TITLE

Imbalance between macrophage migration inhibitory factor and cortisol induces multiple organ dysfunction in patients with blunt trauma

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RUNNING HEAD

Imbalance between MIF and cortisol in blunt trauma

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ABSTRACT- Migration inhibitory factor (MIF) is associated with multiple organ dysfunction syndrome (MODS) in patients with systemic inflammatory response syndrome (SIRS). Our purposes were to determine the serum MIF, cortisol and tumor narcosis factor-α (TNF-α) and to investigate the influences of the balance between the levels of MIF and cortisol in patients with blunt trauma. The cortisol levels were identical between the patients with and without MODS. However, the MIF and TNF-α levels in the patients with MODS were statistically higher than those of the patients without MODS. The cortisol/MIF ratios in the patients with MODS were statistically higher than those of the patients without MODS. The results show that MIF and TNF-α play an important role together in post-traumatic inflammatory response. An excessive serum MIF elevation overrides the anti-inflammatory effects of cortisol and leads to persistent SIRS followed by MODS in blunt trauma patients.

KEY WORDS: Glucocorticoids, multiple organ failure, multiple trauma, tumor necrosis factor-alpha, systemic inflammatory response syndrome.

INTRODUCTION

Systemic inflammatory response syndrome (SIRS) as a result of an excessive inflammation is induced by a wide variety of insults such as infection, pancreatitis and trauma [1]. SIRS is frequently observed in patients with severe blunt trauma [2,3]. Scalea and colleagues indicated that severe SIRS is closely related with poor outcome in trauma patients [2,4,5].

The hypothalamic-pituitary-adrenal axis and glucocorticoids (cortisol) are activated to regulate an excessive inflammation after critical insults [6]. Sustained SIRS without a compensatory anti-inflammatory response induces multiple organ dysfunction syndrome (MODS) [1,7]. Although there are several treatments have been developed to treat trauma, MODS and multiple organ failure are still major complications after severe blunt trauma [3].

Macrophage migration inhibitory factor (MIF) was originally described as a T lymphocyte product that inhibits the random migration of macrophages [8]. Additionally, MIF was rediscovered as an important regulator of inflammation two decades ago [9]. MIF plays a unique role to directly counter-regulate the anti-inflammatory action of glucocorticoids [9,10]. MIF is closely associated with

MODS thus leading to poor outcome in patients with SIRS [11,12]. Chang *et al.* [13] reported that the serum MIF level reflects the severity of blunt trauma. However, the role of MIF in relation to glucocorticoids in post-traumatic inflammation has not yet been sufficiently elucidated.

An imbalance between MIF and glucocorticoids might induce sustained SIRS and MODS leading to poor prognosis in patients with severe blunt trauma. The present study examined levels of serum MIF, cortisol and tumor necrosis factor-α (TNF-α) in patients with severe blunt trauma and their relationship with SIRS and MODS.

MATERIALS AND METHODS

Approval of this study was obtained from the Institutional Review Board. The study followed 45 patients with severe blunt trauma who admitted to the emergency department and intensive care unit. Severe blunt trauma was defined as an injury with Abbreviated Injury Scale (AIS) ≥3. Patients were excluded if they were under 18 years of age, had a terminal illness or were complicated with cardiac arrest. Patients who had been on chronic steroid

treatment, had disorders of hypothalamus-pituitary-adrenal axis, or received exogenous steroids during the observational periods. Etomidate is not available in Japan. Patients with an injury severity score (ISS) [14] ≤8 were also excluded. The sequential organ failure assessment (SOFA) scores were calculated daily [15]. Organ dysfunction was defined as a SOFA score ≥2 in each included organ. MODS was defined as the dysfunction of two or more organs. The patients with MODS and without MODS during the first 5 days after the trauma were defined as the With MODS group and Without MODS group, respectively. The mainly injured body part was defined as a body part with AIS ≥3. When a patient had more than two body parts demonstrating AIS ≥3, then such a patient was defined as multiply injured. The Acute Physiology and Chronic Health Evaluation (APACHE) II scores [16] were used to evaluate the physiological severity of the blunt trauma patients. SIRS was defined according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [1]. A massive transfusion was defined as a transfusion of more than 6 units of packed red blood cells.

Blood samples were collected and placed in a test tube containing

EDTA-2Na using an arterial catheter within 6 hr after the trauma (day 1). Samples were collected daily in the morning on day 2 through day 5. Eight healthy volunteers served as the control group. The blood samples were promptly centrifuged and the separated plasma was frozen at –80 °C until analysis. Serum MIF levels were measured using an enzyme-linked immunosorbent assay. Detailed methods are described elsewhere [17]. Serum cortisol levels were measured by radioimmunoassay (Coritisol Kit TFB, TFB, INC, Tokyo, Japan). Serum TNF-α levels were measured by enzyme immunoassay (Quantikine HS Human TNF-α Immunoassay, R&D SYSTEMS, Minneapolis, USA).

Unless otherwise indicated, all measurements are expressed as the arithmetic mean ± SD. The SPSS 15.0J statistical software package (SPSS Inc., Chicago, Illinois) was used for all statistical calculation analysis. For statistical testing of normality, the Kolmogorov-Smirnov test was used. Logarithmic transformations were made for all variables if needed. Comparisons between the groups were made using Student's *t*-test, the Mann-Whitney U test, the chi square test and the Dunnett's multiple comparison test. A stepwise multiple

linear-regression analysis was used to assess the relationship between the MIF levels and the following variables: gender, age, ISS score, the mainly injured body part and systolic blood pressure on admission to our emergency department. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Forty-five patients with blunt trauma were included in this study. In the first 5 days, 24 patients were complicated with MODS (With MODS group). Twenty-one patients were not complicated with MODS in the first 5 days (Without MODS group). Table 1 presents the characteristics of patients in the two groups. The patients with MODS had higher scores of ISS, SOFA and APACHE II, more transfusion on day 1, and a longer duration with SIRS than those the patients without MODS.

In the patients with MODS, the TNF-α levels on day 3 and day 5 were higher than those in the patients without MODS (Fig. 1). During the study period, the TNF-α levels of the patients with and without MODS were higher than those of the control group. Figure 2 presents the MIF levels, cortisol levels and

cortisol/MIF ratios during the first 5 days. Although the MIF levels on day 1 were not different in the two groups, the MIF levels in the patients with MODS were higher than those in the patients without MODS from day 2 to day 5. In both of the groups, the MIF levels on day 1 were higher than those of the control group and gradually decreased during the study period. Cortisol levels during the study period were not different between the patients with and without MODS and sustained higher levels during the study period in both of the groups than those of the control group. Although the cortisol/MIF ratios in the patients without MODS promptly increased, the cortisol/MIF ratios in the patients with MODS stayed at low levels during the study period.

A stepwise multiple linear-regression analysis revealed that systolic blood pressure on admission to the emergency department (regression coefficient, -0.202; SE, 0.088; standardized coefficient, –0.334; t, -2.3; p = 0.027) was an independent predictor for serum MIF levels on admission (R^2 for the entire model, 0.112). The correlation between serum MIF level and systolic blood pressure on admission is presented in Fig. 3.

DISCUSSION

The present study demonstrated that there were persistently higher levels of serum TNF-α and MIF, imbalance of the MIF and cortisol levels and prolonged duration of SIRS in blunt trauma patients with MODS in comparison with the patients without MODS. Hypotension may be one of the factors that induced MIF secretion after blunt trauma.

TNF-α is central pro-inflammatory cytokine and is associated with poor outcome in critically ill patients [18]. The significantly higher TNF-α levels in patients with MODS in comparison to patients without MODS shown in the present study is consistent with the results of previous studies [18-20]. MIF levels in patients with MODS were significantly higher than those in patients without MODS. MIF is released from the pituitary gland, monocytes and macrophages induced by TNF-α and then MIF initiates TNF-α secretion from macrophages [21-23]. These studies suggest that MIF and TNF-α may act together in a pro-inflammatory loop, contributing to pathogenesis of SIRS and MODS. Calandra *et al.* [24] demonstrated that an antibody against MIF decreased the levels of TNF-α in mice with sepsis. They also observed a direct

contribution of MIF to the pathogenesis of sepsis in TNF-α knockout mice [24]. These results show that MIF may act upstream from TNF-α in the inflammatory response. In clinical settings, as in the present study, MIF and TNF-α may synergistically induce SIRS and subsequent MODS.

Originally, MIF was described as a T lymphocyte product that inhibits the random migration of macrophages [8] and an important regulator of inflammation that plays a unique role to directly counter-regulate the anti-inflammatory action of glucocorticoids [9,10]. Recent studies suggested that MIF is a distinctive cytokine that is expressed in response to clinical insults and is a critical regulator in inflammatory pathways, leading to SIRS and subsequent MODS [9,10]. However, the role of MIF has not yet been sufficiently described in the pathophysiology of blunt trauma. A report by Chuang *et al.* [13] is the only study focused on a role of MIF in blunt trauma [13]. They reported that the MIF level has a close relationship with some physiological measures such as the APACHE II score and without anatomical measures such as the ISS [13]. They also indicated a correlation between the MIF level and the Revised Trauma Score, which was calculated based on the systolic blood pressure, respiratory rate and

consciousness level [13]. The present study also demonstrated the systolic blood pressure on admission, but not the ISS, to be associated with the serum MIF level after the trauma. These results imply that MIF is markedly released by hypoperfusion induced by severe blunt trauma.

The initial release response of MIF after clinical insult is due to the secretion of MIF from stores in various organs [25]. After the initial release response, the stored MIF is completely used up in various tissues [25]. Subsequently, MIF protein is produced in company with the induction of MIF mRNA in various tissues [25]. In the present study, the levels of MIF on day 1 were measured within 6 hr after the trauma. Therefore, the origins of MIF measured on day 1 and after day 1 might be different. For the reasons mentioned above, the initial elevation of the MIF levels on day 1 showed no difference between the patients with and without MODS. Furthermore, persistent high levels of MIF may play a more important role in the induction of SIRS and subsequent MODS than the initial elevation of them just after trauma.

Activation of the hypothalamic-pituitary-adrenal axis resulting in the release of glucocorticoids is a major determinant of the host response to systemic stress

[6]. Glucocorticoids have an important role in the regulation of the host inflammatory and immune response [6]. MIF has a unique role directly counter-regulating the anti-inflammatory action of glucocorticoids [9,10]. In short, MIF and glucocorticoids function as a physiological counter-regulatory dyad that modulates systemic inflammatory and anti-inflammatory responses [6,9,10]. MIF is a critical mediator in patients with SIRS and/or MODS [11,12]. MIF has an important role in the pathogenesis of SIRS and/or MODS and thus correlates with the patient outcome [11,12]. Highly increased MIF levels override the anti-inflammatory effect of cortisol elevated at the same time [11]. Emonts *et al.* [26] also demonstrated a dysfunctional hypothalamic-pituitary-adrenal function and a marked imbalance between the levels of MIF and cortisol in sepsis. The present study showed a persistent imbalance between the inflammatory (MIF) and anti-inflammatory (cortisol) response in blunt trauma patients with MODS. The imbalance suggests that highly increased MIF overrides the anti-inflammatory response of cortisol against systemic inflammation in blunt trauma and leads to sustained SIRS and subsequent MODS.

In conclusion, hypotension after trauma was an independent predictor of

serum MIF levels on admission. Sustained imbalance of MIF and cortisol levels and persistent elevation of TNF-α levels were observed in blunt trauma patients with MODS. The results imply that MIF and TNF-α play an important role together in the post-traumatic inflammatory response. An excessive serum MIF elevation overrides the anti-inflammatory effects of cortisol and leads to persistent SIRS subsequent MODS in blunt trauma patients.

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FIGURE LEGENDS

Fig. 1. Levels of tumor necrosis factor-α (TNF-α) after trauma. Data is presented as the mean ± SD. * $p < 0.05$ vs. the control subjects by the Dunnett's multiple comparison test. *MODS* multiple organ dysfunction syndrome.

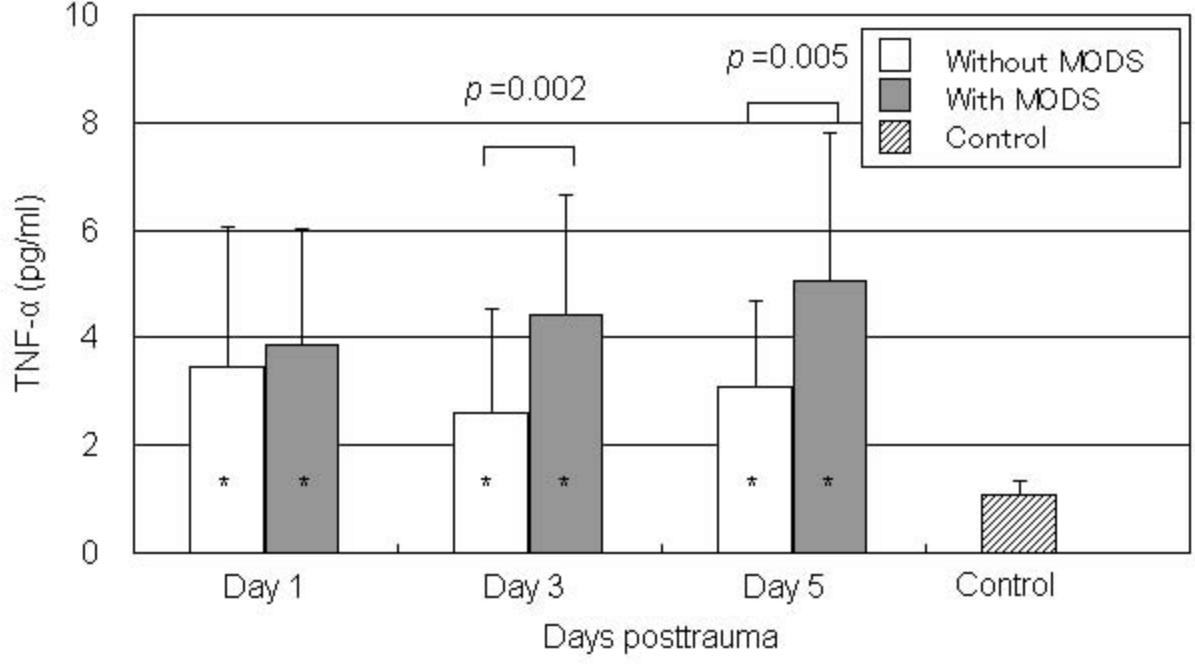
Fig. 2. The levels of macrophage migration inhibitory factor (MIF), cortisol and the MIF/cortisol ratio after trauma. Data is presented as the mean ± SD. * $p < 0.05$ vs. the control subjects by the Dunnett's multiple comparison test. *MODS* multiple organ dysfunction syndrome.

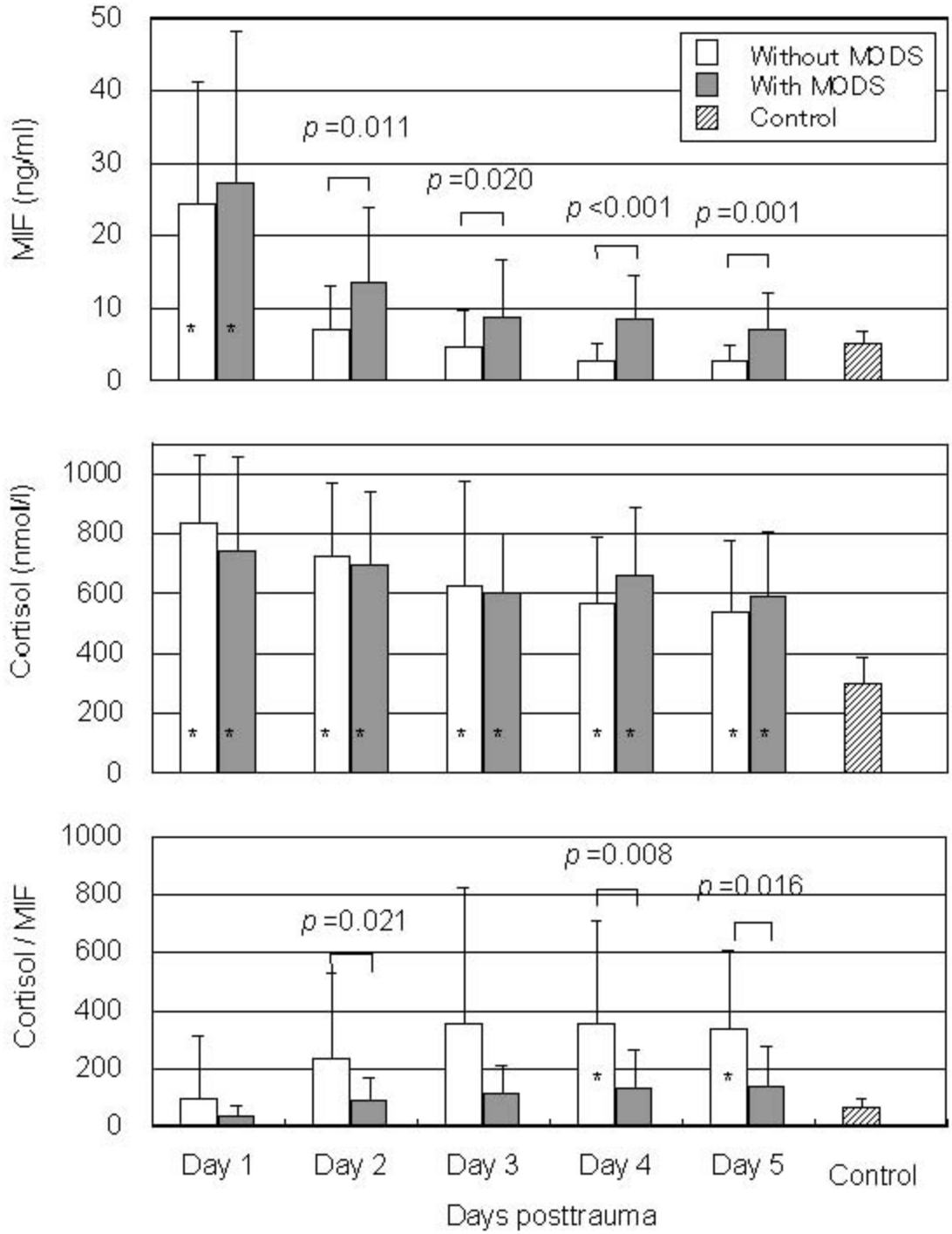
Fig. 3. Correlation between the levels of macrophage migration inhibitory factor (MIF) on day 1 and systolic blood pressure on admission to the emergency department.

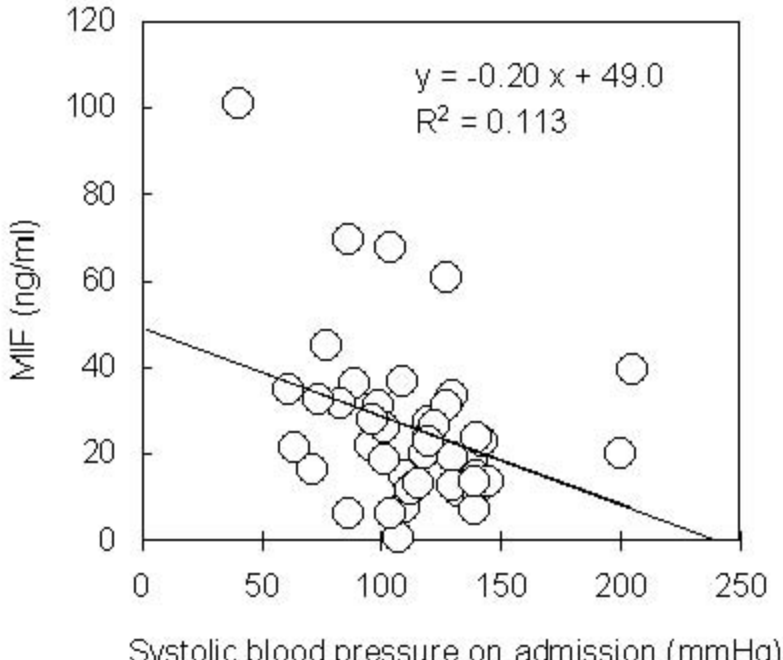
Table 1. Characteristics of the patients

	Without MODS (n=21)	With MODS (n=24)	*p*
Male / Female	13 / 8	16 / 8	ns
Age (years)	41 ± 21	45 ± 18	ns
Systolic blood pressure on admission (mmHg)	116 ± 29	111 ± 33	ns
Massive transfusion on Day 1 (yes / no)	6 / 15	18 / 6	0.002
Injury Severity Score	19 ± 9	27 ± 12	0.025
Mainly injured body part			
Head	9	10	
Chest	3	1	
Abdomen	2	4	ns
Extermity / pelvis	4	3	
Multiply injured	3	6	
Surgery on Day 1 (yes / no)	7 / 14	12 / 12	ns
APACHE II score	9 ± 5	18 ± 6	<0.001
Maximum SOFA score	3.6 ± 1.3	8.6 ± 2.9	<0.001
SIRS (days)	1.1 ± 0.9	2.9 ± 1.9	0.003
Mortality rate in hospital (%)	5	25	ns

MODS multiple organ dysfunction syndrome, *APACHE* acute physiology and chronic health evaluation, *SOFA* sequential organ failure assessment, *SIRS* systemic inflammatory response syndrome, *ns* not significant.


10
8
6
4
2
0
TNF-α (pg/ml)
p =0.002
p =0.005
Without MODS
With MODS
Control
*
Day 1
Day 3
Day 5
Control
Days posttrauma


MIF (ng/ml)
50
40
30
20
10
0
Without MODS
With MODS
Control
p =0.011
p =0.020
p <0.001
p =0.001
Cortisol (nmol/l)
1000
800
600
400
200
0
Cortisol / MIF
1000
800
600
400
200
0
p =0.021
p =0.008
p =0.016
Day 1
Day 2
Day 3
Day 4
Day 5
Control
Days posttrauma


y = -0.20 x + 49.0
R2 = 0.113
MIF (ng/ml)
Systolic blood pressure on admission (mmHg)
0
20
40
60
80
100
120
0
50
100
150
200
250