RESEARCH ARTICLE

Acute Normovolemic Hemodilution Effects on Perioperative Coagulation in Elderly Patients Undergoing Hepatic Carcinectomy

Jian-Rong Guo[1]*, Xiao-Ju Jin[2], Jun Yu[2], Feng Xu[1], Yi-Wei Zhang[1], Hua-Chun Shen[1], Yi Shao[1]

Abstract

Background: Acute normovolemic hemodilution (ANH) has been widely used to prevent the massive blood loss during hepatic carcinoma. The influences of ANH on coagulation function are still controversy, especially in elderly patients. The study observed ANH effects on coagulation function and fibrinolysis in elderly patients undergoing the disease. Materials and Methods: Thirty elderly patients (aged 60-70 yr) with liver cancer (ASA I or II) taken 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 (130/0.4) and Ringer's solution, respectively. Blood samples were drawn from patients in both groups at five different time points: before anesthesia induction (T1), 30 min after ANH (T2), 1 h after start of operation (T3), immediately after operation (T4), and 24 h after operation (T5). Then coagulation function, soluble fibrin monomer complex (SFMC), prothrombin fragment (F1+2), and platelet membrane glycoprotein (CD62P and activated GP IIb/GP IIIa) were measured. Results: The perioperative blood loss and allogeneic blood transfusion were recorded during the surgery. The perioperative blood loss was not significantly different between two groups ($p>0.05$), but the volume of allogeneic blood transfusion in ANH group was significantly less than in control group (350.0±70.7) mL vs. (457.0±181.3) mL ($p<0.01$). Compared with the data of T1, the prothrombin time (PT) and activated partial thromboplastin time (APTT) measured after T3 were significantly longer ($p<0.05$) in both groups, but within normal range. There were no significant changes of thrombin time (TT) and D-dimer between two groups at different time points ($p>0.05$). SFMC and F1+2 increased in both groups, but were not statistically significant. PAC-1-positive cells and CD62P expressions in patients of ANH group were significantly lower than those at T1 ($p<0.05$) and T2-T5 ($p>0.05$). Conclusions: ANH has no obvious impact on fibrinolysis and coagulation function in elderly patients undergoing resection of liver cancer. The study suggested that ANH is safe to use in elderly patients and it could reduce allogeneic blood transfusion.

Keywords: Hepatic carcinectomy - acute normovolemic hemodilution - coagulation function - fibrinolysis

Asian Pac J Cancer Prev, **14 (8)**, 4529-4532

Introduction

Hepatic carcinectomy is always associated with massive blood loss and thus results in increased mortality in this setting (Sriputtha et al., 2013; Wang et al., 2013). In recent years, acute normovolemic hemodilution (ANH) has been widely used to prevent the complications associated with massive homologous blood transfusion. But its influences on coagulation function are still controversial among different coagulation parameters, measuring approaches, and blood sampling time points. Platelet function has currently become one of the important parameters in perioperative monitoring of coagulation function, and the fast developing technique of flow cytometry (FCM) has allowed us to accurately determine the specific parameters for evaluating the activity of platelets *in vivo* (Ou-Yang et al., 2001). Soluble fibrin monomer complex (SFMC) and prothrombin fragment 1+2 (F1+2), active markers of coagulation with high specificity, have been demonstrated to be sensitive parameters of prethrombotic state at early stage (Hafner et al., 1993; Yang et al., 2002). The present study was designed to investigate the feasibility of employing ANH in elderly patients with hepatic carcinoma by observing its influences on perioperative coagulation function, prethrombotic state, and platelet membrane glycoprotein expression.

Materials and Methods

Patient enrollment

A total of 30 elderly patients scheduled to have hepatic carcinectomy from February 2007 to February

*[1]Department of Anesthesiology, Shanghai Gongli Hospital of Pudong New District, Shanghai, [2]Department of Anesthesiology, Yijishan Hospital, Wannan Medical College, Wuhu, China *For correspondence: jrgmail@163.com*

2008 were selected in our study. The inclusion criteria were as follows: ASA I-II; aged 60-70 years with body weight of 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; preoperative 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 Lihuili Hospital, and written informed consents were obtained from the patients or their next of kin.

Anesthesia and intervention

All patients were premedicated with 0.1 g phenobarbital sodium and 0.5 mg atropine for 30 min before surgery. Electrocardiogram, heart rate, blood pressure, and saturation of peripheral oxygen (SpO_2) were monitored routinely with Philips MP60 in operation room. Left radial artery was cannulated for monitoring blood pressure and measuring blood gas. The right internal jugular vein was cannulated for transfusion and central venous pressure (CVP) measurement. Ringer's lactate solution was transfused at 8-10 $mL \cdot kg^{-1} \cdot h^{-1}$ 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 μg/kg fentanyl, 1-2 mg/kg propofol, and 0.6 mg/kg rocuronium. After tracheal intubation, the endotracheal tube was connected to an anesthetic machine (Cicero EM, Made in Germany, Drager Medical, AG & Co, kGaA, D-23542, Lubeck).

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 CO2 pressure ($PetCO_2$) of 30-40 mmHg. 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 blood volume in suction bottle and the weight of gauze pads. 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 the estimated blood volume (EBV), estimated as body weight × 70 mL/kg in male and weight × 60 mL/kg in female), pre-dilution hematocrit (Hctactual), and post-dilution hematocrit (Hctideal), with the following formula: EBV × 2 × (Hctactual-Hctideal)/(Hctactual + Hctideal). In the present study, Hctideal was set at 28%. The collected blood was stored in acid-citrate-dextrose blood bank at room temperature and transfused back to patients in ANH group after operation or when necessary. Control group did not receive hemodilution during operation, but received basic liquid, lactated Ringer's solution and 6% hydroxyethyl starch (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 the right internal jugular vein of all patients at five different time points: before anesthesia induction (T1), 30 min after ANH (T2), 1 h after start of surgery (T3), immediately after surgery (T4), and 24 h after surgery (T5). Each blood sample was added with 0.2 mL citrate sodium to prevent coagulation. The sample was centrifuged at 3 000 r/min (1 006.2×g) for 8 min, then the light yellow supernatant was extracted and stored at -80°C. F1+2 and SFMC were assayed by enzyme-linked immunosorbent assay (ELISA) with a model 680 enzyme-labelling instrument (BIO-RAD, CA, USA) following the manufacturer's instruction (ADL, R&D, USA). Blood samples were prepared according to the instruction. Platelet membrane glycoprotein was determined within 24 h by using a FACSC alibur flow cytometer (BD Biosciences, CA, USA). PAC-1-conjugated with FITC, a monoclonal antibody detecting GP IIb/GP IIIa (fibrinogen receptor); a phycoerythrobilin-labelled monoclonal antibody against CD62P (P-selectin), a phycoerythrobilin-labelled mouse IgG (MIgG-PE), and a fluorescein isothiocyanate-labelled mouse IgM (MIgM-FITC), were purchased from BD Biosciences. The gate was determined with CD61 PerCP-positive platelets and two-parameter analysis of PAC-1-FITC vs. CD62P PE dot plots was performed.

Statistical data analysis

All statistical analyses were conducted with SPSS 11.0. Data was expressed as (x±s). 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

There were no significant differences in age, gender, weight, surgery type, and intraoperative blood loss between two groups ($p>0.05$). But blood transfusion volume in ANH group was significantly smaller than that in control group ($p<0.05$) (Table 1).

Compared with the data before dilution, PT and APTT at T2 and T3 were significantly prolonged in ANH group ($p<0.05$); but they were all within normal range. Inter-group comparisons 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 was still above the lower limit. It was

Table 1. Basic Characteristics of Patients (x±s)

Groups	Gender (male/female)	Age (yr)	Weight (kg)	Blood loss (mL)	Blood transfusion (mL)
Control (n=15)	10/5	64.3±10.1	54.1±9.2	734.7±83.1	457.8±181.3
ANH (n=15)	12/3	65.7±8.1	56.8±8.3	710.9±75.9	350.5±70.7*

ANH, acute normovolemic hemodilution; Compared with control group, *$p<0.05$

Table 2. Changes of SFMC and F1+2 in Two Groups (x±s)

Parameters	Groups	T_1	T_2	T_3	T_4	T_5
SFMC (mg/mL)	Control	52.60±10.10	54.60±11.70	56.60±12.80	57.70±9.20	54.90±10.10
	ANH	48.40±6.20	50.60±10.80	53.90±9.10	55.00±11.90	53.60±11.40
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

SFMC, soluble fibrin monomer complex; F1+2, prothrombin fragment

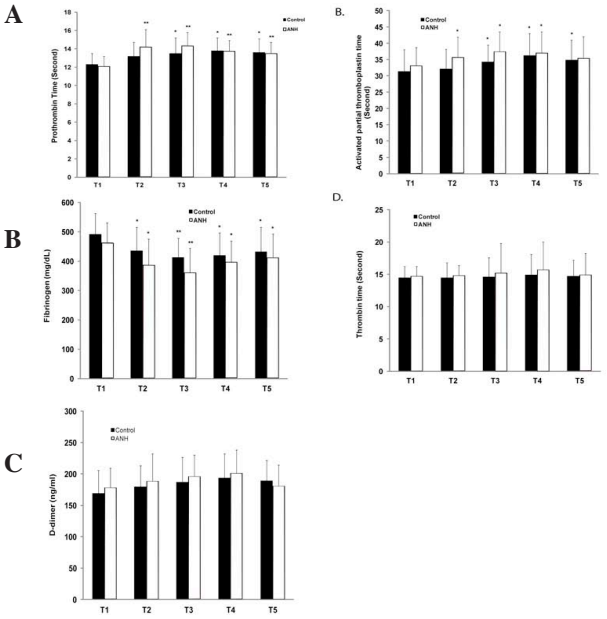

Figure 1. Parameters Reflecting Coagulating Functions in Control and ANH Patients. A. Prothrombin time determined before surgery (T1), 30 min after ANH (T2), during surgery (1 h after the start of surgery, T3), immediately after surgery (T4), and 24 h after surgery (T5); B. Activated partial thromboplastin time (APTT) measured at the above time points; C. Fibrinogen concentration determined at the above time points; D. Thrombin time at the time points; E. Dimer concentration measured at time points. Values are (x±s), one asterisk indicates $p<0.05$, and 2 asterisks indicate $p<0.01$, when compared with the values at the first time point (T1)

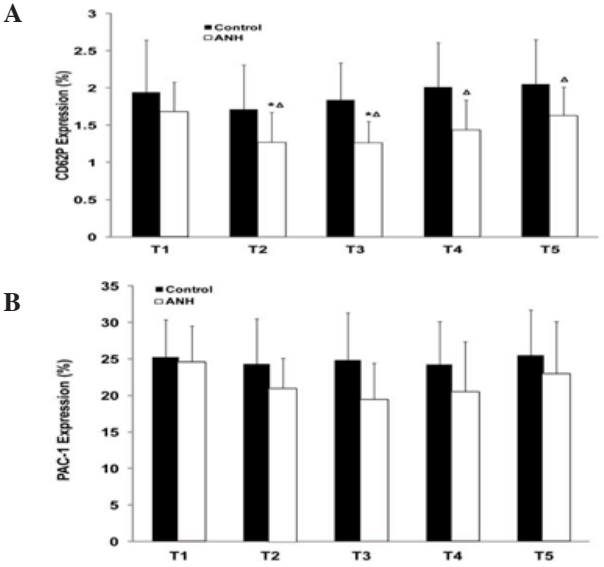

Figure 2. Platelet Activation. A. Expression of CD62P in the control and ANH patients before and after surgery. Values are (x±s). Asterisks indicate significant difference ($p<0.05$), compared with the values at T1, and Δ indicates significant differences ($p<0.05$) between control and ANH patients; B. Expression of PAC-1. Bars are (x±s)

increased after autologous blood transfusion. TT and D-dimer were found similar at different time points and between two groups; both had no intra-group or inter-group differences ($p>0.05$) (Figure 1).

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

After hemodilution, CD62P expression in ANH group was found significantly decreased ($p<0.05$) while activated GP IIb/GP IIIa was only slightly reduced ($p>0.05$). When compared with the control group, CD62P expression in ANH group was significantly reduced at T2-T5 ($p<0.05$) while no such inter-group difference was demonstrated in activated GP IIb/GP IIIa ($p>0.05$) (Figure 2).

Discussion

All coagulation factors other than factor VII are synthesized in the liver, and hepatopathy is usually associated with abnormal coagulation function (Kwon et al., 2013; Saengsawang 2013). Massive bleeding might be encountered during hepatic surgery due to the abundant blood supply to the liver. Blood conservation measures including ANH have been employed in our department to prevent or reduce complications of homologous transfusion. Yet the application of ANH remains controversial because of its potential influence in blood coagulation. It has been demonstrated that rapid transfusion of large dose of hydroxyethyl starch is associated with coagulation suppression by varying degrees, demonstrating as a prolonged APTT (Zhou et al., 2007). But the coagulation status after ANH is generally considered acceptable as long as PLT is maintained at a level over 50×10^9/L (Pisciotto et al., 1995), decrease in coagulation factors is less than 20%-30% of normal levels, FIB is not lower than 0.75 g/L (Murray et al., 1988), and massive blood loss does not occur during surgery. However, the parameters of extrinsic coagulation pathway such as APTT, PT, and TT could only reflect the levels of coagulation factors, not the activation of coagulation pathway (Hafner et al., 1993). To detect the activation of coagulation pathway, ELISA is used in the present study to determine the levels of F1+2 and SFMC, which are molecular fragments generated during the process of coagulation activation and the production of thrombosin 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-thrombosin, which is further involved in the activation of coagulation system

(Pelzer et al., 1991; Bruhn et al., 1992). Therefore, plasma F1+2 could indicate the activities of factor Xa and the coagulation state (Zangari et al., 1997). In the presence of thrombosin, α and β chains of fibrinogen release peptides A and B, and the remaining FB (Fibrinogen) I and II of fibrinogen aggregated together to form SFMC. SFMC could therefore reflect the activity of thrombosin. Elevation of the circulating levels of SFMC and F1+2 indicate the activation of coagulation pathway.

Platelet membrane glycoprotein plays an important role in adhesion, aggregation, and release of platelets. Whole blood FCM was used in the present study to determine the expression of GP IIb/GP IIIa complex and CD62P. GP IIb/GP IIIa complex is formed on the surface of platelet membrane during platelet activation. The activated GP IIb/GP IIIa complex that can be detected by PAC-1, is an early marker of platelet activation. Increased level of PAC-1 suggests the enhancement of platelet adhesiveness. Another membrane glycoprotein CD62P, also called P-selectin, is a later marker of platelet activation (Michelson et al., 2006). It was 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 h. Since the survival time of activated platelets in circulation was 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 al. (2003) have measured some parameters such as thrombin-antithrombin-III complex(TAT), plasmin- 2-antiplasmin complex (PAP), F1+2, and DD in a study on ANH during hip arthroplasty, and found that ANH did not influence intraoperative coagulation and fibrinolysis, while the incidence of postoperative thrombosis was reduced. The present study demonstrated that SFMC and F1+2 were not significantly changed after surgery in both groups, but there seemed to be an increasing trend, indicating the activation of coagulation and fibrinolysis during surgery. In the study, the levels of 2 parameters were peaked after surgery and returned to the normal 24 h after surgery. The FCM analysis results demonstrated that expression of PAC-1 and CD62P was reduced after ANH with HES (130/0.4) compared with that before hemodilution or in control group, suggesting that HES may inhibit the over-activation of platelets during hepatic carcinectomy. It has been suggested that HES molecules binding to platelets may prevent platelet stimulators from binding to their receptors on the surface of platelet membrane, blocking the transduction of activation signals, and thereby reducing the activation products (Liang et al., 2006). The present study demonstrated that although ANH with HES (130/0.4) could inhibit expression of membrane glycoprotein transiently with some effects on fibrinolysis, it did not increase blood loss, and its impact on coagulation was very limited.

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

Acknowledgements

The authors are grateful to Dr. Hao Wang for statistical support and Dr. Cai-De Lu for data collection. They also thank Ping Duan for editorial assistance and Song Li for revision work. The study is supported by Ningbo Medical Technology Foundation 200612.

References

Bruhn HD, Conard J, Mannucci M, et al (1992). Multicentric evaluation of a new assay for prothrombin fragment F1+2 determination. *Thromb Haemost,* **68**, 413-7.

Hafner G, Schinzel H, Ehrenthal W, et a1 (1993). Influence of blood sampling from venipunctures and catheter systems on serial determinations of prothrombin activation fragment 1+2 and thrombin-antithrombin III complex. *Ann Hematol*, **67**, 121-5.

Kwon HT, Ma GX, Gold RS, et a1 (2013). Primary care physicians' cancer screening recommendation practices and perceptions of cancer risk of Asian Americans. *Asian Pac J Cancer Prev*, **14**, 1999-2004.

Liang H, Yang CX, Zeng YM (2006). Effect of acute hypervolemic hemodilution with different colloids on GP IIb/GP IIIa and P-selectin expression in patients with colon cancer during perioperative period. *Chin J Anesthesiol*, **26**, 898-900.

Michelson AD (2006). Evaluation of platelet function by flow cytometry. *Pathophysiol Haemost Thromb*, **35**, 67-82.

Murray DJ, Olson J, Strauss R (1988). Coagulation changes during packed red cell replacement of major blood loss. *Anesthesiology*, **69**, 839-45.

Ou-Yang SJ, Cui M (2001). Detection of coagulation parameters in elderly. *Shanghai J Med Lab Sci*, **21**, 498-500.

Pisciotto PT, Benson K, Hume H, et al (1995). Prophylactic versus therapeutic platelet transfusion practices in hematology and/or oncology patients. *Transfusion*, **35**, 498-502.

Pelzer H, Schwarz A, Stüber W (1991). Determination of human prothrombin activation fragment F1+2 in plasma with an antibody against a synthetic peptide. *Thromb Haemost*, **65**, 153-9.

Piecuch W, Sokołowska B, Dmoszyńska A (2003). Evaluation of selected parameters of blood coagulation and fibrinolysis system in patients undergoing total hip replacement surgery with normovolemic hemodilution procedure and standard enoxaparine prophylaxis. *Chir Narzadow Ruchu Ortop Pol,* **68**, 95-9.

Saengsawang P, Promthet S, Bradshaw P (2013). Infection with opisthorchis viverrini and use of praziquantel among a working-age population in northeast Thailand. *Asian Pac J Cancer Prev*, **14**, 2963-6.

Sriputtha S, Khuntikeo N, Promthet S, et al (2013). Survival rate of intrahepatic cholangiocarcinoma patients after surgical treatment in Thailand. *Asian Pac J Cancer Prev*, **14**, 1107-10.

Wang X, Song ZF, Xie RM, et al (2013). Analysis of death causes of in-patients with malignant tumors in Sichuan Cancer Hospital of China from 2002 to 2012. *Asian Pac J Cancer Prev,* **14**, 4399-402.

Yang XY, Shan HW (2002). Progression in diagnosis and treatment of coronary artery disease: acute coronary syndrome. *J Postgrad Med*, **23**, 1-4.

Zangari M, Lockwood CJ, Scher J, et al (1997). Prothrombin activation Fragment (F1.2) is increased in pregnant patients with antiphospholipid antibodies. *Thromb Res*, **85**, 177-83.

Zhou X, Zhang XL (2007). Advances in clinical studies of hydroxyethyl starch. *Chin Pharm J*, **42**, 646-9.