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Minimal hemolytic effect of veno-venous bypass during liver transplantation

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Abstract. During liver transplantation, extracorporeal veno-venous bypass (VVBP) from the lower to the upper part of the body is used to prevent the negative circulatory effects otherwise seen on clamping the inferior caval vein during the actual change of livers. Extracorporeal circulation can sometimes be complicated by hemolysis. Therefore, we wanted to determine whether the technique used during liver transplantation might also cause hemolysis. This was measured as plasma hemoglobin using a spectrophotometric method including tetramethylbenzidine. Of 11 patients tested, 5 showed an increase in plasma hemoglobin during the use of VVBP; in no case, however, was this clinically significant. Three patients showed short peaks of hemoglobin during bypass. In two patients the rise continued after bypass and they required higher pump speed than the six patients without hemolysis (mean ± SD 1900 ± 150 RPM vs 1700 ± 60 RPM, respectively). Pump flow, time of VVBP, and numbers of transfusions during the transplantation did not differ between the groups. We conclude that VVBP used during liver transplantation may cause hemolysis, but with low frequency and low clinical significance. We further conclude that the spectrophotometric method used is reliable and sensitive enough to determine the degree of hemolysis.

Key words: Liver transplantation, veno-venous bypass – Veno-venous bypass, liver transplantation – Hemolytic effect, veno-venous bypass

Most liver transplant centers use a veno-venous bypass (VVBP) system during liver transplantation. The rationale behind the use of extracorporeal circulation is the necessity to clamp the inferior caval and portal veins during the phase of the operation in which the old and diseased liver is taken out and the new liver is not yet in place. This clamping causes great disturbances in the central circulation with a 50% reduction in venous return and, as a result, a secondary reduction in blood pressure, central venous pressure, and cardiac output.

Furthermore, the clamping causes a pooling of blood in the lower part of the body with stasis in varicose veins that may increase bleeding diathesis and cause a rise in potassium and a fall in pH. This, in turn, may result in cardiac arrest when the circulation through the new liver is opened again.

The most commonly used veno-venous bypass is one in which the blood is recirculated from the lower part of the patient via the femoral and portal veins to the central circulation via the axillary vein. The blood is pumped using a centrifugal pump and the bypass flow is usually 1–3 l/min. This method is routinely used in many centers during liver transplantation and was first described by Shaw et al. [4]. However, this technique may itself cause problems. For example, when using extracorporeal bypass during cardiac surgery, the destruction of red cells with hemolysis is sometimes seen [1].

This study was performed in order to investigate whether VVBP used during liver transplantation may also cause hemolysis, tested as a rise in plasma hemoglobin.

Materials and methods

Eleven liver transplant recipients (six men and five women) were included in the study. Their age was 41 ± 15 (mean ± SD) years and their weight 61 ± 11 kg. Nine patients were transplanted because of chronic liver failure and two because of metabolic diseases.

VVBP was performed using a centrifugal blood pump (Bio-Pump, Bio-Medicus, Eden Prairie, Minn., USA). This system utilizes the constrained forced-vortex pumping principle, where the blood is allowed to pass through a vortex created by the rotation of a series of cones. This nonocclusive hemodynamic design reduces trauma on blood elements and should thereby decrease hemolysis. In all but one patient, the tubing used was of a type with heparin covalently bound to all the plastic surfaces in contact with the blood. The binding was performed by Carmeda (Stockholm, Sweden). In the first patient we tested, only the tubing in contact with the vessels was heparinized. VVBP was used during the anhepatic phase of the operation for 84 ± 27 min.

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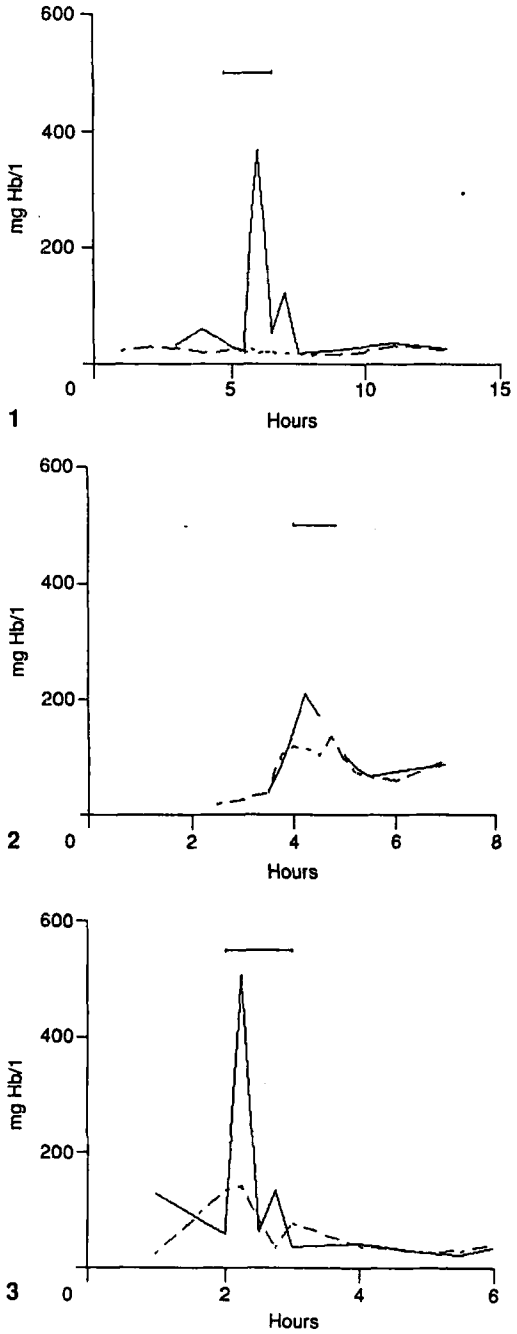

Figs. 1–3. Three patients with temporarily increased levels of plasma hemoglobin during VVBP. *Solid lines* indicate pulmonary artery samples. *Broken lines* indicate radial artery samples. VVBP time during operation is indicated as a *straight line*

Blood samples were obtained without pressure stasis from the radial and pulmonary arteries every 15 min from 1 h before starting VVBP until 1 h after terminating VVBP. During the rest of the operation, samples were obtained every hour. In two patients samples were obtained only from the pulmonary artery and in one patient, only from the radial artery.

The samples were collected in 5-ml vials with sodium citrate as anticoagulant. The vials were immediately gently mixed and within 8 h centrifuged at 800 *g* for 10 min. The supernatant plasma was removed and centrifuged at 800 *g* for 5 min to assure that all erythrocytes had been removed. The plasma samples were stored at −70 °C until analyzed.

Hemoglobin assay

We used the spectrophotometric method described by Standefer and Vanderjagt [5] with the modifications of Geissler and Stith [2]. Hemoglobin catalyzes the oxidation of 3,3′-5,5′-Tetramethylbenzidine (TMB) in the presence of hydrogen peroxide (H_2O_2). The reaction produces a green color, the intensity of which is directly related to the concentration of hemoglobin in the sample.

The normal value of plasma hemoglobin is less than 50 mg/l if pressure stasis is used during sampling and less than 5 mg/l without stasis. These low values necessitate a method of analysis that is sensitive and precise in order to be able to study variations during a course like VVBP.

Ten microliters of the plasma sample was added to 0.5 ml of a solution containing 5 g/l of crystalline 3,3′-5,5′-TMB (Sigma, St. Louis, Mo., USA) in acetic acid, 90% (v/v). The solution was gently mixed and the oxidation process was initiated by adding 0.5 ml of a 1% hydrogen peroxide solution. After 25 min of incubation, the reaction was stopped by adding 5 ml of a diluent solution, acetic acid, 10% (v/v).

The samples were then analyzed in a spectrophotometer (DMS 100, Varian, Australia) at wavelength 375 nm. The blank contained 0.5 ml TMB solution, 0.5 ml peroxide solution, and 5 ml diluent solution. The plasma hemoglobin concentration was calculated from a standard curve prepared as described by Geissler and Stith [2].

Statistical analysis

Results are presented as mean ± SD. Pulmonary artery and radial artery sample results have been compared statistically using the *t*-statistic evaluation for paired observations. Differences have been considered statistically significant if $P < 0.05$.

Results

Before VVBP

All patients were screened for irregular erythrocyte antibodies. Only one patient had a hemolytic erythrocyte antibody of type anti-Kell (Fig. 5). All but one patient had a stable level of plasma hemoglobin with no signs of progressive hemolysis. One patient, in whom only pulmonary artery samples were collected, showed an increased level in plasma hemoglobin with 360 mg/l 90 min before starting VVBP. The peak was seen in a single sample and the concentration was normal at the start of VVBP. There were no significant differences between pulmonary and radial artery samples in the other patients.

During VVBP

Six patients had stable levels of plasma hemoglobin throughout VVBP with no signs of hemolysis. Three patients showed a temporarily increased level in pulmonary artery samples with maximum levels of 368 mg/l, 210 mg/l, and 508 mg/l, respectively (Figs. 1–3). VVBP flow and speed data did not differ between these patients (Table 1).

Two patients showed a continuously increasing level of plasma hemoglobin beginning 1 h after the start of VVBP (Figs. 4, 5). One of these two patients had a clearly observable increase in both the pulmonary and radial artery sam-

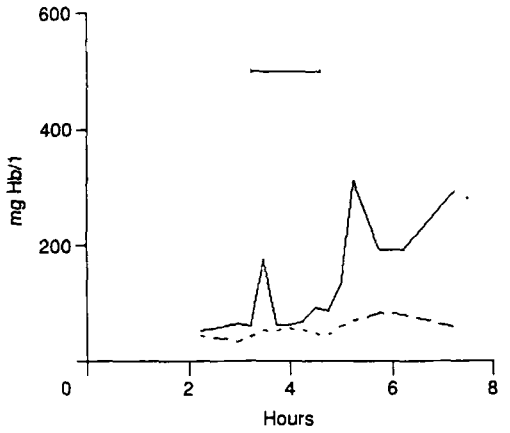

Fig. 4. Patient with increased level of plasma hemoglobin in the pulmonary artery samples. The maximum peak (313 mg/l) is seen immediately after terminating VVBP

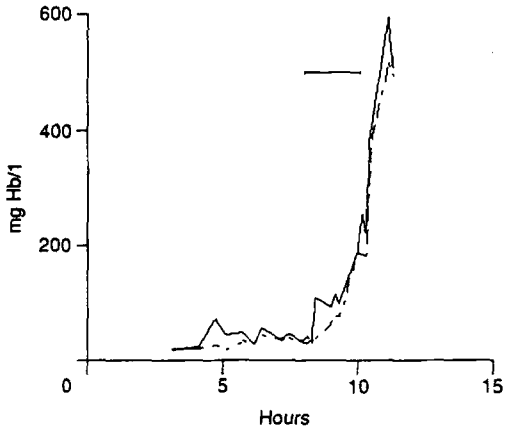

Fig. 5. Patient with increased level of plasma hemoglobin in both the pulmonary and the radial artery samples showing clinical signs of hemolysis. Maximum peak (594 mg/l) is seen 1 h after terminating VVBP

ples (Fig. 5), and this patient also had clinical signs of hemolysis with red urine. Unfortunately, the urine was not tested for plasma hemoglobin.

VVBP speed was higher in these two patients than in the six patients without hemolysis. Pump speed in the two groups was 1900 ± 150 RPM and 1700 ± 60 RPM, respectively. Time on VVBP, pump flow, and intraoperative blood transfusion did not differ between the groups (Table 1).

There were no significant differences between pulmonary and radial artery samples, except for the patient shown in Fig. 1 and for the patient with clinically noticed hemolysis (Fig. 5), where levels in the pulmonary artery were significantly higher.

After VVBP

Immediately after VVBP was terminated, a small peak was seen in the plasma hemoglobin level in most of the patients, especially in the pulmonary artery samples (Figs. 1–3). However, there were no significant differences between pulmonary and radial artery samples. The two

Table 1. Veno-venous bypass and transfusion data

Plasma hemoglobin	Pump flow (l/min)	Pump speed (RPM)	Time on VVBP (min)	Intraoperative transfusion (units)
Stable ($n = 6$)	1.8 ± 2	1700 ± 60	88 ± 23	10.8 ± 5.5
Temporary increase ($n = 3$)	1.6 ± 0.3	1700 ± 130	89 ± 22	17.7 ± 6.8
Continuous increase ($n = 2$)	1.7 ± 0.2	1900 ± 150	116 ± 12	18.5 ± 16.3

patients showing a continuous increase in the plasma hemoglobin level during VVBP both showed a further increase to maximum levels of 313 mg/l and 594 mg/l, respectively. The levels in the pulmonary artery samples were significantly higher than those in the radial artery samples in both of these cases.

Discussion

In this study we measured plasma hemoglobin levels in 11 patients before, during, and after the use of veno-venous bypass during liver transplantation to evaluate any hemolysis caused by the procedure. Extracorporeal circulation may cause hemolysis when, for example, roller pumps are used in cardiac surgery. One could, however, speculate that hemolysis might also appear in centrifugal pumping when high pump speed is required. High pump speed may be required for acceptable pump flows, especially if the axillary vein is narrow. This might have been the case in two of our patients with hemolysis, as both patients required a higher pump speed.

The difference in plasma hemoglobin concentration between the samples from pulmonary and radial artery could be the effect of dilution in the systemic circulation or pulmonary uptake of free hemoglobin. There are other reasons for elevations in plasma hemoglobin levels during liver transplantation besides their being caused by VVBP. The slight rise seen in most patients immediately after terminating VVBP coincides with unclamping of the new liver graft and may eventually be due to remnants of the preservation solution used for the liver (UW solution, DuPont, The Netherlands and Perfadex, Pharmacia, Uppsala, Sweden) entering the circulation. The preservation solutions contain many different substances released from the liver during storage, and some of them may be hemolytic. Another cause may be that residual erythrocytes in the new liver graft have been hemolyzed during storage and that hemoglobin has been released into the circulation.

An additional possible cause is irregular erythrocyte antibodies in the recipient. Liver recipients are usually multitransfused before the operation. In our study, 64% of the patients received transfusions prior to the operation.

Yet another cause may be that the new liver contains immunologically active cells directed towards the reci-

pient's erythrocyte antigen. It is clearly shown that the liver is immunologically capable. In ABO-compatible transplantations, i.e., a blood group 0 to A transplantation, as many as 25% of the patients develop postoperative hemolysis after some weeks by a graft-versus-host (GVH) reaction [3].

In two patients we found a significant increase in plasma hemoglobin levels starting during VVBP and continuing after termination of VVBP (Figs. 4, 5). The first of these patients showed no clinical signs of hemolysis. The donor and the recipient were both of blood group A_1, Rh-pos, and neither of them had any irregular erythrocyte antibodies.

The second patient had a hemolytic, irregular erythrocyte antibody identified as anti-Kell. By tracing all blood units transfused during the operation, we could eliminate the possibility that Kell-positive blood was given as a reason for the hemolysis. The donor was Kell-negative, so Kell-antigen from the donor is also ruled out as a reason for hemolysis. The donor liver was of the same blood group as that of the recipient (B, Rh-pos) and there were no signs of irregular erythrocyte antibodies in the donor. This speaks strongly against a hemolysis caused by a GVH reaction, together with the fact that this reaction usually appears later in the course, after some weeks have passed. There might have been some differences in minor blood groups between the donor and the recipient, but these minor blood groups seldom cause hemolysis.

In sum, we found that extracorporeal veno-venous bypass used during the anhepatic phase of liver transplantation may have caused laboratory hemolysis in 5 of our 11 patients. In none of these patients did the hemolysis cause any clinical problems. We therefore conclude that veno-venous bypass can safely be used in liver transplantation.

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