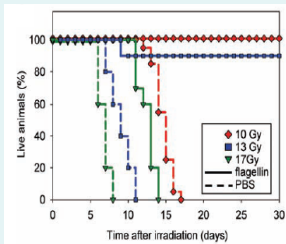
Introduction

Radiation therapy is widely used to treat malignant tumors. However, exposure to high-dose ionizing radiation (IR) leads to acute genotoxic stress for the hematopoietic (HP) system and the gastrointestinal (GI) tract, determining the adverse side effects of anticancer radiation therapy.

In this study, Burdelya *et al.* investigated whether radioprotection can be achieved through suppression of apoptosis, which is the major mechanism underlying massive cell loss in the radiosensitive tissues. For this purpose, the authors aimed to induce an antiapoptotic mechanism often acquired by tumor cells: the activation of the nuclear factor- κ B (NF- κ B), by stimulating Toll-like receptor (TLR) signaling. Indeed, TLR signaling triggered by commensal microflora plays a protective role in the GI tract and the resulting activation of NF- κ B does not induce acute inflammatory responses.

TLR5 was focused on as it is expressed on enterocytes, dendritic cells, and endothelial cells of the small intestine lamina propria.

TLR5 Agonist Bacterial Flagellin Has *in vivo* Radioprotective Activity

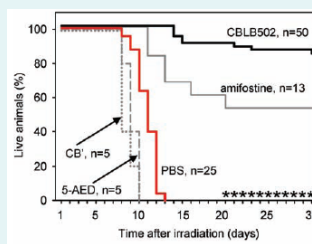


Flagellin purified from *Salmonella enterica* serovar Dublin is injected into NIH-Swiss mice 30 min before total-body γ irradiation (TBI).

Mice treated with 0.2 mg/kg of body weight of flagellin were rescued from the IR doses of 10 and 13 Gy which induce mortality from both HP and GI acute radiation syndromes.

Flagellin prolonged the median survival of mice exposed to 17 Gy TBI from 7 to 12 days.

CBLB502, an Engineered Flagellin Derivative, Retains the Radioprotective Efficacy of Flagellin

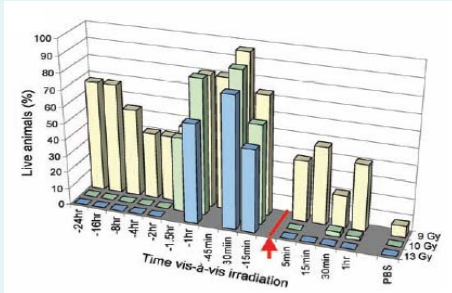


CBLB502, the most potent activator of NF- κ B among the different engineered flagellin derivatives tested, was administered by single injection (0.2 mg/kg) to NIH-Swiss mice 30 min before 13 Gy TBI.

More than 87% of the CBLB502-treated mice were rescued from radiation-induced death. This protective effect was significantly stronger than the one provided by amifostine, one of the most powerful previously described radioprotectant (54% of the mice treated with 150 mg/kg amifostine were rescued).

The dose used for CBLB502 was less than 1% of its maximum tolerated dose (MTD), in contrast to the dose used for amifostine which was close to its MTD (200 mg/kg).

CBLB502 is Effective as Both Protects Against and Mitigates Radiation-Induced Injury



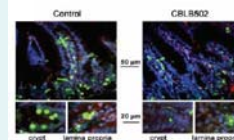
The time frame for effective administration of CBLB502 was investigated at different radiation doses.

Mice were protected against TBI-induced lethal HP or combined HP and GI syndromes (10 Gy and 13 Gy, respectively) only when treated with CBLB502 15 to 60 min before TBI.

However, at the lower dose of 9 Gy TBI, over 90% of the control mice were killed, whereas CBLB502 provided radioprotective benefits when administered as early as 24 hours before, or up to 1 hour after irradiation (40% of the treated mice were rescued).

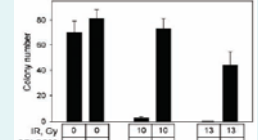
CBLB502 Provides an Antiapoptotic Effect in Both GI Tract and HP System

NIH-Swiss mice treated with 0.2 mg/kg CBLB502 30 min before 15 Gy TBI exhibited a dramatic reduction of the proportion of apoptotic cells in the lamina propria of the small intestine, including vascular endothelial cells.



Apoptotic endothelial cells displayed yellow fluorescence as a combination of green TUNEL staining and red endothelial marker (CD31-specific antibody) staining.

CBLB502 injection 1 hour before lethal TBI (10 or 13 Gy) protected NIH-Swiss mouse HP progenitor cells from apoptosis, as judged by preservation of the number of granulocyte/macrophage colony forming cells.

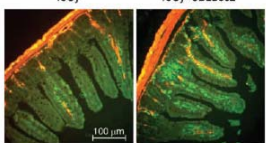
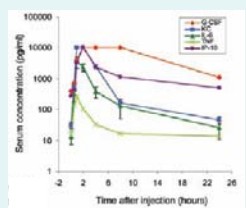


Granulocyte/macrophage colony forming units were quantified in bone marrow cells obtained from NIH-Swiss mice.

CBLB502-Mediated Radioprotection Involves Several NF- κ B-Responsive Factors

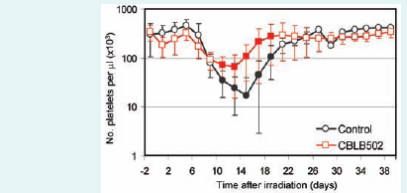
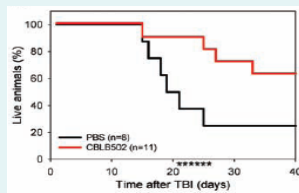
NIH-Swiss mice pretreated with CBLB502 (0.2 mg/kg) 30 min before 15 Gy TBI exhibited an enhanced expression of superoxide dismutase 2 (SOD2, green staining) in the lamina propria of the small intestine. SOD2 is a radioprotective antioxidant induced by NF- κ B.

CBLB502 injection (in the absence of irradiation) induced the release of multiple cytokines regulated by NF- κ B in mouse plasma.



A Pilot Study of Nonhuman Primates Confirms Effectiveness of CBLB502 as Radioprotectant

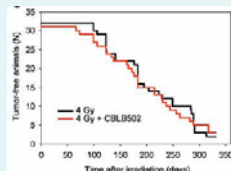
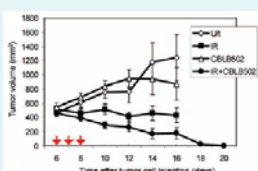
Rhesus monkeys (*Macaca mulatta*) were subjected to a single intramuscular injection of CBLB502 (0.04 mg/kg, resulting in a blood concentration of CBLB502 equivalent to 0.2 mg/kg in mice) 45 min before 6.5 Gy TBI (a dose lethal for 70% of monkeys). In CBLB502-treated monkeys, the onset of radiation-induced mortality was delayed by 10 days and the 40-day survival rate was increased from 25 to 64%. Thrombocytopenia (reduced platelet count), a key predictor of primate death after lethal irradiation, was less protracted and less severe in CBLB502-treated monkeys than in control monkeys.



CBLB502 Treatment Does Not Affect the Radiosensitivity of Tumors and Does Not Enhance the Carcinogenicity of Irradiation

Tumor-bearing mice were used as experimental radiotherapy model. They were subjected to 3 daily 4 Gy TBI (as radiotherapy is commonly applied as fractionated irradiation) and were treated 1 hour before each radiation with CBLB502 (0.2 mg/kg) or were untreated (U). CBLB502 protected mice from radiation-induced mortality (figure not shown) but did not interfere with the killing of the tumors (as judged by tumor volume).

In cancer-prone p53^{-/-} mice, 100% of which develop lymphomas and sarcomas within 1 year after sublethal 4 Gy TBI, CBLB502 treatment 30 min before TBI had no effect on the timing and frequency of tumor appearance.



Concluding Remarks

- > The radioprotective effect of CBLB502 demonstrated in this study suggests a potential application for this peptide as an adjuvant for anticancer radiotherapy.
- > Notably, CBLB502 reduces radiation toxicity in healthy tissue without compromising the therapeutic antitumor efficacy of radiation and without promoting radiation-induced carcinogenicity.
- > The mechanisms underlying the differential radioprotective effect of CBLB502 in healthy tissues versus tumors remain unclear. The authors suggest that it could be due to the constitutive activation of NF- κ B in cancer cells and/or inhibition of downstream TLR5 signaling by the activated phosphatidylinositol-3 kinase present in many tumors.
- > In a recent study, Rhee *et al.* (Gastroenterology, 2008) showed that TLR5 activation by peritumoral flagellin treatment substantially increased tumor necrosis, leading to significant tumor regression in a mouse xenograft model of human colon cancer. Could then CBLB502-dependent activation of TLR5 signaling have an antitumor effect *per se*? This would potentially increase the efficacy of anticancer therapy.
- > CBLB502 could also have a biodefense application as a protectant or mitigator for radiation emergencies.