Free hemoglobin concentrations in patients receiving massive blood transfusion during emergency surgery for trauma

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Purpose: To determine free hemoglobin concentration in patients who received massive blood transfusion during emergency surgery for trauma with consideration of the storage of the transfused blood.

Methods: Fifteen patients undergoing emergency surgery for multiple trauma and who received blood transfusion of more than 5,000 mL were studied. Transfusion of the stored whole blood in citrate-phosphate glucose solution using a micropore filter was started before surgery. Serum concentrations of hemoglobin (total:THb and free:fHb) and total haptoglobin (THp) were measured until 5,000 mL of blood had been transfused. Serum free haptoglobin (fHp) concentration was calculated. The correlation between the changes in hemoglobin or haptoglobin concentrations and total storage days of the transfused blood was analyzed by a simple regression analysis.

Results: Free hemoglobin was detected after 2,000 mL transfusion. The THp and fHp decreased after 1,000 mL transfusion. Total storage time (days) of transfused blood had correlated with the changes of THp ($P < 0.0001$) and fHp ($P = 0.0027$) but not with the changes of THb ($P = 0.984$) and fHb ($P = 0.834$).

Conclusion: After blood transfusion during surgery for trauma, serum haptoglobin concentration decreased with transfusion of ≥ 1,000 mL of whole blood with mean storage time of 12.2 dy. Free hemoglobin was detected after 2,000 mL transfusion when THp decreased to 1,000 mg·L^{-1}. Serum haptoglobin concentrations correlated negatively with storage time (days) of transfused blood.

Objectif : Déterminer la concentration d'hémoglobine libre chez des patients qui reçoivent une transfusion sanguine massive, pendant une opération urgente pour trauma, en considérant le temps de conservation du sang transfusé.

Méthode : L'étude a porté sur 15 patients polytraumatisés, opérés d'urgence, qui ont reçu une transfusion de plus de 5 000 mL de sang. Avant l'intervention, on a commencé la transfusion de sang complet, conservé dans une solution de glucose citrate-phosphate, en utilisant un filtre micropore. Les concentrations sériques d'hémoglobine (totale : HbT et libre : Hbl) et d'haptoglobine totale (HpT) ont été mesurées jusqu'à ce que 5 000 mL de sang aient été transfusés. On a aussi noté l'haptoglobine sérique libre (Hpl). La corrélation entre les changements de concentrations d'hémoglobine ou d'aptoglobine et le nombre de jours de conservation du sang transfusé a été analysée par une analyse de régression simple.

Résultats : L'hémoglobine libre a été détectée après 2 000 mL de transfusion. L'HpT et l'Hpl ont baissé après 1 000 mL de transfusion. Le temps total de conservation (jours) du sang transfusé était en corrélation avec les modifications d'HpT ($P < 0,0001$) et d'Hpl ($P = 0,0027$), mais non avec les changements d'HbT ($P = 0,984$) ni d'Hbl ($P = 0,834$).

Conclusion : Après la transfusion de sang pendant une opération pour trauma, la concentration sérique d'haptoglobine décroît avec une quantité ≥ 1 000 mL de sang complet transfusé et selon un temps moyen de conservation de 12,2 jours. L'hémoglobine libre est détectée après la transfusion de 2 000 mL tandis que l'HpT baisse à 1 000 mg·L^{-1}. Les concentrations sériques d'haptoglobine sont en corrélation négative avec le temps de conservation du sang transfusé.

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TANSFUSION of large volumes of blood is often necessary in patients after major trauma,. Transfused blood contains free hemoglobin, as well as fragile erythrocytes that produce additional free hemoglobin.[1] Hemolysis of stored blood progresses with storage.[2] In addition, trauma causes intravascular hemolysis.[3] Hemolysis due to massive blood transfusion accompanied by hemolysis induced by trauma may have deleterious effects on organ function including the kidney.

Haptoglobin combines with free hemoglobin and the complex is not filtered at the glomerulus but is metabolized in the liver.[4] Thus, haptoglobin may prevent renal damage induced by free hemoglobin. Haptoglobin concentrations decrease with transfusion, and after trauma.[5] In our previous study,[6] 2,600 mL blood transfusion with mean storage time of 12.5 dy decreased serum total haptoglobin concentration to < 1,000 $mg \cdot L^{-1}$ and increased the detection of free hemoglobin in patients undergoing elective surgery. In trauma patients, more severe hemolysis, i.e. increase of free hemoglobin, may occur than in elective patients. Only one previous study has reported the effects of massive blood transfusion on hemolysis in trauma patients.[7] However, in that study, Gando and Tedo did not refer to the storage time of the transfused blood that might have a great effect on hemolysis. The purpose of the present study was to determine the concentrations of hemoglobin and haptoglobin (total and free) in patients receiving massive blood transfusion during surgery for trauma, with consideration of the storage days of the transfused blood.

Methods

The protocol was approved by the Institutional Review Board and written informed consent was obtained from patient's family. Fifteen patients undergoing emergency surgery after multiple trauma (seven with fractures of legs and pelvis, eight with abdominal hemorrhage) who were anticipated to receive > 5,000 mL of blood transfusion were included. Patients who had liver or renal damage or coagulopathy before study, or who underwent hepatic or renal surgery were excluded.

Before transfusion, a control blood sample (5 mL) was drawn from the catheter in the radial artery. Transfusion of the stored whole blood in citrate-phosphate glucose solution (CPD, supplied by Japanese Red Cross Society) started before surgery. All blood preparations were transfused with the use of micropore filters (pore size 40 μm). Arterial blood was again drawn to measure serum concentrations of hemoglobin and haptoglobin after 1,000, 2,000, 3,000, 4,000, and 5,000 mL of blood had been transfused. The sampled arterial blood was centrifuged to separate the serum which was freeze-stored at -20°C. Serum total hemoglobin concentration was measured by tetramethyl benzidine method (detection limit 0.775 $\mu mol \cdot L^{-1}$). Serum free hemoglobin concentration was measured by enzyme linked immunosorbent assay (detection limit 0.775 $\mu mol \cdot L^{-1}$). Serum total haptoglobin concentration was measured by immuno-nephelometry (detection limit 50 $mg \cdot L^{-1}$). All these measurements were done at the laboratory of the Green Cross Co. Ltd., Osaka, Japan. Serum free haptoglobin concentration was calculated by using the serum concentrations of total haptoglobin and total hemoglobin according to the method by Oomura *et al.*,[8] as follows: When serum total haptoglobin concentration > (1.42 × serum total hemoglobin concentration): serum free haptoglobin concentration = serum total haptoglobin concentration - 1.42 × serum total hemoglobin concentration. When serum total haptoglobin concentration ≤ (1.42 × serum total hemoglobin concentration): serum free haptoglobin concentration = 0.

The data are expressed as mean ± standard deviation. Statistical analysis was performed with one way repeated measures analysis of variance followed by the Student-Newman-Keuls test for the changes of hemoglobin and haptoglobin concentrations. The correlation between the % changes of serum concentrations of hemoglobin or haptoglobin and the total storage time (days) of the transfused blood was analyzed by a simple regression analysis. The total storage time of the transfused blood was the mean of the sum of days in storage of 5,000 mL (1 unit is 200 mL) of blood each patient received. P values less than 0.05 were considered statistically significant.

Results

There were eleven male and four female patients aged 57 ± 26 (range: 19~71) yr old. The duration of surgery was 383 ± 139 (155~625) min. Total volume of transfused blood was 6,990 ± 1,656 (5,000~9,800) mL and of infused crystalloid was 6,573 ± 1,278 (4,550~8,725) mL during surgery. Fresh frozen plasma of 1,020 ± 358 (400~1,600) mL was transfused. Platelets were not administered. Hematocrit before surgery was 26.2 ± 4.9 (19.0~33.8) % and after surgery was 36.5 ± 3.7 (30.2~40.8) %. The storage time of transfused blood averaged 12.2 ± 7.4 (4~20) days.

Serum total hemoglobin concentration increased in accordance with increased transfusion volume, $P <$ 0.05 after 3,000 mL (Figure 1, upper). Free hemo-

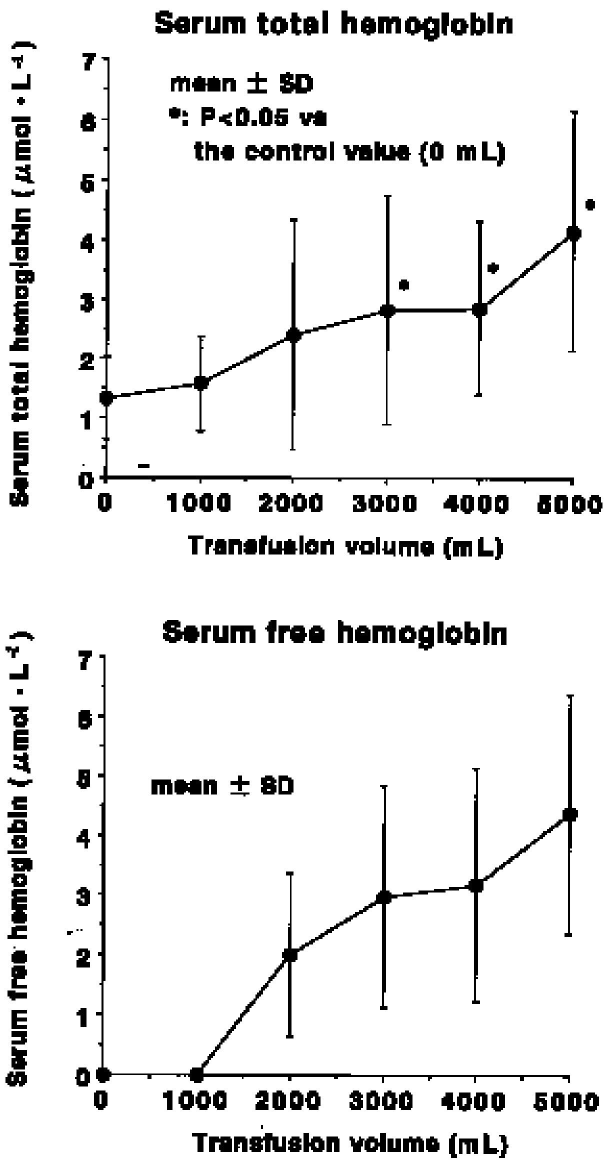

FIGURE 1 Serum total hemoglobin (upper) and free hemoglobin (lower) concentrations after blood transfusion.
Bars indicate standard deviation (SD). *: $P < 0.05$ *vs* the control value at 0 mL transfusion. In the lower figure, the control value (0) was excluded from the linear regression.

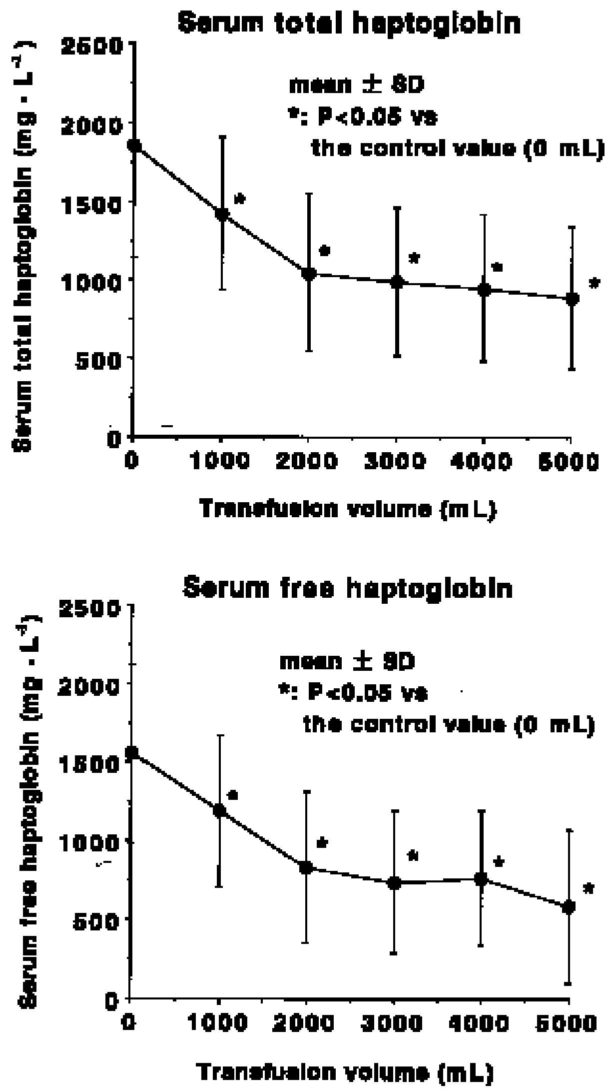

FIGURE 2 Serum total haptoglobin (upper) and free haptoglobin (lower) concentrations after blood transfusion.
Bars indicate standard deviation (SD). *: $P < 0.05$ *vs* the control value at 0 mL transfusion.

globin was detected after 2,000 mL transfusion (Figure 1, lower). Serum concentrations of total and free haptoglobin decreased with 1,000 mL of transfusion (Figure 2). Serum total haptoglobin concentration decreased to 1,000 $mg \cdot L^{-1}$ after 2,000 mL transfusion. After 5,000 mL transfusion, % changes of serum total haptoglobin concentration ($P < 0.0001$) and serum free haptoglobin concentration ($P = 0.0027$) correlate with the storage time of transfused blood (Figure 3). There was no correlation of % changes of serum total ($P = 0.984$) or free ($P = 0.834$) hemoglobin concentrations with storage time.

Discussion

During surgery after major trauma, serum haptoglobin concentration decreased after transfusion of 1,000

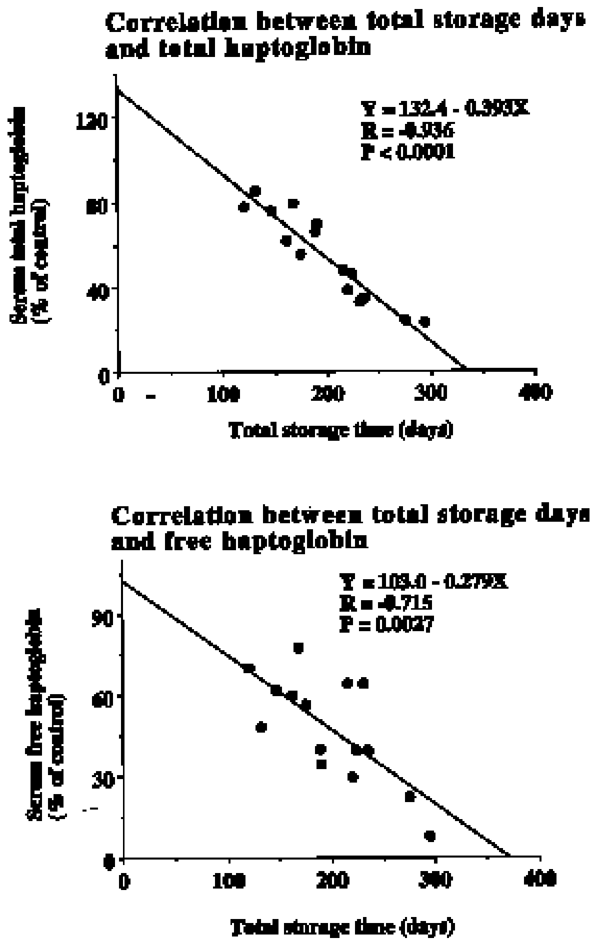

FIGURE 3 Correlation between total storage days of the transfused blood and serum total (upper) or free (lower) haptoglobin concentrations at 5,000 mL of transfusion.
Serum concentrations of both total and free haptoglobin have negative correlation with total storage.

mL whole blood with mean storage time of 12.2 days. Free hemoglobin was detected after 2,000 mL blood transfusion, when serum total haptoglobin concentration decreased to 1,000 mg·L^{-1}. Serum haptoglobin concentrations correlated negatively with storage time of transfused blood.

With massive blood transfusion, there is a risk of renal damage due to hemolysis. Red blood cells in the storage blood are fragile and are easily broken to produce free hemoglobin. Free hemoglobin has a potential to reduce glomerular filtration rate.[9] Heme proteins, metabolites of hemoglobin, damage renal tubular cells.[10] Renal tubular blockage by precipitated hemoglobin casts induces excretory failure.[6] Thus, increasing hemolysis, as shown by increasing free hemoglobin is deleterious to renal function. Massive blood transfusion accelerates the increase of hemoglobin concentration, and the adverse effects of hemolysis following massive blood transfusion add to the hemolysis caused by trauma itself.[12]

Factors such as circulatory insufficiency, hypoxemia, endotoxin, and disseminated intravascular coagulation following trauma have adverse effects on renal function. Free hemoglobin cannot be the cause of renal failure by itself:[13] red cell stroma causes renal failure following hemolysis.[14] However, the complex of haptoglobin with free hemoglobin is not filtrated in the glomerulus[4] which may precipitate and cause renal damage. That is also the reason why hemolysis may be deleterious for renal function. In the present study, we did not include renal function test data because it might be difficult to explore the relation of renal function and hemolysis due to the different time course of renal damage and hemolysis and also because there are many factors influencing renal function in major trauma.

The decrease of haptoglobin concentration may depend on the concentration of free hemoglobin which might increase according to hemolysis following damage of the vessel or tissue or to hemolysis by the absorption of hematoma. Free hemoglobin normally forms a complex with haptoglobin. This complex is degradable by hepatocytes and reticuloendothelial cells. The elimination half-life of the complex is about 20 hr.[7] Therefore, when we measured the serum concentration of haptoglobin just after blood transfusion, the complex might be detected as part of total haptoglobin. The effects of liver damage on haptoglobin concentration could not be neglected because in major trauma patients, liver itself or hepatic blood flow might be injured even though those who had liver damage were not included in the study.

In the present study of trauma patients, free hemoglobin was detected with transfusion of 2,000 mL of whole blood with mean storage time of 12.2 days, while the serum total haptoglobin concentration decreased to 1,000 mg·L^{-1}. These values were consistent with our previous study in elective surgery.[6] We expected that, in major trauma patients, hemolysis might occur more severely than in elective surgical patients previously reported[6] because major trauma might injure more epithelium of blood vessels, induce more cytokines to break erythrocyte, need more rapid blood transfusion. However, it was surprising that the present results showed little differences between the hemolysis in major trauma patients and in elective surgical patients. In addition, serum haptoglobin concentrations correlate well with the total storage days of transfused blood. It was suggested that consumption of haptoglobin was due to blood transfusion. However, serum hemoglobin concentrations did not correlate with the storage time of blood products. That might be due to hemolysis following damage of the vessel or tissue or to hemolysis by the absorption of hematoma rather than to hemolysis in the transfused blood alone.

Free hemoglobin increases and haptoglobin decreases with storage of blood.[2] In whole blood stored for 12.5 days, free hemoglobin concentration of 2.84 $\mu mol \cdot L^{-1}$ and total haptoglobin concentration of 880 $mg \cdot L^{-1}$ have been reported.[2] Since both the aging of the stored blood and the quantity of the blood transfused might be determinants of the serum concentrations of hemoglobin and haptoglobin, the total storage time of the transfused blood would have an impact on the hemoglobin and haptoglobin concentrations after transfusion. Without specifying how long the transfused blood had been in storage and without control values of haptoglobin, Gando and Tedo reported a serum total haptoglobin concentration of 694 $mg \cdot L^{-1}$ after 3,477 mL with blood transfusion in trauma patients.[7]

Haptoglobin therapy has been reported to be effective in the prevention of renal failure due to intravascular hemolysis and hemoglobinemia by thermal injury,.[5] However, Gando and Tedo reported that the administration of 4,000 units haptoglobin did not improve either the severity of hemolysis or the deterioration in renal tubular function caused by massive transfusion.[7] In their study, the control group was transfused 3,477 mL while the haptoglobin group was transfused 10,146 mL. It is, therefore, improper to compare the data between these two groups. In our present study patients were not treated with haptoglobin. Further study is necessary to confirm the effect of haptoglobin therapy.

In conclusion, during surgery for major trauma, serum haptoglobin concentration decreased after blood transfusion of 1,000 mL or more whole blood with a mean storage time of 12.2 dy. Free hemoglobin was detected after transfusion of 2,000 mL when total haptoglobin concentration decreased to 1,000 $mg \cdot L^{-1}$. Serum haptoglobin concentrations correlated negatively with storage time (days) of transfused blood. Therefore, transfusion of old blood might decrease serum haptoglobin, which increases free hemoglobin.

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