Abstract

**Purpose.** High-mobility group box 1 (HMGB-1) is a late-phase cytokine, which is released extracellularly in response to systemic inflammation caused by infection, shock, or trauma. We examined the plasma levels of HMGB-1 to clarify its role in surgical stress.

**Methods.** The subjects of this study were 37 patients who underwent elective general surgery. We measured plasma concentrations of HMGB-1 over time using a quantitative enzyme-linked immunosorbent assay. We then analyzed the relationships between the change in HMGB-1 concentration and perioperative factors, including postoperative complications. Statistical analyses were performed using two-way repeated-measures analysis of variance.

**Results.** The HMGB-1 level was higher on postoperative day 3 in patients with a prolonged operative time or large intraoperative blood loss ($P = 0.048$ and $P = 0.041$, respectively). The HMGB-1 level also remained high over time in patients with postoperative complications ($P = 0.037$).

**Conclusions.** These results show that the plasma HMGB-1 level is related to surgical stress such as operative time and blood loss. The level remained high over time in patients with postoperative complication, suggesting progression of the complication.

**Key words** Plasma high-mobility group box 1 · Surgical stress · Perioperative period · Postoperative complications

Introduction

Several cytokines, such as interleukin (IL)-1, tumor necrotizing factor (TNF)-α, IL-6, and IL-8, are involved in systemic inflammation caused by infection, shock, trauma, and surgery. Recent advances in cytokine biology and clinical immunology have led to widespread acceptance of the idea that cytokines can function as systemic mediators. High-mobility group box 1 (HMGB-1, also referred to as amphoterin) is a nuclear protein, which acts as a cytokine once released extracellularly. Extracellular HMGB-1 acts as an alarm signal to induce inflammation, proliferation, and migration of cells. Although HMGB-1 can also cause tissue regeneration after inflammation, an excessive amount of HMGB-1 prolongs inflammation and lowers the possibility of survival in septic situations. Elevated plasma HMGB-1 concentrations have been found in clinical syndromes such as sepsis, ischemia/reperfusion injury, infection, and arthritis.

Unlike other proinflammatory cytokines, HMGB-1 is a “late-appearing” inflammatory mediator, so it provides a wider time frame for clinical intervention against progressive inflammatory disorders. HMGB-1, produced by macrophages, mature dendritic cells, and natural killer cells, appeared in plasma between 8 and 32 h after lipopolysaccharide (LPS) infusion into mice, and blockade with antibodies, even after LPS infusion, improved survival. Therefore, we examined the plasma level of HMGB-1 during the perioperative period, hoping that inhibition of excessive HMGB-1 activity promptly after surgery may reduce postoperative complications.

Patients and Methods

**Patients**

Thirty-seven patients who underwent elective surgery for various disorders between April 2005 and August
Peripheral venous blood samples were collected over time from all patients: preoperatively and then on postoperative days (PODs) 1, 3, and 7. Samples were collected into endotoxin-free tubes (Falco, Tokyo, Japan) and the plasma concentration of HMGB-1 was measured. We analyzed interactions between HMGB-1, the white blood cell count (WBC), and serum C-reactive protein (CRP) levels, and perioperative parameters with the postoperative outcome, especially infectious complications. Perioperative parameters included age, gender, surgical procedure, operative time, blood loss, and blood transfusion during surgery. The definitions of postoperative infection were as follows: wound infection, defined as a wound broken down, gaping, or completely dehisced; pneumonia, defined as radiologically proven pulmonary consolidation with culture-positive sputum; atelectasis, defined as radiologically proven atelectasis requiring airway cleaning under bronchoscopy; pyothorax, defined as culture-positive pleural effusion; and enteritis, defined as diarrhea more than 10 times a day. Written informed consent was obtained from all patients.

Measurement of Plasma HMGB-1 Concentration

The plasma concentration of HMGB-1 was measured using a quantitative enzyme-linked immunosorbent assay kit purchased from Shino-Test (Tokyo, Japan), according to the manufacturer’s instructions. The detection threshold of this assay is <1 ng/ml and the between-assay coefficient of variation is <8%. Plasma samples were stored at −80°C before measurement.

Statistical Analysis

The relationships among perioperative parameters, HMGB-1, WBC, CRP, and postoperative outcome were analyzed using a two-way analysis of variance with repeated measures over time. A P value of less than 0.05 was considered significant.

Results

Transition of Postoperative WBC, CRP, and HMGB-1

Figure 1 shows the mean peripheral WBC count and the mean concentrations of plasma HMGB1 and CRP. The WBC count peaked on POD 1 and decreased after POD 3. The plasma CRP level peaked on POD 3 and decreased on POD 7. The plasma HMGB-1 level, which has a wider standard deviation than the other two measures, decreased slightly after peaking on POD 1.

Relative Shifts of WBC, CRP, and HMGB-1

Because the baseline plasma HMGB-1 concentrations varied greatly (day 0 in Fig. 1), the relative shifts from the baseline were calculated after withdrawing the log value of the baseline (day 0) from the log values of PODs 1, 3, and 7. Those data were sorted according to perioperative measures and postoperative outcome.