International Journal of
Radiation Oncology
biology • physics

www.redjournal.org

Biology Contribution

Irradiation Alters MMP-2/TIMP-2 System and Collagen Type IV Degradation in Brain

Won Hee Lee, M.S.,* Junie P. Warrington, B.A.,‡ William E. Sonntag, Ph.D.,‡ and Yong Woo Lee, Ph.D.*,†

**School of Biomedical Engineering and Sciences, †Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia; and ‡Reynolds Oklahoma Center on Aging, Department of Geriatric Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma*

Received Jul 30, 2010, and in revised form Dec 9, 2010. Accepted for publication Dec 15, 2010

Summary

The present study was designed to investigate the effects of whole-brain irradiation on matrix metalloproteinases (MMPs)/tissue inhibitors of metalloproteinases (TIMPs) and extracellular matrix (ECM) degradation in the brain. We demonstrated for the first time that a whole brain irradiation-induced imbalance between MMP-2 and TIMP-2 expression may have an important role in the pathogenesis of brain injury by degrading ECM components of the blood–brain barrier (BBB) basement membrane.

Purpose: Blood-brain barrier (BBB) disruption is one of the major consequences of radiation-induced normal tissue injury in the central nervous system. We examined the effects of whole-brain irradiation on matrix metalloproteinases (MMPs)/tissue inhibitors of metalloproteinases (TIMPs) and extracellular matrix (ECM) degradation in the brain.

Methods and Materials: Animals received either whole-brain irradiation (a single dose of 10 Gy γ-rays or a fractionated dose of 40 Gy γ-rays, total) or sham-irradiation and were maintained for 4, 8, and 24 h following irradiation. mRNA expression levels of MMPs and TIMPs in the brain were analyzed by real-time reverse transcriptase-polymerase chain reaction (PCR). The functional activity of MMPs was measured by *in situ* zymography, and degradation of ECM was visualized by collagen type IV immunofluorescent staining.

Results: A significant increase in mRNA expression levels of MMP-2, MMP-9, and TIMP-1 was observed in irradiated brains compared to that in sham-irradiated controls. *In situ* zymography revealed a strong gelatinolytic activity in the brain 24 h postirradiation, and the enhanced gelatinolytic activity mediated by irradiation was significantly attenuated in the presence of anti-MMP-2 antibody. A significant reduction in collagen type IV immunoreactivity was also detected in the brain at 24 h after irradiation. In contrast, the levels of collagen type IV were not significantly changed at 4 and 8 h after irradiation compared with the sham-irradiated controls.

Conclusions: The present study demonstrates for the first time that radiation induces an imbalance between MMP-2 and TIMP-2 levels and suggests that degradation of collagen type IV, a major ECM component of BBB basement membrane, may have a role in the pathogenesis of brain injury. © 2012 Elsevier Inc.

Keywords: Collagen type IV, Extracellular matrix, Matrix metalloproteinases, Tissue inhibitor of metalloproteinases, Whole-brain irradiation

Reprint requests to: Yong Woo Lee, Ph.D., Department of Biomedical Sciences and Pathobiology, School of Biomedical Engineering and Sciences, ICTAS Building (MC-0298), Virginia Tech, Stanger Street, Blacksburg, VA 24061. Tel: (540) 231-8484; Fax: (540) 231-9738; E-mail: ywlee@vt.edu

This project was supported by National Institute of Neurological Disorders and Stroke grant R01NS056218.

Conflict of interest: none.

Int J Radiation Oncol Biol Phys, Vol. 82, No. 5, pp. 1559–1566, 2012
0360-3016/$ - see front matter
doi:10.1016/j.ijrobp.2010.12.032

Introduction

Radiation therapy continues to be the main treatment modality in the therapeutic management of brain tumors (1). Approximately 200,000 individuals are treated with either partial large-field or whole-brain irradiation every year in the United States (2). The use of radiotherapy for treatment of brain tumors, however, is limited by the risk of radiation-induced injury to normal brain tissue, which can subsequently lead to devastating functional deficits several months to years after treatment (3). Recent randomized, prospective trials also provide evidence that the addition of whole-brain radiation therapy to stereotactic radiosurgery may cause a significant reduction in learning and memory in patients with brain metastasis (4). Presently, there is sparse information about the etiology of radiation-induced damage to normal tissue in brain.

Extracellular matrix (ECM) is a complex of various proteins and proteoglycans, including collagens, laminin, fibronectin, and tenascin, which constitute the basal lamina of the blood−brain barrier (BBB) (5). In addition to acting as a physical barrier to the passage of macromolecules and cells, ECM separates adjacent tissues, provides mechanical support for cell attachment, and serves as a substratum for cell migration and a medium of communication between cells (5). A number of studies have demonstrated that degradation and consequent rearrangement of ECM are critically involved in the breakdown of the BBB (6, 7). Despite a crucial role for ECM degradation in the BBB breakdown, the involvement of ECM remodeling in the pathophysiology of radiation-induced brain injury has not yet been investigated.

Matrix metalloproteinases (MMPs), a large family of ECM-degrading enzymes, have been implicated in the pathophysiological processes of neurodegenerative diseases by causing BBB disruption (8). The potential role of MMPs in brain injury in response to irradiation, however, remains largely unknown, whereas evidence demonstrates that MMPs are associated with radiation-induced damage to various other tissues (8−11).

In the present study, we examined the critical role of MMPs in radiation-induced ECM degradation in brain to define the molecular mechanisms of BBB disruption and subsequent brain injury by whole-brain irradiation. Our results provide the first novel evidence to demonstrate that MMP-2 plays a pivotal role in radiation-induced ECM degradation in brain.

Methods and Materials

Animals

Fisher 344-Brown Norway (F344×BN) male rats and C57BL/6 male mice were purchased from Harlan Laboratories (Indianapolis, IN) and Jackson Laboratory (Bar Harbor, ME), respectively. Animals were housed under a 12-h light:12-h dark cycle with food and water provided *ad libitum*. Animal care was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and this study was approved by the Institutional Animal Care and Use Committee.

Whole-brain irradiation and tissue sample preparation

A single-dose whole-brain irradiation procedure was carried out as described previously (12). Briefly, rats were anesthetized with ketamine-xylazine (intraperitoneal, 80−12 mg/kg) and received a single dose of 10 Gy (rate of 4.23 Gy/min) using a ^{137}Cs γ irradiator. Control rats were anesthetized but not irradiated. Animals were maintained for 4, 8, and 24 h postirradiation. For real-time reverse transcriptase-polymerase chain reaction (RT-PCR), rat brains were rapidly removed, and two different brain regions (hippocampus and cortex) were dissected and immediately frozen in liquid nitrogen. For immunofluorescent staining and *in situ* zymography, whole brains were rapidly removed after perfusion and immediately frozen in liquid nitrogen.

Fractionated whole-brain irradiation has been performed in mice as described previously (13). Briefly, mice were anesthetized with ketamine-xylazine (intraperitoneal, 100−15 mg/kg) and received a clinical fractionated dose of whole-brain irradiation (total cumulative dose of 40 Gy in 8 fractions of 5 Gy each, twice per week for 4 weeks) using a ^{137}Cs γ irradiator. Mice in the control group were only anesthetized. The mice were maintained for 4, 8, and 24 h after the last fractionated dose of whole-brain irradiation. Mouse brains were rapidly removed after perfusion and hemisected at the midline. Brains were then immediately frozen in liquid nitrogen.

Real-time RT-PCR

Quantitative real-time RT-PCR using TaqMan probes and primers (Applied Biosystems, Foster City, CA) were used for gene expression analyses as described previously (12). Amplification of individual genes was performed with Applied Biosystems 7300 real-time PCR system using TaqMan Universal PCR Master Mix and a standard thermal cycler protocol. TaqMan Gene Expression Assay Reagents for rat MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-12; and rat TIMP-1 and TIMP-2; rat glyceraldehydes-3-phosphate dehydrogenase (GAPDH); and mouse MMP-2, TIMP-2, and GAPDH were used for specific probes and primers of PCR amplifications. Cycle threshold cycle (C_T) was determined, and relative quantification was calculated by the comparative C_T method as described previously (12).

In situ zymography

To detect and localize net gelatinolytic activity of MMPs in brain sections, we carried out *in situ* zymography as described previously (14). Briefly, 100 μg/ml fluorescein-conjugated DQ gelatin (Molecular Probes, Eugene, OR) was mixed with 0.2% agarose melted in reaction buffer at pH 7.6 (50 mM Tris-HCl, pH 7.5, 0.15M NaCl, 5 mM $CaCl_2$ and 0.2 mM sodium azide). Brain sections (20 μm) were incubated with the reaction mixture prepared above for 24 h at 37°C in a moist dark chamber. Sections were then briefly washed with ice-cold phosphate-buffered saline (PBS) and distilled water. Slides were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and examined using a Zeiss AXIO Imager A1m fluorescence microscope (Carl Zeiss MicroImaging, Thornwood, NY). Negative controls were prepared by incubation of tissue sections with nonimmune rabbit serum (normal rabbit immunoglobulin G [IgG; Santa Cruz Biotechnology, Santa Cruz, CA]) instead of the primary antibody, and rabbit anti-MMP-2 and rabbit anti-MMP-9 polyclonal antibodies (Santa Cruz Biotechnology) were added to the reaction mixture to inhibit metalloproteinase activities. Images were acquired with 200× objective, using an AxioCam MRc5