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BRAIN RESEARCH

Research Report

Quantitative analyses of matrix metalloproteinase activity after traumatic brain injury in adult rats

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ABSTRACT

Recent laboratory evidence implicates matrix metalloproteinases (MMPs) as playing a pivotal role in ischemic and traumatic brain injuries (TBI). Here, quantitative real-time PCR analyses revealed that brains from TBI rats displayed significantly elevated MMP-9 expression at 24 h post-TBI, which remained upregulated at least until 48 h after injury. Immunohistochemical analyses similarly revealed significantly increased MMP-9 immunoreactivity at 24 and 48 h post-TBI. These results demonstrate that alterations in MMPs (i.e., MMP-9) commenced immediately after TBI, suggesting that treatment strategies designed to maintain MMP integrity should be initiated in the acute phase of injury.

1. Introduction

Traumatic brain injury (TBI) is a serious public health problem in the United States, with annual estimates of 5 million new head injuries and 2 million succumbing to life-long difficulties in daily activities. The economic cost for TBI is $56 billion annually (Mammis et al., 2008). The last 2 decades witnessed an increase in TBI cases due to combat injuries, including blast, impact or acceleration/deceleration injuries to the head, sustained in Iraq and Afghanistan may result in TBI characterized by damaged to the frontal and temporal lobes (Chuck, 2008; Inglese et al., 2005; Kraus et al., 2007; Suh et al., 2006). Unfortunately, there is currently no proven effective therapy for TBI. Accordingly, based on the significant economic burden and the lack of therapies, urgent research is warranted to elucidate TBI pathophysiology and its treatment.

Animal models of TBI have recently focused on two reproducible techniques namely fluid percussion and controlled cortical impact (CCI) injury models (LaPlaca et al., 2007; Tehranian et al., 2008). The brain injury produced by the CCI model replicates many clinical pathologic features of TBI, including an initial necrotic cell death in the underlying cortical tissue and white matter axonal injury, followed by an apoptotic cell death in surrounding tissue due to multiple subsequent events such as edema, ischemia, excitotoxicity and altered gene expression (Dikranian et al., 2008; Riess et al., 2002; Sandhir et al., 2008; You et al., 2008).

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Recent experimental studies have suggested the participation of matrix metalloproteinases (MMPs) in TBI (Falo et al., 2006, 2008; Hu et al., 2008). Indeed, elevated MMP-9 levels have been detected in the plasma or serum of TBI patients (Suehiro et al., 2004; Vajtr et al., 2008; Vilalta et al., 2008) and in the cortex and hippocampus of TBI animals (Falo et al., 2006, 2008; Kim et al., 2005; Truettner et al., 2005). In addition, treatment strategies for abrogating the TBI-induced MMP destabilization have been examined, such as hypothermia and hyperbaric oxygen therapy, with encouraging outcomes (Hu et al., 2008; Truettner et al., 2005; Vlodavsky et al., 2006). In order to enhance the success of therapeutic interventions directed at MMPs, elucidating the temporal acute profile of MMP activity may prove beneficial in guiding treatment initiation. Thus, the present study employed quantitative analyses of MMP-9 expression using quantitative real-time PCR verified with routine immunohistochemistry.

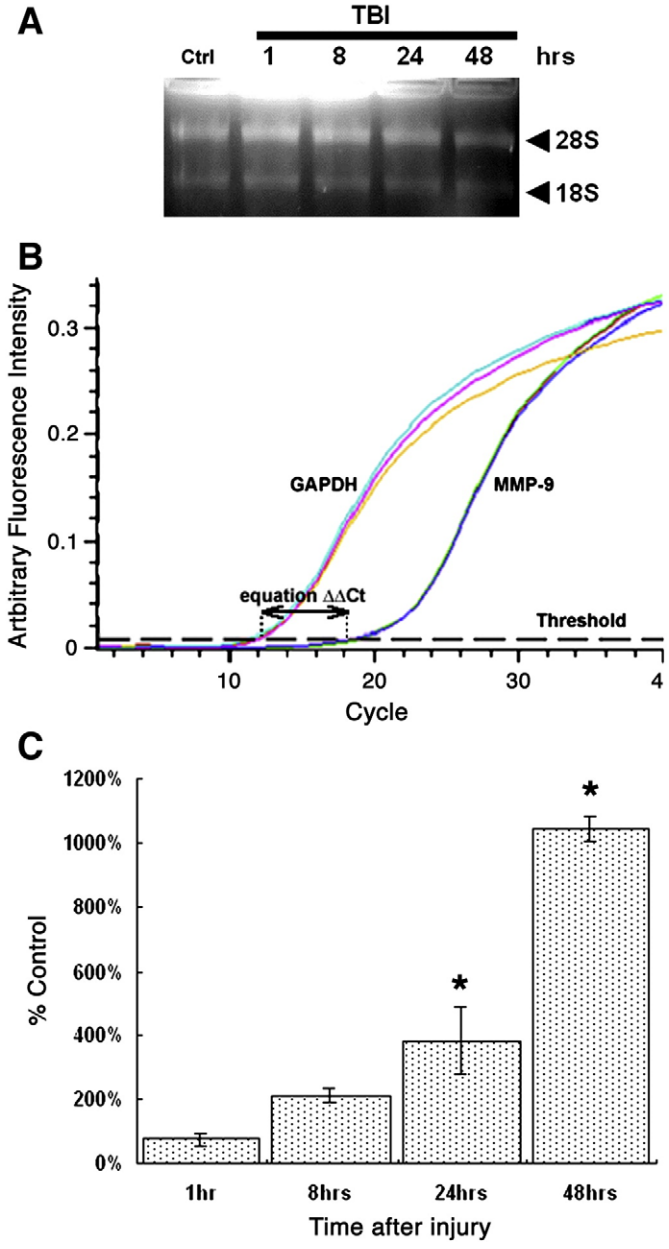

Fig. 1 – QRT-PCR analyses of MMP-9 expression in TBI brains. (Panel A) Confirms RNA integrity under UV light by visualization of 28S- and 18S-rRNA bands on a denaturing gel containing ethidium bromide. (Panel B) A logarithmic plot of fluorescence signal, in triplicates, for MMP-9 and GAPDH mRNA expression in the brain post-TBI. Threshold cycle (Ct) values were calculated by the equation DDCt = $\Delta Ct_{MMP\text{-}9} - \Delta Ct_{GAPDH}$ where ΔCt is the difference in Ct values between MMP-9 and the GAPDH. (Panel C) QRT-PCR analyses of *MMP-9* gene expression in the injured hemispheres ($n = 3$–5 from triplicate independent experiments) were performed 1, 8, 24, and 48 h after brain injury. Bars represent mean values ± SE. Asterisks indicate statistical significance: $^{*}p < 0.05$ versus control.