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The Effects of Massive Transfusion and Haptoglobin Therapy on Hemolysis in Trauma Patients

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Abstract: A retrospective study was conducted on 53 patients who suffered severe trauma to determine the severity of intravascular hemolysis, the variations of renal function after trauma, and the effects of transfusion and haptoglobin therapy on these factors. Serum total haptoglobin, total hemoglobin, and urine free hemoglobin were measured 0, 1, 3, and 5 days after the trauma and renal tubular function was evaluated by the urinary *N*-acetyl-β-D-glucosaminidase (NAG) index. Patients were divided into two groups depending on whether or not haptoglobin was given: group A ($n = 34$) did not receive haptoglobin, and group B ($n = 19$) was administered 4,421 ± 245 U haptoglobin based on clinical indications. The total transfusion volumes were 3,477 ± 594 ml and 10,146 ± 1,794 ml, in groups A and B, respectively ($P < 0.01$). In group A, total haptoglobin was remarkably decreased to 69.4 ± 11.6 mg/dl on day 0, but recovered to within the normal range on day 3, while the total hemoglobin was increased and the urine hemoglobin was positive in 61.8% of the patients. In group B, decreases in total haptoglobin and increases in total hemoglobin were more remarkable, and 84.2% had a positive urine hemoglobin. On day 5, groups A and B had NAG indices of 18.8 ± 3.3 and 133.6 ± 33.8 U/L/creatinine respectively ($P < 0.01$). These findings led us to conclude that trauma caused hemolysis and that the administration of 4,000 U haptoglobin did not improve either the severity of hemolysis or the deteriorated renal tubular function caused by massive transfusion.

Key Words: trauma, hemolysis, transfusion, haptoglobin

Introduction

Trauma is known to cause intravascular hemolysis[1,2] and patients with major abdominal trauma[3] have been reported to show decreased serum haptoglobin levels. It has also been demonstrated that massive transfusion is followed by hemolysis due to weakened old banked red blood cells.[4] Although the effectiveness of haptoglobin therapy for the prevention of renal failure due to intravascular hemolysis and hemoglobinuria caused by thermal injury has been reported,[5,6] the relationships among the severity of intravascular hemolysis after trauma, serum haptoglobin and hemoglobin levels, and urine free hemoglobin levels, and the changes in renal function, remain unclear. Furthermore, it has not yet been elucidated how massive transfusion or haptoglobin therapy affect intravascular hemolysis.

Assuming that hemolysis due to massive transfusion accompanied by trauma-induced hemolysis leads to a drop in serum haptoglobin and the appearance of urine free hemoglobin, which has a deleterious effect on renal function, we hypothesized that haptoglobin therapy might be effective for the prevention of renal failure. To test this hypothesis, serum haptoglobin, hemoglobin and urine free hemoglobin levels, and renal function after trauma were measured, and the effects of massive transfusion and the administration of haptoglobin on these factors were evaluated.

Materials and Methods

This study was approved by the Ethics Committee of the Sapporo City General Hospital and informed consent was obtained from the patients or their closest relatives. It was conducted over an 18-month period from September, 1989, through the end of March, 1991.

Patients

A total of 53 trauma patients were divided into two groups according to whether or not haptoglobin was

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administered. Thus, group A was comprised of 34 patients who did not receive haptoglobin, and group B of 19 patients who did receive haptoglobin. The severity of trauma was evaluated based on the Injury Severity Score (ISS)[7,8] and Acute Physiology and Chronic Health Evaluation II (APACHE II).[9] The clinical data of both groups are shown in Table 1.

Measurement and Protocols

Immediately after the patients were conveyed to the emergency room, defined as day 0, and on days 1, 3, and 5, blood and urine samples were collected. Blood was taken by allowing it to flow into a syringe through the arterial catheter to prevent hemolysis. Other measurements included serum total haptoglobin by single radial immunodiffusion (SRID) (M-partigen, Hoechst, Frankfurt, Germany) using a reference value for normal adults of 100–230 mg/dl; serum total hemoglobin, by the tetramethyl benzidine method (Hitachi 105 spectrophotometer Hitachi, Tokyo, Japan) at 2–12 mg/dl; and urine free hemoglobin, by the tetramethyl benzidine method (not detected). Serum free hemoglobin levels were obtained by the following equations:[10]

1. When total haptoglobin / 1.42 > total hemoglobin: Free hemoglobin = 0
2. When total haptoglobin / 1.42 < total hemoglobin: Free hemoglobin = total hemoglobin − total haptoglobin / 1.42

As indices of renal glomerular function and renal tubular function, serum and urine creatinine, serum beta-2 microglobulin [Beta2MG, radioimmunoassay, Pharmacia Uppsala, Sweden], and urine *N*-acetyl-β-D-glucosaminidase (NAG, m-cresol purple, Shionogi, Osaka, Japan) were determined to calculate the creatinine clearance and the NAG index (urine NAG / urine creatinine). Increases in serum creatinine of 2.0 mg/dl and over, or a 24-h urine output of below 500 ml were defined as evidence of acute renal failure. The total transfusion volume for the first 5 days after trauma was also calculated.

Statistics

All data obtained were expressed as means ± SEM. Statistical analyses were made by one-way analysis of variance (ANOVA) with repeated measures, Student's paired and unpaired *t*-tests (two-tailed), and the chi-square test. Significance was confirmed at $P < 0.05$.

Results

The total transfusion volume of group B was significantly greater than that of group A, but no significant differences could be found among the other clinical data between the two groups (Table 1). On day 0 or 1 after trauma, 4,000 U of haptoglobin was administered to the 19 group B patients. On day 4, an additional 2,000 U of haptoglobin was given to two of these patients, and 4,000 U more was given to one patient.

Table 2 shows the changes in levels of serum total haptoglobin, total hemoglobin, and urine free hemoglobin. The total haptoglobin levels began to fall immediately after the trauma, but then increased to within normal limits on day 3. Haptoglobin therapy accelerated the recovery, but did not significantly decrease the increased levels of total hemoglobin and urine free hemoglobin. Urine free hemoglobin was detected in 61.8% (21/34) of the group A patients and 84.2% (16/19) of the group B patients.

The changes in serum free hemoglobin are shown in Fig. 1. In group B, the administration of haptoglobin led to a decrease in serum free hemoglobin, which

Table 1. Clinical data of the patients, treatment, and outcome

	Group A No haptoglobin (n = 34)	Group B Haptoglobin (n = 19)	P value
Age (years)	34.1 (2.8)	36.5 (4.3)	NS
Sex (male/female)	25/9	11/8	NS
Injury Severity Score	27.1 (2.0)	26.1 (3.1)	NS
APACHE II score	17.8 (1.8)	15.5 (1.9)	NS
Transfusion (ml)	3,447 (594)	10,146 (1,749)	$P < 0.01$
Surgery (yes/no)	21/13	13/6	NS
ARF (yes/no)	6/28	4/15	NS
DIC (yes/no)	8/25	5/14	NS
Outcome (survived/died)	23/11	11/8	NS

SEM are in parentheses
APACHE, Acute Physiology and Chronic Health Evaluation; ARF, acute renal failure; DIC, disseminated intravascular coagulation; NS, not significant

Table 2. Variations of serum total haptoglobin, hemoglobin, and urine free hemoglobin after trauma

		Day 0	Day 1	Day 3	Day 5	ANOVA
Serum total Haptoglobin (mg/dl)	A	69.4 (11.6)	93.3 (15.7)	128.6 (19.2)†	144.9 (20.3)†	$P < 0.01$
	B	73.2 (10.2)	196.5 (21.4)^{†,c}	165.8 (21.9)†	162.4 (28.8)**	$P < 0.001$
Serum total Hemoglobin (mg/dl)	A	16.8 (3.1)	8.8 (2.4)*	7.8 (1.4)**	8.1 (2.6)*	$P < 0.05$
	B	21.5 (5.3)	16.9 (5.7)^{a}	13.9 (3.4)	14.6 (3.0)^{b}	NS
Urine free Hemoglobin (mg/dl)	A$^{\#}$	8.9 (5.2)	4.0 (1.3)	2.2 (0.6)	10.6 (5.8)	NS
	B$^{\#}$	10.3 (5.5)	8.4 (4.7)	6.0 (2.6)	10.5 (6.3)	NS

SEM are in parentheses
A, no haptoglobin group (n = 34); A$^{\#}$ (n = 21); B, haptoglobin group (n = 19), B$^{\#}$ (n = 16); NS, not significant
* $P < 0.05$, ** $P < 0.01$, † $P < 0.001$ vs. Day 0, ^{a} $P < 0.05$, ^{b} $P < 0.01$, ^{c} $P < 0.001$ between A and B

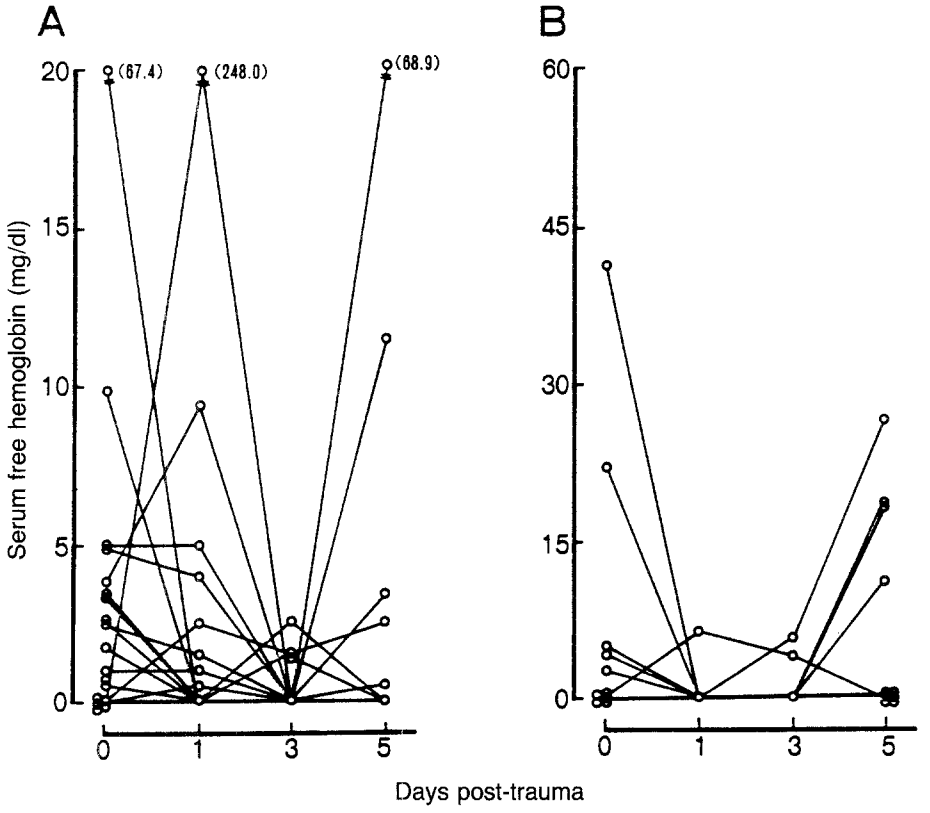

Fig. 1A,B. Changes in serum free hemoglobin levels after trauma. **A** group A (n = 18); **B** group B (n = 10). The data point for 0 mg/dl on days 1, 3, and 5 represent some cases

tended to rise again on day 3 or 5. When correlation was made by way of rectilinear regression, using the least squares methods, no definite correlation could be found between serum free hemoglobin and urine free hemoglobin in either group.

The effect of transfusion was investigated by dividing the total number of patients by the transfusion volumes: namely, those given transfusions of less than 1,000 ml, being 21 patients from group A who received 392 ± 95 ml, and 4 patients from group B who received 450 ± 250 ml; and those given transfusions over 1,000 ml being 13 patients from group A who received 5,942 ± 951 ml, and 15 patients from group B who received 12,460± 2,563 ml. In patients who received a transfusion volume of over 1,000 ml, the total haptoglobin remained at decreased levels, even on day 5, and urine free hemoglobin was still detected in those patients from group A (Fig. 2). In contrast, when the transfusion volume was low, the administration of haptoglobin inhibited the increase of total serum hemoglobin, and urine free hemoglobin was not detected at all (Fig. 3). When the transfusion volume was high, haptoglobin did not affect the changes in serum and urinary hemoglobin (Fig. 2).

Table 3 shows the changes in the various indices of renal function. All parameters, except for creatinine, became worse on day 5. Moreover, the NAG index, an index of renal proximal tubular function, was sig-

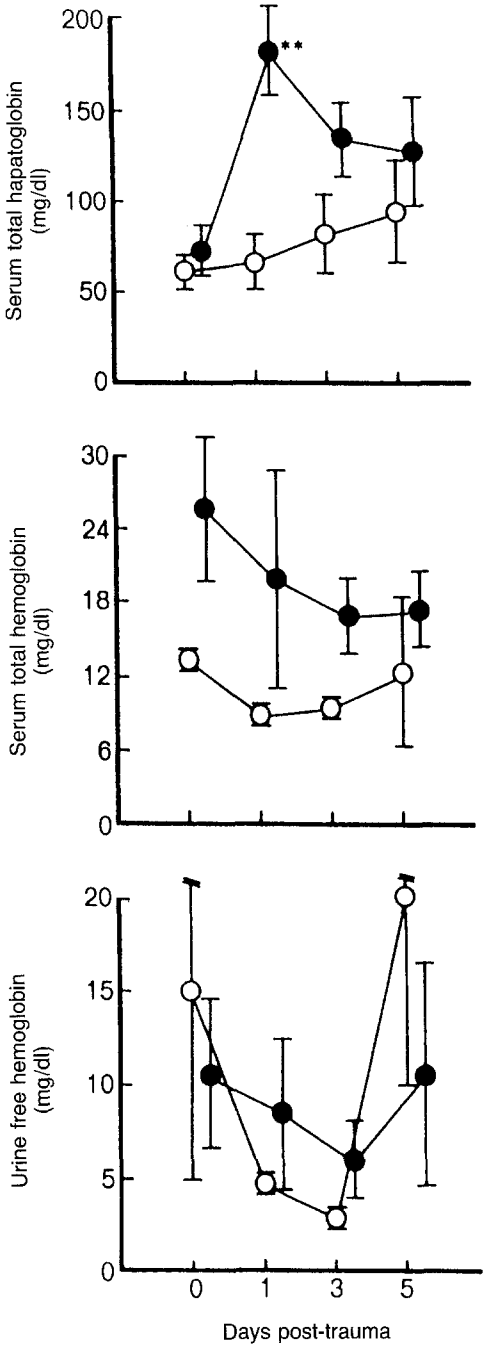

Fig. 2. Changes in serum total haptoglobin, total hemoglobin and urine free hemoglobin in patients who received a total transfusion volume of over 1,000ml. *Open circles*, group A (n = 13); *closed circles*, group B (n = 15). $^{**}P < 0.01$ vs. day 0

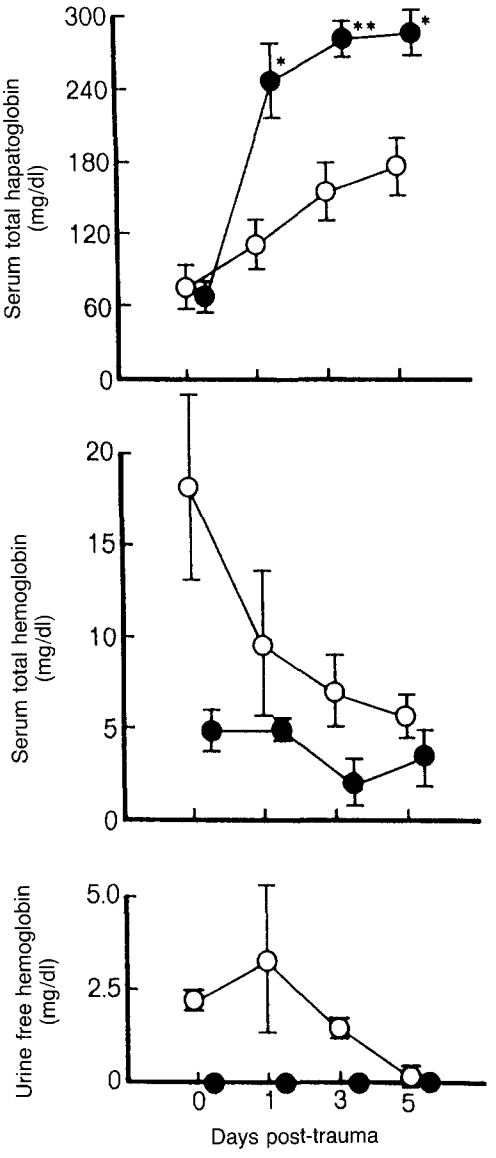

Fig. 3. Changes in serum total haptoglobin, total hemoglobin and urine free hemoglobin in patients who received a total transfusion volume of less than 1,000 ml. *Open circles*, group A (n = 21); *closed circles*, group B (n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. day 0

nificantly higher on days 0 and 5 in group B than in group A.

Discussion

The present study involved a detailed examination of the relationship between transfusion and haptoglobin therapy and the changes in haptoglobin and hemoglobin levels caused by trauma-induced hemolysis. The clinical application of haptoglobin preparations was first granted approval in Japan in 1985, and since then it has become widely used in the treatment of hemolysis due to burn injuries, massive transfusion, and extracorporeal circulation. It works on the basis that one unit of haptoglobin combines with 1 mg of hemoglobin, with a plasma elimination half-life ($t_{1/2}$) of about 20 h.

The changes in serum total haptoglobin observed in this study are consistent with the results reported

Table 3. Changes in renal function parameters

		Day 0	Day 5	*P* value
Creatinine (mg/dl)	A	1.2 (0.05)	1.4 (0.2)	NS
	B	1.1 (0.05)	1.4 (0.3)	NS
Creatinine clearance (ml/min)	A	107.2 (9.4)	75.9 (8.3)	$P < 0.05$
	B	94.9 (14.4)	66.7 (15.6)	$P < 0.05$
Serum β_2 microglobulin (μg/ml)	A	1.9 (0.5)	4.3 (1.9)	$P < 0.05$
	B	1.9 (0.2)	4.7 (1.3)	$P < 0.05$
Urine NAG index (U/L/Cr)	A	11.8 (1.7)	18.8 (3.3)	$P < 0.05$
	B	31.4 (8.0)*	133.6 (33.8)**	$P < 0.05$

SEM are in parentheses
NAG, *N*-acetyl-β-D-glucosaminidase; A, no haptoglobin group; B, haptoglobin group; NS, not significant
* $P < 0.05$, ** $P < 0.01$ between A and B

by Stahl[3] whose subjects suffered major abdominal trauma, although his report did not detail whether transfusion was performed or not, or how severe the major abdominal trauma was. In the present study, similar changes were observed in patients on whom the effect of transfusion could be neglected. It was also found that haptoglobin was decreased after severe trauma and that serum total hemoglobin levels changed reciprocally to the changes in haptoglobin, while urine free hemoglobin was detectable in many patients. These results indicate that the decrease in haptoglobin was caused by:

1. Mechanical hemolysis following damage of the vessel or tissues, or hemolysis caused by the absorption of a hematoma and ecchymosis via damaged blood vessels or lymphatics
2. The release of free hemoglobin and the formation of its complex with haptoglobin
3. Degradation of the complex by hepatocytes and reticuloendothelial cells

Thus, free hemoglobin exceeding the amount of haptoglobin passed through the renal glomerulus to become urine free hemoglobin.

About 30% of the subjects suffered complications consisting of disseminated intravascular coagulation (DIC). It is well known that, although infrequent, DIC follows intravascular hemolysis, or vice versa; however, it could not be concluded how DIC was related to hemolysis after trauma in this study. No definite correlation between serum free hemoglobin and urine free hemoglobin was found, which might be attributed to the fact that serum free hemoglobin was not directly determined but calculated. In fact, the serum free hemoglobin obtained in this study by haptoglobin based on the SRID method and equation 2 was relatively lower than that obtained by direct measurement using the enzyme-linked immunosorbent assay (ELISA) method, or by using haptoglobin based on the turbidimetric immunoassay and equation 2.[11]

Massive transfusion after trauma accelerated the increase in hemoglobin levels, and the decrease in haptoglobin levels, and the adverse effects of hemolysis following massive transfusion[4] can be considered to have added to the hemolysis caused by the trauma itself. Haptoglobin was effective for the treatment of hemolysis due to trauma; however, following massive transfusion, the administration of about 4,000 U of haptoglobin only tentatively increased the serum haptoglobin, while the increase of hemoglobin in the serum and urine persisted, which may be attributed to the incorrect determination of serum free hemoglobin, the low dose of haptoglobin, and the short plasma half-life of haptoglobin preparations.

The remarkable increase in the NAG index of group B could not be definitively explained, but of course the NAG index is not a specific indicator of proximal tubular dysfunction secondary to hemolysis. It is also well known that factors such as circulatory insufficiency (including dehydration and hypovolemic shock), hypoxemia, endotoxin, and DIC following trauma have an adverse affect on renal function. Therefore, the apparent change in renal tubular function could be attributed to these adverse factors; however, considering that there were no between-group differences in clinical data such as the ISS and APACHE II scores, haptoglobin may not have been sufficient to suppress the release of free hemoglobin caused by trauma and massive transfusion. Because there is no definite correlation between serum free hemoglobin and urine free hemoglobin, when serum free haptoglobin and free hemoglobin can not be accurately determined, the dose of haptoglobin should be decided by monitoring the disappearance of urine free hemoglobin.

Although the precise mechanism involved in the association of hemolysis with acute renal failure remains unknown, the mechanism of the occurrence of renal tubular disturbance due to free hemoglobin is assumed to involve cytotoxity of heme, a metabolite of hemoglobin,[12] and the formation of hemoglobin

casts.[13] In contrast, it is thought that free hemoglobin can not be the cause of renal failure by itself,[14] and that red cell stroma causes renal failure following hemolysis.[15] Rubiner et al.[16] also suggested that if the hemoglobin can be rendered stroma free, many or all of its deleterious effects on renal function may be prevented. The effects of hemolysis on renal function are probably due to the activation of blood coagulation which could be mediated by the stromal fraction of the red blood cells; which might be another reason why haptoglobin therapy did not improve deteriorated renal tubular function caused by massive transfusion after trauma.

In conclusion, the changes in serum haptoglobin, and serum and urine free hemoglobin after trauma were precisely monitored in this study. Our results indicated that massive transfusion sometimes results in increasing hemolysis due to trauma, leading to a raised free hemoglobin, which in turn may result in a deterioration of renal tubular function. Interestingly, haptoglobin therapy did not improve these changes. Nevertheless, a prospective randomized study is mandatory to further investigate these phenomena.

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