Abstract

Advancing radiotherapy requires improving the therapeutic ratio by minimizing normal tissue toxicity while maximizing tumor control. Preclinical evidence shows that ultra-high dose-rate (UHDR) irradiation represents a promising strategy to achieve this goal. UHDR spares normal tissue while maintaining tumor control. This phenomenon is named the FLASH effect. However, the mechanisms driving the FLASH effect remain incompletely understood. This thesis integrates radiation chemistry, radiobiology, computational modeling, and translational veterinary implementation to elucidate fundamental drivers of UHDR-induced tissue sparing and to establish the groundwork for clinical translation via analysis of clinical fractionation's impact on the FLASH effect.

In vitro radiochemical studies using electron and proton UHDR beams revealed that key radiolysis products, such as hydrogen peroxide, non-hydroxyl radicals, and oxygen consumption, are consistently reduced under UHDR conditions compared to conventional dose rates (CDR), in both aqueous and protein rich environments. These findings indicate that UHDR modifies radiation chemistry in a manner that is beamline-dependent and sensitive to oxygen tension. Despite these substantial radiochemical differences, plasmid DNA assays demonstrated no significant dose-rate dependence in DNA strand break formation, suggesting that downstream biochemical processes or tissue level factors mediate the FLASH effect. A complementary physicochemical modeling framework was developed to interrogate radical reaction kinetics under varying dose-rates and oxygenation conditions. These simulations revealed that radical–radical recombination

contributes minimally to UHDR associated *in vitro* chemistry, while dose-rate dependent modulation of peroxyl-forming reactions represent a more plausible contributor to tissue sparing.

In vivo studies of acute murine skin toxicity demonstrated a robust FLASH sparing at single high dose delivery; however daily fractionation was found to suppress the FLASH effect. Building on these findings, a blinded, two-arm, randomized veterinary clinical trial was designed and implemented to compare UHDR and CDR electron therapy in dogs with appendicular osteosarcoma. A comprehensive UHDR dosimetry workflow was developed, incorporating active and passive dosimeters with real time charge monitoring on a UHDR enabled Mobetron system. Early results demonstrate safe delivery, stable beam performance, and the feasibility of large animal dose escalation studies essential for future human translation.

Together, these studies provide a multi-scale examination of the FLASH effect, spanning fundamental radiochemistry to translational radiobiology and clinical workflow development. By linking dose-rate dependent chemical alterations, oxygen mediated biological responses, and practical considerations for clinical implementation, this work establishes a mechanistic and operational foundation for optimizing UHDR delivery in future preclinical and clinical radiotherapy applications.