Acute Hyperglycemia Is A Treatable Risk Factor For Spinal Cord Injury: Animal Experiment And Human Cohort Study

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Introduction: Traumatic SCI can cause severe motor/sensory dysfunction, resulting in a significant reduction in the quality of life. Mechanical trauma rapidly leads to blood-spinal cord barrier disruption, neuronal cell death, edema, axonal damage and demyelination, followed by a cascade of secondary injuries that expand the inflammatory reaction around the epicenter of the original injury. The central nervous system (CNS) has a limited capacity for endogenous regeneration and repair; therefore, it is necessary to identify factors that exacerbate SCI in order to prevent any further deterioration of the neurological function and improve the outcomes of injuries. Although age, blood pressure and infection are each considered to be prognostic factors in patients with SCI, exacerbating factors that are amenable to treatment remain to be elucidated.

Microglial cells, the resident immune cell in the CNS, form the first line of defense after being stimulated by exposure to invading pathogens or tissue injury. Immediately after SCI, activated microglia enhance and propagate the subsequent inflammatory response by expressing cytokines, such as TNF-α, IL-6 and IL-1β. Recently, we demonstrated that the activation of microglia is associated with the neuropathological outcomes of SCI. Although the precise mechanisms of microglial activation remain elusive, several basic research studies have reported that hyperglycemia is involved in the activation of resident monocytic cells, including microglia. For example, the number of pancreatic resident monocytes is increased in hyperglycemic rodents, leading to the upregulation of islet-derived inflammatory factors, such as IL-6 and IL-8. In addition, peritoneal monocytes are activated under conditions of hyperglycemia, subsequently inducing greater production of TNF-α than that associated with a normoglycemic state. Furthermore, hyperglycemia correlates with the worsening of tactile allodynia accompanied by the hyperactivation of dorsal horn microglia. Because microglial activation is associated with secondary injury after SCI, we hypothesized that hyperglycemia may also influence the pathophysiology of SCI by altering microglial responses.

Methods: The mice were anesthetized with pentobarbital (75 mg/kg i.p.) and were subjected to a contusion injury (70 kdyn) at the 10th thoracic level using an Infinite Horizons Impactor (Precision Systems Instrumentation). For flow cytometry, the samples were stained with the antibodies and analyzed using a FACS Aria II flow cytometer and the FACSDiva software program (BD Biosciences). We retrospectively identified 528 SCI patients admitted to the Department of Orthopaedic Surgery at the Spinal Injuries Center (Fukuoka, Japan) between June 2005 and May 2011. The patients’ data were obtained from their charts.

Results: Under hyperglycemic conditions, both in vivo and in vitro, inflammatory reactivity was exponentially enhanced with the promotion of NF-κB nuclear translocation in microglial cells. ChIP-PCR analysis identified NF-κB-dependent downstream gene expressions. After SCI, the hyperglycemic mice
exhibited the progressive expansion of neural damage, with more severe motor deficits than that noted in the normoglycemic mice. Especially, microglia isolated from injured spinal cords of hyperglycemic mice after SCI exhibited markedly upregulated gene expressions of inflammatory cytokines. Consistent with the animal study findings, a Pearson chi-square analysis of the data from 528 SCI patients indicated that hyperglycemia on admission (glucose level ≥ 126 mg/dl) was a significant risk predictor of a poor functional outcome (Odds ratio, 2.66; 95% CI, 1.52 to 4.72; P <0.001). Moreover, a multiple linear regression analysis showed admission hyperglycemia to be a powerful independent risk factor for a poor motor outcome, even excluding diabetes mellitus cases associated with chronic hyperglycemia (regression coefficient: -1.37, 95% CI: -2.65 to -0.10, P < 0.05). Manipulating the blood glucose level in acute SCI rescued the exacerbation of the pathophysiology and the motor functional outcomes of the hyperglycemic mice. Our findings reveal that hyperglycemia in acute SCI is a novel prognostic factor with a negative impact on the motor function, highlighting the importance of achieving tight glycemic control after central nervous system injury.

Discussion: In this study, the microglial activation was accompanied by immediate nuclear translocation of NF-κB. Considering our finding that the cellular and nuclear expression of NF-κB was comparable between the microglia cultured under hyperglycemic and normoglycemic conditions before LPS stimulation, acute hyperglycemia alone does not appear to be involved in microglial activation. However, in the inflammatory state, acute hyperglycemia enhanced the nuclear translocation of NF-κB, which resulted in the increased expression of proinflammatory cytokines. In fact, a ChIP-PCR analysis demonstrated NF-κB binding to the TNF-α promoter, indicating that NF-κB directly regulates TNF-α transcription in microglial cells. TNF-α is reported to induce the apoptosis of neural cells, including neurons and oligodendrocytes, after SCI. Therefore, the excessive TNF-α expression observed in the microglia brought about by the activation of NF-κB in the hyperglycemic state is expected to contribute to a poor functional outcome after SCI, with increased apoptosis of neural cells.

Tight glycemic control during the acute clinical presentation is reported to improve the prognoses of diseases such as myocardial infarction, kidney damage, brain ischemia and liver dysfunction. In addition, achieving tight glycemic control (BGL < 110 mg/dl) reduces both the morbidity and mortality of critically ill patients. Nevertheless, in the management of acute SCI, inadequate attention has been paid to the BGL, and, in fact, to date, there have been no reports of the effects of acute glycemic control on the SCI outcomes. Moreover, although it is recommended to maintain the SBP, which was revealed to not be an independent predictor of the functional outcome, over 90 mmHg in the acute phase of SCI, guidelines on the management of SCI have not indicated the need for glycemic control. However, the results of the present cohort study suggest that achieving strict glycemic control is more important than controlling the SBP in patients with acute SCI. Therefore, a prospective and randomized study is needed to confirm the clinical efficacy of glycemic control in patients with acute hyperglycemia after SCI.

Significance: A human cohort study and animal experiment revealed that acute hyperglycemia exacerbates the functional outcomes of spinal cord injury via microglial overactivation, irrespective of a history of diabetes mellitus, and indicate that tight glycemic control may improve the patient outcomes of acute SCI.
Direct isolation and gene expression analysis of microglia from injured spinal cord. (A) After SCI, the resident microglial fraction was distinguishable from the monocyte/macrophage fraction by a flow cytometric analysis. (B) The immunocytochemical analysis of FACS-sorted microglia one day after isolation. (C) The mRNA levels of pro-inflammatory cytokines increased in the microglia of HG mice 12 hours after SCI (n = 6/group). Scale bars: 20 μm (B). *P < 0.05, Wilcoxon rank-sum test (C). The error bars indicate the SEM.
The high glucose condition resulted in increased nuclear translocation of NF-κB p65 in the activated microglial cells. (A) The immunocytochemical analysis of the p65 translocation into the nuclei of microglial cells 30 min after LPS stimulation. (B) A schematic diagram illustrating the primer set sites in relation to the TNF-α promoter. ATG indicates the gene transcription start site. The negative control is located upstream of the promoter. (C) In the ChIP-PCR analysis, the binding of p65 was observed at the putative NF-κB binding site following LPS stimulation. (D, E) The densitometric scanning of the immunoblots of BV-2 cells showed no significant changes in the cellular p65 levels among the groups, whereas there were significant increases in the p65 translocation into the nuclei in the hyperglycemic medium (12.5 mM or 25 mM) after LPS stimulation compared with that in the normoglycemic medium (5.6 mM) (n = 6/group).
Glycemic control in the hyperglycemic mice prevented deterioration of the pathophysiology and functional outcomes after SCI. (A) Schedules of glycemic control with insulin injection. (B) Time course of the blood glucose levels in the HG mice after insulin treatment. The black (insulin 1d) and gray (insulin 4d) arrows indicate the time of insulin injection. (C) LFB-stained cross-sections of tissue from the HG mice and HG mice treated with 1d insulin injection seven days after SCI. (I-K) The BMS, footprint analysis and the grip walk tests.