Role of plasminogen activator inhibitor type-1 in radiation-induced endothelial cell apoptosis.

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Normal tissue toxicity still remains a dose-limiting factor in clinical radiation therapy. Intestinal radiation toxicity is characterized by mucosal injury, inflammation, vascular activation followed by development of progressive vascular fibrosis/sclerosis and radiation enteritis. The endothelium is known to play a critical role in radiation-induced intestinal injury. Previous studies showed that endothelial cell (EC) apoptosis plays a central role in early radiation-induced intestinal injury. Recently, we demonstrated that plasminogen activator inhibitor type 1 (PAI-1) is an essential mediator of late intestinal radiation toxicity. PAI-1 knockout mice (PAI-1 -/-) are protected against intestinal radiation-induced damage with increased survival and better intestinal function compared with wild type mice (Wt). However, it is not clear whether PAI-1 plays a role in acute radiation-induced intestinal damages. We hypothesized that PAI-1 could contribute to the radiosensitivity of the endothelium in acute phases of radiation enteropathy. In vitro, irradiation stimulates PAI-1 expression (mRNA and protein) in EC 4 hours to 48 hours after irradiation. Moreover, FACS analyses (Sub-G1 and AnnexinV) and caspase assay showed that apoptosis is rapidly induced 4h to 24h after irradiation. These results suggest that PAI-1 could play a key role in radiation-induced EC cell death. To prove that PAI-1 is involved, molecular modulation of PAI-1 expression was performed using an expression vector and a RNA interference strategy. In EC overexpressing PAI-1 (pCMV PAI-1), radiation-induced apoptosis is increased 6h after 10Gy irradiation compared with EC transfected with a control vector. 48 hours after PAI-1 siRNA transfection, PAI-1 mRNA and protein levels are decreased by 85%. Sub-G1 and Annexin V flow cytometry analyses showed that radiation-induced apoptosis is decreased in siRNA-PAI-1 transfected EC. These preliminary results show that PAI-1 plays a role in the radiosensitivity of EC. To prove the relevance of our in vitro results, radiation-induced EC apoptosis in vivo was monitored in a model radiation enteropathy. After exposure of an intestinal segment to 19 Gy radiation, acute intestinal radiation injury are assessed in Wt and PAI-1 -/- mice 4 hours to 3 days after irradiation. Radiation injury score (RIS) is monitored and radiation-induced EC apoptosis is followed by double immunolabelling TUNEL or caspase-3 / CD31. RIS is reduced in PAI-1 -/- mice compared to Wt mice suggesting that acute EC apoptosis is reduced in PAI-1 -/- mice. This current and ongoing work should allow to determine the putative role of PAI-1 in radiation-induced endothelial cells apoptosis and consequently in intestinal radiation injury.

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