Evidence for a role of kallikrein-kinin system in patients with shock after blunt trauma

KATSUHIKO SUGIMOTO,1 MITSUHIRO HIRATA,2 MASATAKA MAJIMA,3 MAKOTO KATORI,3 AND TAKASHI OHWADA2
1Department of Emergency and Critical Care Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142–8666; and Departments of 2Traumatology and Critical Care Medicine and 3Pharmacology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan

Sugimoto, Katsuhiro, Mitsuhiro Hirata, Masataka Majima, Makoto Katori, and Takashi Ohwada. Evidence for a role of kallikrein-kinin system in patients with shock after blunt trauma. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1556–R1560, 1998.—Bradykinin (BK) is activated via plasma and/or tissue kallikrein-kinin (K-K) system pathways during hypotension after blunt trauma. The precise role of the K-K system in human subjects has not been defined. We developed a new method for measuring levels of BK in the blood and examined the role of the K-K system in patients with shock after trauma. Eight patients were entered into this study. We measured the levels of a high-molecular-weight kininogen (HMWK), a low-molecular-weight kininogen (LMWK), BK, and (1–5)-BK in the blood of patients in an unstable state (Pre) and a stable state (Post). At Pre, the blood BK level was significantly elevated, the HMWK and LMWK levels were significantly lower, and the (1–5)-BK level was significantly higher than the respective levels at Post. Our data suggest a significant role for the K-K system in the pathogenesis of shock after blunt trauma. This newly developed method for determination of the activation of the plasma K-K system appears to be useful for determining the severity of a trauma.

SHOCK REMAINS A MAJOR CAUSE of morbidity and mortality after blunt trauma (22). The roles of recently discovered cytokines, such as tumor necrosis factor and the interleukins, have been the focus of recent scientific research (6, 9). Moreover, in trauma patients, activation of the kallikrein-kinin (K-K) system, which generates bradykinin (BK), has been suspected for several decades, although its precise role in shock after trauma has not been determined (5). Since BK was discovered by M. Rosha in 1949, there has been speculation that BK induces pathological conditions such as endotoxin shock because of its potent hypotensive effect (11, 20). Although BK has been suspected of mediating the hypotensive responses during some kinds of shock, all results appearing in previous reports were supported by indirect evidence, and there was no clear, direct evidence to indicate that BK induces hypotension after trauma (14). In 1970, Beery et al. (2) determined that BK itself acted directly in the blood and reported an increase in the blood BK levels during hypotension due to hemorrhage. But the sensitivity of the BK bioassay method used (which used feline jejunum) is low, making the method unsuitable for a clinical setting. Quantitation of the components of this system, several of which are unstable and short-lived in vivo, has long been difficult, and the lack of specific antagonists of BK has impeded precise definition of its action during shock after trauma (11). Recently, some BK antagonists, including oral active compounds, were developed and used to evaluate the role of the BK system in pathological conditions, but these antagonists were used only in experimental studies (17). We recently developed a new method of measuring BK experimentally (16). In the present prospective clinical study, we used this new method to measure the levels of BK and the components of the K-K system in the blood of patients with shock after trauma to examine the role of the K-K system after trauma in a clinical setting.

PATIENTS AND METHODS

From patients admitted to the intensive care unit of Kitasato University Hospital (Kanagawa, Japan) with hemorrhagic shock after blunt trauma, we chose eight whose lowest systolic blood pressure on admission was <100 mmHg. We excluded patients with sustained tension-pneumothorax, cardiac tamponade, spinal shock, accidental hypothermia, and drug abuse, all of which can produce hypotension. The blood samples were collected from these patients to measure the levels of BK and components of the K-K system under unstable conditions after injury (Pre; namely, at the earliest possible time after injury, during initial evaluation in the emergency room) and under stable conditions before discharge (Post). Blood samples were taken from 10 age-matched normal healthy volunteers (5 male, 5 female), as normal control, to measure the levels of the same components of BK. To quantitate BK and the components of the K-K systems, we measured the levels of 1) BK itself, 2) high-molecular-weight kininogen (HMWK) as a precursor to BK in the plasma K-K system (PKKS), 3) low-molecular-weight kininogen (LMWK) as a precursor to BK in the tissue K-K system (TKKS), and 4) (1–5)-BK as a product of BK degradation in the blood. Nineteen milliliters of blood were collected from each patient’s femoral artery. Ten milliliters of each sample was placed in a plastic tube that contained 40 ml of ice-cold absolute ethanol (HPLC grade; Wako Pure Chemicals, Osaka, Japan). The remainder of each sample (9 ml) was placed in a plastic tube that contained 1 ml of sodium citrate. The first blood samples (10 ml of blood with ethanol) were centrifuged at 3,000 revolutions/min for 30 min at 4°C, and the supernatants were transferred to other plastic tubes for measurement of the levels of BK and (1–5)-BK. The other samples (9 ml of blood with sodium citrate) were centrifuged at 3,000 revolutions/min for 30 min at 4°C, and plasma from each was transferred to another plastic tube for measurement of the levels of components of the K-K system.

Determination of kininogen levels in plasma. The plasma levels of HMWK and LMWK were determined by the method reported previously, in which kininogens were converted to...
BK, and the amounts of kinin generated were measured with a BK ELISA kit (Dainippon Pharmaceutical, Osaka, Japan) (18, 24). Kininogen levels were expressed as nanograms BK equivalent per milligrams plasma protein (24).

Determination of BK and a stable BK metabolite (1—5)-BK, in the circulation. The ethanol extracts (supernatants) were evaporated to dryness and washed with diethylether to remove the lipids. The washed samples were dissolved in 4 ml of distilled water that had been acidified with 0.2 ml of 0.01 N HCl and were applied to a Sep-Pak C18 cartridge column. After being washed with 12 ml of distilled water and 4 ml of 0.1 M acetic acid, BK and its degradation products were eluted with 6 ml of 80% (vol/vol) acetonitrile contained in 0.1 M acetic acid. The kinin fraction was evaporated under reduced pressure, and the residue was dissolved in 800 ml of the assay buffer. The levels of BK and (1—5)-BK were determined with a newly developed ELISA kit for BK (Dainippon) and an ELISA kit for (1—5)-BK (Dainippon) (18). Using the patients’ notes and the levels of BK and components of the K-K system in the blood samples, we made comparisons between the Pre and Post stage parameters in the surviving patients and between parameters in the patients who survived and those who did not. The parameters were levels of BK, HMWK, LMWK, and (1—5)-BK; injury severity score (ISS), according to the abbreviated injury score-90 (4); alveolar-arterial oxygen differences (A-aO2); total blood transfusion (ISS), according to the abbreviated injury score-90 (4); alveolar-arterial oxygen difference (mmHg); BE, base excess (mmol); Hb, minimum hemoglobin concentration on admission (g/dl); Plat, minimum platelet count on admission; minimum platelet counts on admission; and base excess.

All data are expressed as means ± SD. The statistical analysis of the results was performed by one-way analysis of variance and Student’s t-test for paired or unpaired data. A probability level of <0.05 was considered to be significant.

The protocol for this clinical study was reviewed and approved by the Ethics Committee of Kitasato University Hospital. Informed consent was obtained from each patient and legal guardian before the study was initiated and after the risks and benefits involved had been explained.

RESULTS

The characteristics and physiological parameters of patients are presented in Table 1. The total number of injured organs in these patients was 34 (mean number of organ injuries per patient, 4.25). The most frequently injured organs were the extremities, including pelvic fractures (14/34, 41.2%).

The changes in the various clinical parameters in surviving patients from the Pre to the Post stage were significant, with the exception of the change in arterial pH between the Pre and Post stages (Table 1).

Between the Pre and Post stages, the levels of BK and (1—5)-BK in the blood decreased significantly. The changes in levels of HMWK and of LMWK in the blood were also significant. The levels of HMWK, LMWK, BK, and (1—5)-BK at Post were almost the same as those in normal healthy volunteers (Table 2).

The difference in respective parameters for patients with blunt trauma between surviving (n = 4) and nonsurviving (n = 4) groups was not significant, with the exception of the difference in B/W (surviving group, 137.7 ± 75.9 ml/kg; nonsurviving group, 340.1 ± 93.3 ml/kg, P < 0.05). However, the levels of BK and

### Table 1. Characteristics of patients in shock after blunt trauma and comparison of physiological parameters in four surviving patients with shock between Pre and Post stages

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (n = 8)</th>
<th>Pre</th>
<th>Post</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.8 ± 6.0</td>
<td>47.3 ± 18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>4/4</td>
<td>2/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISS</td>
<td>33.5 ± 3.1</td>
<td>27.0 ± 7.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/W</td>
<td>238.9 ± 47.3</td>
<td>137.7 ± 75.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prop</td>
<td>4/4</td>
<td>4/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBP</td>
<td>45.5 ± 7.3</td>
<td>47.0 ± 29.4</td>
<td>88.7 ± 5.7</td>
<td>0.0461</td>
</tr>
<tr>
<td>SI</td>
<td>1.6 ± 0.4</td>
<td>1.4 ± 0.5</td>
<td>0.7 ± 0.1</td>
<td>0.0494</td>
</tr>
<tr>
<td>a-aO2</td>
<td>108.9 ± 24.6</td>
<td>83.3 ± 20.3</td>
<td>23.3 ± 2.9</td>
<td>0.0085</td>
</tr>
<tr>
<td>BE</td>
<td>-8.4 ± 2.3</td>
<td>-5.3 ± 3.7</td>
<td>2.9 ± 0.5</td>
<td>0.0198</td>
</tr>
<tr>
<td>Hb</td>
<td>6.8 ± 1.5</td>
<td>7.6 ± 1.4</td>
<td>11.0 ± 1.4</td>
<td>0.3195</td>
</tr>
<tr>
<td>Plat</td>
<td>9.1 ± 0.5</td>
<td>8.4 ± 5.4</td>
<td>37.3 ± 13.6</td>
<td>0.0444</td>
</tr>
<tr>
<td>APACHE II</td>
<td>11.6 ± 1.7</td>
<td>13.0 ± 3.8</td>
<td>2.7 ± 1.9</td>
<td>0.0466</td>
</tr>
</tbody>
</table>

Values are means ± SE except male/female, proportion (Prop: survivors/dead), and P values. Pre, unstable conditions after trauma; Post, stable conditions before discharge; ISS, injury severity score by abbreviated injury score-90; B/W, total blood transfusion volume/body weight (ml/kg); MBP, mean arterial blood pressure (mmHg); SI, shock index (pulse rate/systolic blood pressure); a-aO2, alveolar-arterial oxygen difference (mmHg); BE, base excess (mmol); Hb, minimum hemoglobin concentration on admission (g/dl); Plat, minimum platelet count on admission (×10⁶/ml); APACHE II; acute physiology and chronic health evaluation.

(1—5)-BK in the blood from nonsurviving patients at Pre were significantly higher than the respective value in the blood from survivors at Pre. Moreover, the levels of HMWK and of LMWK were significantly lower in the blood of nonsurvivors than in the blood of survivors at Pre, respectively (Table 2).

The correlation between levels of BK in the blood and three indicators of injury severity, namely, a-aO2, APACHE II, and ISS, were measured. The relationship between APACHE II and the levels of BK in the blood was significant (r = 0.876, P < 0.05; Fig. 1), but no other relationships between the other two indicators and BK levels were significant. Other components of BK [HMWK, LMWK, (1—5)-BK] yielded the same results (data not shown).

### Table 2. Comparison of levels of BK and components of K-K system in surviving patients after blunt trauma between Pre and Post stages and between Presurvivors and Pre nonsurvivors on admission

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HMWK</th>
<th>LMWK</th>
<th>(1—5)-BK</th>
<th>BK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>7.9 ± 1.3</td>
<td>26.2 ± 3.2</td>
<td>1,217.5 ± 460.2</td>
<td>27.1 ± 4.2</td>
</tr>
<tr>
<td>Post</td>
<td>13.4 ± 3.6</td>
<td>40.3 ± 6.6</td>
<td>262.6 ± 259.1</td>
<td>4.3 ± 4.7</td>
</tr>
<tr>
<td>P value</td>
<td>0.028</td>
<td>0.008</td>
<td>0.007</td>
<td>0.001</td>
</tr>
<tr>
<td>NS</td>
<td>5.2 ± 1.6</td>
<td>11.9 ± 3.1</td>
<td>2,565.5 ± 443.6</td>
<td>348.3 ± 12.9</td>
</tr>
<tr>
<td>P value</td>
<td>0.040</td>
<td>0.001</td>
<td>0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>NV</td>
<td>16.6 ± 0.8</td>
<td>51.3 ± 1.5</td>
<td>ND</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

Values for Pre (n = 4), Post (n = 4), nonsurvivors (NS; n = 4), and normal volunteers (NV) are means ± SE. HMWK, high-molecular-weight kininogen (ng bradykinin (BK) eq/mg protein); LMWK, low-molecular-weight kininogen (ng BK eq/mg protein); (1—5)-BK, degradation products of BK (pg/ml); ND, not determined. Values for BK are pg/ml.
DISCUSSION

Although the hypovolemia that follows hemorrhage due to organ injury is the main cause of the hypotension and/or shock that occurs after blunt trauma, many chemical mediators or cytokines, which are activated by endogenous endotoxins, hypoxia, acidosis, or reperfusion injury, are suspected of being involved in the induction of these conditions (21). In particular, it has been postulated that BK may play a role in shock, but there has been no clear evidence to date indicating that it is strongly correlated with shock in the clinical setting (5). A major reason for this lack of evidence is that measurements of the components, many of which are unstable and short-lived (~30 s) in vivo, of the K-K system, including BK itself, have proved difficult to quantitate. Moreover, the lack of specific inhibitors of BK has impeded precise definition of its action in shock after trauma (12). Other authors have reported the role of BK in shock using a newly developed anti-BK-antagonist, but only in experimental studies (17). To overcome the difficulties of determining the active form of BK, we proposed other approaches to demonstrate activation of the K-K system. The determination of the kinin precursor proteins HMWK and LMWK was one approach, and selective reductions of the values of these parameters provided information on the activation of the PKKS in inflammation models (24). As an alternative method, an assay system of the stable metabolite of BK, (1—5)-BK, was developed. This was also successfully used in the inflammation models, and the levels of (1—5)-BK were well correlated with the severity of inflammation (18). Recently, we developed a highly sensitive assay system for BK itself (16). The results of the present clinical study, using this new method, show that the levels of BK in the blood were significantly elevated in human subjects during the unstable stage (shock or hypotension, systolic blood pressure <100 mmHg) after blunt trauma compared with the levels at the stable stage (systolic blood pressure >100 mmHg), whereas the levels of the precursors to BK (HMWK and LMWK) were decreased and that of the degradation product of BK [(1—5)—BK] was elevated (Table 2). This was direct evidence of activation of the K-K system. Therefore, with the use of this new assay system, the activation of the K-K system can be detected more precisely and the role of BK in pathological conditions can be defined.

BK is a potent pharmacological agent that produces hypotension in experimental studies. The levels of BK in the blood that induce hypotensive responses were tested not only in experimental animals, but also in human volunteers (3, 17, 20). These reports indicated that the arterial blood kinin level that would reduce the MBP was ~10—100 pg/ml. In this clinical study, the levels of BK were 20–300 pg/ml, sufficient to have this effect.

There are two pathways for production of the active forms of BK, one being the PKKS, and the other, the TKKS (16). The PKKS is part of the contact system of plasma protease, related to the complement and clotting cascades. Factor XII (Hageman factor) can be activated either directly or indirectly by damage to the endothelium and by exposure of the basement membrane. Activated factor XIIa hydrolyzes circulating prekallikrein to generate kallikrein, which, in turn, cleaves HMWK to yield BK. BK is a nonapeptide with a short half-life (~30 s) that is rapidly destroyed in the pulmonary vascular beds by angiotensin-converting enzyme (kininase II) or by a circulating enzyme, kininase I. When tissue damage and/or hemorrhage occurs as a result of blunt trauma, factor XII from the injured tissue or hemorrhagic site is converted to the active form, factor XIIa, which then activates PKKS to produce BK in the blood. Other factors, including endotoxins, can also activate this PKKS. Many authors have proposed that endogenous endotoxin can be translocated to the circulating blood after trauma or during shock (23). Thus shock after trauma might activate the
PKKS, via the action of translocated endotoxins, to generate BK. Blood transfusion and large-volume crystalloid infusion, which are generally used for patients with trauma, may activate this PKKS. Therefore, in this clinical study, we collected blood samples from patients before initial volume replacement with crystalloid or blood products, or both, at the hospital. Also, none of the patients in this study ever received crystalloid or blood products before arrival at the hospital. Therefore, we can exclude this factor which might have activated PKKS. We also determined the plasma kininogen levels in terms of milligrams plasma protein (Table 2). Thus the effects of any kind of dilution of plasma were minimized in the present study. From the results of our clinical study, it is unclear which factors activate the PKKS to generate BK after trauma. We suspect that factors including tissue damage, hemorrhage, hypotension itself, or translocated endotoxin after trauma might activate PKKS to generate BK in the blood after blunt trauma. Our results indicate that not only the PKKS but also the TKKS was activated after blunt trauma to generate BK. The level of LMWK in the blood under unstable conditions (Pre) was significantly lower than that under stable conditions (Post), similar to the HMWK level (Table 2). In the TKKS, glandular kallikrein stimulates LMWK to generate BK in the tissue, circulating blood, or both (10). The active BK in the blood is generated from the nonactive HMWK in the PKKS or from the LMWK in the TKKS. It has been established that many organs and tissues, including the pancreas, kidney, intestine, and saliva glands, contain TKKS (7). Many authors have noted the possibility that the PKKS might be involved in pathological changes, such as shock, sepsis, or adult respiratory distress syndrome, but little is known about the relationship between TKKS and such pathological conditions (19). Although it is unclear from this study how active BK was generated from the TKKS after blunt trauma, we suspect that organ damage by the trauma may have activated the TKKS directly. The rate of decrease in the level of LMWK from Post to Pre was not significantly different from the rate of decrease in the level of HMWK during the same time. Thus, it was unclear which K-K system, the PKKS or the TKKS, was mainly involved in generating the BK in the blood after trauma.

There were no significant differences in this study, in terms of clinical parameters, between the surviving and nonsurviving groups of patients on admission. However, there were significant differences between surviving and nonsurviving groups in the levels of BK and the components of K-K systems at the Pre stage in the blood (Table 2). From other previous experimental studies, the effects of vasodilatation and blood pressure changes were dependent on the levels of BK, but this clinical study showed no relationship between levels of BK and changes of blood pressure (2). The reason for this result may have been that the activity of BK in the blood drawn from nonsurvivors on admission was extremely high, and the vascular reactivity to BK may have reached its plateau level. On the other hand, in a clinical setting like this study, not only BK, but also other substances that could be activated after trauma, may affect the circulatory system and induce hypotension. Therefore, we could not clearly define the relationship between the degree of hypotension and the level of BK after trauma. Not only hypotension, but also other pathological changes, including increased hypervascular permeability, are induced by BK. Thus post-traumatic complications, such as pulmonary edema and other organ dysfunction, may result from BK activation after trauma. Therefore, the level of BK in the blood after trauma may be a useful parameter not only for judging the severity of the injury, but also for predicting the outcome of trauma patients in the earlier stages. We believe that this is the first report to describe a significant correlation between hypotension and elevated levels of BK in the blood of human subjects after trauma.

Perspectives

We used a new method to examine the role of the K-K system in patients after trauma. We showed that elevated levels of BK, with decreasing levels of precursors of BK and increasing levels of the products of BK degradation in the blood of survivors, were strongly correlated with hypotension after blunt trauma in human subjects. Apart from the K-K system, other cytokines or mediators or both may be activated after trauma, and this cytokine network is bound to be complicated. This clinical study was carried out in a small sample of patients, and so further clinical studies are required to determine the relationship between the K-K system and other cytokines after trauma, as well as the relationship between activation of the K-K system after trauma and other late complications in a larger number of subjects.

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