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Effects of Acute Normovolemic Hemodilution on Perioperative
Coagulation and Fibrinolysis in Elderly Patients
Undergoing Hepatic Carcinectomy[△]

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Objective To observe the effects of acute normovolemic hemodilution (ANH) on coagulation function and fibrinolysis in elderly patients undergoing hepatic carcinectomy.

Methods Thirty elderly patients (aged 60-70 years) with liver cancer (American Society of Anesthesiologists physical status I-II) scheduled for hepatic carcinectomy from February 2007 to February 2008 were randomly divided into ANH group ($n=15$) and control group ($n=15$). After tracheal intubation, patients in ANH group and control group were infused with 6% hydroxyethyl starch (HES) (130/0.4), and basic liquid containing 6% HES and routine Ringer's solution, respectively. In all the studied patients, blood samples were drawn at five different time points: before anesthesia induction (T_1), 30 minutes after ANH (T_2), 1 hour after start of operation (T_3), immediately after operation (T_4), and 24 hours after operation (T_5). Then coagulation function, soluble fibrin monomer complex (SFMC), prothrombin fragment (F1+2), and platelet membrane glycoprotein (activated GPIIb/GPIIIa and P-selectin) were measured.

Results The perioperative blood loss was not significantly different between the two groups ($P>0.05$). The volume of allogeneic blood transfusion in ANH group was significantly smaller than that in control group (350.5 ± 70.7 mL vs. 457.8 ± 181.3 mL, $P<0.01$). Compared with the data of T_1 , prothrombin time (PT) and activated partial thromboplastin time in both groups prolonged significantly after T_3 ($P<0.05$), but still within normal range. There were no significant changes in thrombin time and D-dimer between the two groups and between different time points in each group (all $P>0.05$). SFMC and F1+2 increased in both groups, but without statistical significance. P-selectin expression on the platelet surface of ANH group was significantly lowered at T_2 and T_3 compared with the level at T_1 ($P<0.05$). Compared with control group, P-selectin was sig-

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nificantly lower in ANH group at T_2 - T_3 (all $P < 0.05$).

Conclusions In elderly patients undergoing resection of liver cancer, ANH may not hamper fibrinolysis and coagulation function. It could therefore be safe to largely reduce allogeneic blood transfusion.

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HEPATIC carcinectomy is always associated with massive blood loss. In recent years, acute normovolemic hemodilution (ANH) has been widely used to prevent the complications associated with massive homologous blood transfusion. But the influences of ANH on coagulation function are still controversial with different coagulation parameters, measuring approaches, and time points. The influence on platelet function has currently become one of the important parameters in perioperative monitoring of coagulation function, and the fast developing technique of flow cytometry allowed us to obtain accurate specific parameters for evaluating activity of platelet *in vivo*.¹ Soluble fibrin monomer complex (SFMC) and prothrombin fragment 1+2 (F1+2), being active markers of coagulation with higher specificity, were demonstrated to be sensitive parameters of prethrombotic state in early stage.^{2,3} The present study was designed to investigate the feasibility of ANH in elderly hepatoma patients by observing its influences on perioperative coagulation function, prethrombotic state, and platelet membrane glycoprotein.

PATIENTS AND METHODS

Patient enrollment

A total of 30 elderly patients scheduled to have hepatic carcinectomy from February 2007 to February 2008 were selected in our study. The inclusion criteria were as follows: American Society of Anesthesiologists (ASA) physical status I-II; aged 60-70 years; weighing 45-74 kg; no severe dysfunction of liver, kidney, or coagulation system; no severe pulmonary or cardiovascular diseases; no anti-coagulation medication in the previous 2 weeks; pre-operative hematocrit (Hct) $> 35\%$ and hemoglobin (Hb) > 120 g/L. The patients were randomly divided into ANH group ($n=15$) and control group ($n=15$). The protocol of this trial was approved by the Ethic Committee of Lihuli Hospital, and written informed consents were obtained from the patients or their next of kin.

Anesthesia and intervention

All the patients were premedicated with 0.1 g phenobar-

bital sodium and 0.5 mg atropine 30 minutes before surgery. Electrocardiogram, heart rate, blood pressure (BP), and saturation of peripheral oxygen were monitored routinely with Philips MP60 in the operation room. Left radial artery was cannulated for BP monitoring and blood gas detection, and right internal jugular vein was cannulated for transfusion and central venous pressure monitoring, respectively. Ringer's lactate solution was transfused at $8-10 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ to compensate the loss of body fluid due to deprivation of water and food. Anesthesia was induced with intravenous administration of 0.03-0.05 mg/kg midazolam, 3-5 $\mu\text{g/kg}$ fentanyl, 1-2 mg/kg propofol, and 0.6 mg/kg rocuronium. After tracheal intubation, the endotracheal tube was connected with anesthetic machine (Cicero EM, Lubeck, Germany). The tidal volume of mechanical ventilation was set at 8-10 mL/kg and the frequency at 12-14 breath per minute to maintain an end-tidal CO_2 pressure of 30-40 mm Hg. Anesthesia was stabilized with propofol, fentanyl, atracurium, and intermittent inhalation of isoflurane at a low concentration. Bispectral index (BIS) value was controlled within the range of 45-60.

Volume of blood loss was estimated based on the blood volume in suction bottle and the weight of bandage. Intraoperative transfusion volume was the sum of physiological demand, loss volume after fasting, loss volume of the third space, and intraoperative loss volume. Patients in ANH group were treated with ANH in which whole blood was collected from internal jugular vein (200-300 mL/min) and equal volume of 6% medium-molecular-weight hydroxyethyl starch (HES) 130/0.4 (Voluven, WC730206, Fresenius Kabi, Bad Homburg, Germany) was transfused. The volume of whole blood collection was calculated based on estimated blood volume (EBV, body weight $\times 70 \text{ mL/kg}$ in male and body weight $\times 60 \text{ mL/kg}$ in female), pre-dilution Hct ($\text{Hct}_{\text{actual}}$), and post-dilution Hct ($\text{Hct}_{\text{ideal}}$), with the following formula: $\text{EBV} \times 2 \times (\text{Hct}_{\text{actual}} - \text{Hct}_{\text{ideal}}) / (\text{Hct}_{\text{actual}} + \text{Hct}_{\text{ideal}})$. In the present study, $\text{Hct}_{\text{ideal}}$ was set at 28%. The collected blood was stored in acid-citrate-dextrose blood bank at room temperature and transfused back to the patients in ANH group after the operation or when necessary. Control group did not re-

ceive hemodilution during operation but basic liquid containing lactated Ringer's solution and 6% HES (130/0.4). Intraoperative hemodynamic parameters were monitored continuously. Homologous packed red blood cells could be transfused properly when Hb was lower than 8 g/L and Hct lower than 25%.

Measuring parameters

Blood samples were collected from right internal jugular vein at five different time points: before anesthesia induction (T_1), 30 minutes after ANH (T_2), 1 hour after start of surgery (T_3), immediately after surgery (T_4), and 24 hours after surgery (T_5). The collected blood was then anti-coagulated with 0.2 mL citrate sodium. The anti-coagulated blood was centrifuged at $1\,006.2 \times g$ for 8 minutes, then the light yellow supernatant was extracted and stored at -80°C . F1+2 and SFMC were assayed by enzyme-linked immunosorbent assay (ELISA) in Model 680 enzyme-labelling instrument (BIO-RAD, CA, USA) following the manufacturer's instruction (ADL, USA). Platelet membrane glycoprotein was determined within 24 hours with a FACSCalibur flow cytometer (BD Biosciences, CA, USA). PAC-1 FITC was fluorescein isothiocyanate-labelled monoclonal antibody of activated GPIIb/GPIIIa (fibrinogen receptor); CD62P PE was phycoerythrobilin-labelled monoclonal antibody against P-selectin; MIgG PE was phycoerythrobilin-labelled mouse IgG; and MIgM FITC was fluorescein isothiocyanate-labelled mouse IgM. All those four antibodies were produced by BD Biosciences. The gate was determined with CD61 peridinin chlorophyll protein (PerCP)-positive platelets and two-parameter analysis of PAC-1-FITC vs. CD62P PE dot plots was performed.

Statistical analysis

The measurement data were expressed as means $\pm$ SD. All statistical analyses were conducted with SPSS 11.0. Group comparison was analyzed by *t*-test and intra-group comparison by two-way analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

General data including age, gender, weight, surgery type, and intraoperative blood loss were not significantly different between the two groups ($P > 0.05$). But blood

transfusion volume in ANH group was significantly smaller than that in control group ($P < 0.01$) (Table 1).

Compared with the figures before dilution, PT and APTT at T_2 and T_3 were significantly prolonged in ANH group (all $P < 0.05$); PT and APTT in both groups prolonged significantly after T_3 (all $P < 0.05$), but they were all within normal range. Inter-group comparison of these two parameters did not exhibit significant difference ($P > 0.05$). The concentration of fibrinogen (FIB) was significantly reduced after ANH ($P < 0.05$), but much higher than the lower limit. It was increased after autologous blood transfusion. Thrombin time (TT) and D-dimer were found similar in comparison among different time points and between the two groups; both had no significant intra-group or inter-group difference (all $P > 0.05$) (Table 2).

SFMC and F1+2 were not significantly increased after surgery and there was no significant inter-group difference ($P > 0.05$) (Table 2).

After hemodilution, P-selectin expression in ANH group was found significantly decreased ($P < 0.05$) while activated GPIIb/GPIIIa expression was only slightly reduced ($P > 0.05$). When compared with control, P-selectin expression in ANH group was significantly reduced at T_2 - T_5 (all $P < 0.05$) while no such inter-group difference was demonstrated in activated GPIIb/GPIIIa expression ($P > 0.05$) (Table 2).

DISCUSSION

All coagulation factors other than factor VII are synthesized in liver, and hepatopathy is usually associated with abnormal coagulation function. Massive bleeding might be encountered during hepatic surgery due to the abundant blood supply to liver. Blood conservation measures including ANH have been applied in our department to prevent or reduce complications of homologous transfusion. Yet the application of ANH remained controversial because of its influence on coagulation function. It was reported that rapid transfusion of large dose HES was closely associated with coagulation suppressing effect to different degrees, demonstrating as prolonged APTT.⁴ But the coagulation status after ANH was generally considered acceptable as long as platelet was maintained at a level over $5 \times 10^{10}/\text{L}$,⁵ coagulation factors did not reduce by 20%-30% compared with normal levels, FIB was not lower than 0.75 g/L,⁶ and massive blood loss could be pre-

Table 1 . General information and intraoperative data in two groups[§] ($n=15$)

Group	Gender (male/female)	Age (yr)	Weight (kg)	Blood loss (mL)	Blood transfusion (mL)
Control	10/5	64.3 $\pm$ 10.1	54.1 $\pm$ 9.2	734.7 $\pm$ 83.1	457.8 $\pm$ 181.3
ANH	12/3	65.7 $\pm$ 8.1	56.8 $\pm$ 8.3	710.9 $\pm$ 75.9	350.5 $\pm$ 70.7*

§: Plus-minus values are means±SD.

ANH: acute normovolemic hemodilution.

* $P<0.01$ compared with control.

Table 2. Parameters of coagulation function, changes of SFMC and F1+2, and changes of platelet membrane glycoproteins in two groups[§]

Item	Group	T ₁	T ₂	T ₃	T ₄	T ₅
Coagulation function Parameters						
PT (s)	Control	12.3 ± 1.2	13.2 ± 1.5	13.5 ± 1.7*	13.8 ± 1.4*	13.6 ± 1.5*
	ANH	12.1 ± 1.1	14.2 ± 1.9**	14.3 ± 1.5**	13.7 ± 1.2**	13.5 ± 1.2**
APTT (s)	Control	31.4 ± 6.6	32.1 ± 6.1	34.3 ± 5.2*	36.3 ± 6.7*	34.9 ± 6.1*
	ANH	33.1 ± 5.6	35.7 ± 6.2*	37.4 ± 6.1*	37.0 ± 6.5*	35.3 ± 6.7*
FIB (mg/dL)	Control	492 ± 71	435 ± 81*	413 ± 65**	419 ± 77*	432 ± 83*
	ANH	463 ± 68	385 ± 91*	361 ± 82**	397 ± 72*	411 ± 82*
TT (s)	Control	14.5 ± 1.7	14.5 ± 2.3	14.6 ± 3.0	14.9 ± 3.2	14.7 ± 2.5
	ANH	14.7 ± 1.5	14.8 ± 1.6	15.2 ± 4.6	15.7 ± 4.3	14.9 ± 3.4
DD (ng/mL)	Control	169 ± 36.5	180 ± 33.4	187 ± 39.5	194 ± 38.1	189 ± 32.6
	ANH	178 ± 31.3	188 ± 44.1	195 ± 35.3	201 ± 37.6	181 ± 33.5
Markers of activated coagulation						
SFMC (mg/mL)	Control	52.6 ± 10.1	54.6 ± 11.7	56.6 ± 12.8	57.7 ± 9.2	54.9 ± 10.1
	ANH	48.4 ± 6.2	50.6 ± 10.8	54.0 ± 9.1	55.0 ± 11.9	53.6 ± 11.4
F1+2 (nmol/L)	Control	0.73 ± 0.28	0.78 ± 0.24	0.86 ± 0.30	0.90 ± 0.26	0.85 ± 0.30
	ANH	0.70 ± 0.20	0.77 ± 0.33	0.86 ± 0.32	0.91 ± 0.38	0.87 ± 0.41
Platelet membrane glycoproteins						
P-selectin (%)	Control	1.9 ± 0.7	1.7 ± 0.6	1.8 ± 0.5	2.0 ± 0.6	2.1 ± 0.6
	ANH	1.7 ± 0.4	1.3 ± 0.4* [#]	1.3 ± 0.3* [#]	1.4 ± 0.4 [#]	1.6 ± 0.4 [#]
Activated GPIIb/GPIIIa (%)	Control	25.2 ± 5.2	24.3 ± 6.2	24.8 ± 6.5	24.2 ± 5.9	25.5 ± 6.2
	ANH	24.7 ± 4.8	21.0 ± 4.1	19.5 ± 4.9	20.6 ± 6.8	23.1 ± 7.0

§: Plus-minus values are means±SD.

T₁: before anesthesia induction; T₂: 30 minutes after ANH; T₃: 1 hour after start of surgery; T₄: immediately after surgery; T₅: 24 hours after surgery.

PT: prothrombin time; APTT: activated partial thromboplastin time; FIB: fibrinogen; TT: thrombin time; DD: D-dimer; SFMC: soluble fibrin monomer complex; F1+2: prothrombin fragment.

* $P<0.05$, ** $P<0.01$ compared with T₁; [#] $P<0.05$ compared with control.

vented during surgery. But the parameters of extrinsic coagulation pathway such as APTT, PT, and TT could only reflect the levels of coagulation factors, but not the activation of coagulation pathway.³ To detect the activation of coagulation pathway, the present study used ELISA to determine the amounts of F1+2 and SFMC. The two substances are molecular fragments generated during the process of coagulation activation and the production of thrombin and fibrinogen. Thrombinogen is split under the action of factor Xa at the amino terminal into fragments of F1+2 and coagulation prozymogen 2, and the latter is transformed into α -thrombin, which is further involved in the activation of coagulation system.^{7,8} Therefore, plasma F1+2 could demonstrate both the activity of factor Xa and coagulation state.⁹ Under the effect of thrombin, α and β chains of FIB release peptides A and B, and the remaining FIB I and II aggregate together to form SFMC, thus named because of its solubility in 5 mol·g⁻¹·L⁻¹ urea. SFMC could therefore reflect the activity of thrombin. Elevation of the circulating levels of SFMC and F1+2 thus indicated the

activation of coagulation pathway.

Platelet membrane glycoprotein plays an important role in adhesion, aggregation, and release of platelets. Whole blood flow cytometry was applied in the present study to determine the expression of activated GPIIb/GPIIIa and P-selectin. GPIIb/IIIa complex forms on the surface of platelet membrane during platelet activation, and the activated GPIIb/IIIa complex is an early marker of platelet activation. Increased molecular amount of activated GPIIb/GPIIIa suggests the enhancement of platelet adhesiveness. Another membrane glycoprotein, P-selectin, is a later marker of platelet activation.¹⁰ It is found in A2 particles within platelet cytoplasm after the activation of platelets. Expressed on the surface of platelets, this type of glycoprotein bound specifically with activated platelets for at least 1 hour. Since the survival time of activated platelets in circulation is very short, the hemostatic function of platelets could be heavily affected in over-activation. Therefore, it is important to determine the activation degree of platelets based on the expression level

of membrane glycoprotein.

Piecuch et al¹¹ measured some parameters such as thrombin-antithrombin-III complex, plasmin- α_2 -antiplasmin complex, F1+2, and DD in a study on ANH during hip arthroplasty, and found that ANH did not influence intraoperative coagulation and fibrinolysis, and the incidence of postoperative thrombosis was reduced. The present study demonstrated that SFMC and F1+2 in both ANH and control groups did not change significantly after surgery, but they demonstrated an increasing trend, indicating the activation of coagulation and fibrinolysis during surgery. Their amounts peaked immediately after surgery and recovered to the normal level 24 hours after surgery. The flow cytometry analysis was not influenced by hemodilution and the results demonstrated that expressions of activated GPIIb/GPIIIa and P-selectin were reduced after ANH with HES (130/0.4) compared with those before hemodilution or in control group, indicating that HES might inhibit the over-activation of platelets during hepatic carcinectomy. It was possibly because HES molecules binding with platelets might prevent platelet stimulators from binding with receptors on the surface of platelet membrane, blocking the transduction of activation signals, and thereby reducing the activation products.¹² The present study demonstrated that although ANH with HES (130/0.4) could inhibit expression of membrane glycoprotein transiently with some effects on fibrinolysis, the blood loss was similar with control group and the impact on coagulation was very limited.

In summary, ANH with HES 130/0.4 could be a safe blood conservation measure to reduce homologous transfusion, with limited influence on coagulation and fibrinolysis.

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