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ORIGINAL RESEARCH

Elevated Circulating Levels of the Pro-Inflammatory Cytokine Macrophage Migration Inhibitory Factor in Individuals With Acute Spinal Cord Injury

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Abstract

Objective: To test the hypothesis that macrophage migration inhibitory factor (MIF) is elevated in the circulation of individuals with acute spinal cord injury (SCI) compared with uninjured individuals.

Design: Prospective, observational pilot study.

Setting: Academic medical center.

Participants: Adults with acute traumatic SCI (n = 18) and uninjured participants (n = 18), comparable in age and sex distribution.

Interventions: Not applicable.

Main Outcome Measures: The primary outcome measure was the plasma MIF levels. Potential correlations were examined between MIF and clinical/demographic variables. The secondary outcome was to determine if other immune mediators were elevated in participants with acute SCI and if their levels correlated with the MIF.

Results: MIF was significantly elevated in subjects with acute SCI compared with control subjects at 0 to 3 ($P<.0029$), 4 to 7 ($P<.0001$), and 8 to 11 ($P<.0015$) days postinjury (DPI). At 0 to 3 DPI, levels of cytokines interleukin-6 ($P<.00017$), interleukin-9 ($P<.0047$), interleukin-16 ($P<.007$), interleukin-18 ($P<.014$), chemokines growth-related oncogene α/chemokine (C-X-C motif) ligand 1 ($P<.0127$) and macrophage inflammatory protein 1-β/chemokine (C-C motif) ligand 4 ($P<.0015$), and growth factors hepatocyte growth factor (HGF) ($P<.0001$) and stem cell growth factor-β ($P<.0103$) were also significantly elevated in subjects with acute SCI. With the exception of interleukin-9, all of these factors remained significantly elevated at 4 to 7 DPI; a subset (interleukin-16, HGF, stem cell growth factor-β) remained elevated throughout the study. Within individuals, MIF levels correlated with HGF ($P<.018$) and interleukin-16 ($P<.01$).

Conclusions: These data demonstrate that MIF is significantly elevated in subjects with acute SCI, supporting further investigation of MIF and other inflammatory mediators in acute SCI, where they may contribute to primary and secondary functional outcomes.

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Traumatic spinal cord injury (SCI) occurs in approximately 12,000 Americans annually and greatly impacts daily living.[1,2] To identify potential strategies to promote recovery after SCI, many studies have investigated acute responses to SCI, including biochemical cascades that promote secondary damage and scar formation.[3] In particular, there is interest in understanding secondary damage triggered by acute inflammation after SCI, which deleteriously affects long-term functional outcomes.[4,5]

A recent study demonstrated that within participants with acute SCI, circulating inflammatory mediators were highest in participants with motor and sensory complete (ASIA [American Spinal Injury Association] Impairment Scale [AIS] grade A) injuries.[6] Significantly, a subset of acutely elevated biomarkers was predictive of motor recovery at 6 months post-SCI.[6] These data support the important concept that in human SCI, at clinically relevant acute time points, elevated inflammatory and other biomarkers may be proportional to injury severity and/or predictive of functional outcomes.[6-8]

The focus here is macrophage migration inhibitory factor (MIF), a proinflammatory cytokine released by activated immune cells.[9-14] MIF is elevated acutely in preclinical models of SCI and is neurotoxic to spinal neurons in vitro.[15-17] Mice that genetically lacked MIF had enhanced neuronal survival after SCI and more robust functional recovery than wild-type mice.[18] In humans, plasma MIF is elevated in subjects with chronic SCI.[19] Here, we tested the hypothesis that MIF is elevated in individuals with acute SCI and explored whether MIF levels correlate with clinical or demographic variables. As a secondary outcome, we investigated the extent of inflammation in individuals with SCI by measuring a broad panel of inflammatory mediators.

Methods

Participants

This study was performed in accordance with ethical standards of and approved by the local institutional review board. Written, informed consent was obtained from participants (recruited from 2009 to 2013) prior to enrollment. Inclusion criteria for participants with SCI were as follows: ≥18 years old and an initial traumatic SCI that had occurred ≤1 week prior. Traumatic etiologies were falls, motor vehicle collisions, or sports. Injuries sustained by violence (n=1) or by force of a blunt object (n=1) were categorized as other (n=2). Participants were excluded if they had a documented medical history of cancer, autoimmune disease, or another neurologic disease. Most participants (n=17/18) were recruited from the trauma center, where a surgeon, board certified in trauma and critical care, assessed clinical variables, including the Glasgow Coma Scale (GCS) and Injury Severity Score (ISS), which was calculated using the abbreviated injury scale 90 system (TraumaOne Software[a]). ISS scores were severe (>15), moderate (9−15), or mild (<9). GCS scores were severe (<8), moderate (9−12), or mild (>13). One participant was recruited from the department of emergency medicine. The presence of an SCI was determined by clinical and radiographic presentation. For most participants (n=10/18), a physiatrist board certified in SCI medicine evaluated the SCI according to the International Standards for Neurologic Classification of Spinal Cord Injury (ISNCSCI). Two participants died prior to any neurologic examination, 4 participants died prior to an ISNCSCI examination, and 4 surviving participants did not receive an ISNCSCI examination. Clinical/demographic variables were obtained from the New York State Pathway Trauma Registry abstraction form and participants' medical records. These factors included age, sex, mechanism of injury, ISS, GCS, injury completeness, AIS grade (when available), length of stay (LOS), and survival status (table 1). Steroids were administered to a minority of participants (n=6/18), consistent with its use as a treatment option during the study.

Blood samples from participants with SCI were obtained as soon as possible after hospital admission. Sampling occurred once daily, when possible, throughout the participant's first 2 weeks post-SCI or LOS, whichever was shortest. For uninjured controls, blood was drawn once from participants who were ≥18 years old, without history of SCI, and within an age range and sex distribution similar to the participants with SCI (see table 1). Samples from 18 subjects per group were analyzed.

List of abbreviations:

AIS	ASIA (American Spinal Injury Association) Impairment Scale
CCL4	chemokine (C-C motif) ligand 4
CXCL1	chemokine (C-X-C motif) ligand 1
DPI	days postinjury
GCS	Glasgow Coma Scale
GRO	growth-related oncogene
HGF	hepatocyte growth factor
IL	interleukin
ISNCSCI	International Standards for Neurologic Classification of Spinal Cord Injury
ISS	Injury Severity Score
LOS	length of stay
MIF	macrophage migration inhibitory factor
MIP1-β	macrophage inflammatory protein 1-β
SCGF-β	stem cell growth factor-β
SCI	spinal cord injury

Biochemical analysis

Multiplex assays (Bio-Plex Pro Human Cytokine 21-Plex, Group II, #MFO-005KMII and 27-Plex, Group I #M500KCAF0Y[b]) were used to measure MIF and 47 other factors (table 2). Assays were performed on a Luminex MAGPIX Multiplex Platform[b] and analyzed using Bio-Plex Manager software (Bio-Rad[b]). Assays were conducted according to manufacturer's protocol; each sample was assayed at least in duplicate. Assay ranges were essentially as specified by the manufacturer. For statistical analyses, measurements below the limit of detection were assigned a value of half of the lowest detectable value.[19] Data are reported as mean ± SEM, unless otherwise noted.

Statistics

In individuals with acute SCI, circulating inflammatory proteins peak at 0 to 3 days postinjury (DPI) and then decline.[6] Therefore, to structure the data and analysis, data binning was applied. Data were aggregated as bins I (0−3), II (4−7), III (8−11), and IV (12−15) DPI. The mean was calculated for each participant per bin and was used for all subsequent analyses. Table 1 shows samples collected in each bin for individuals with SCI. The number of samples (n=78) obtained from the individuals with SCI are as follows: bins I (n=31), II (n=24), III (n=15), and IV (n=8).

The Mann-Whitney *U* test was used to compare levels of biochemical factors in each bin for uninjured participants and subjects with SCI. The Mann-Whitney *U* test was also used to