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

Jian-Rong Guo, Xiao-Ju Jin, Jun Yu, Feng Xu, Yi-Wei Zhang, Hua-Chun Shen, Yi Shao

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 controversial, 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 carcinoma - 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...
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 (SpO₂) 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·kg⁻¹·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 (PetCO₂) 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 an 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 FACSCalibur flow cytometer (BD Biosciences, CA, USA). PAC-1-conjugated with FITC, a monoclonal antibody detecting GP Ib/IX (glycoprotein 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 gates were 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)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Blood loss (mL)</th>
<th>Blood transfusion (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=15)</td>
<td>10/5</td>
<td>64.3±10.1</td>
<td>54.1±9.2</td>
<td>734.7±83.1</td>
<td>457.8±181.3</td>
</tr>
<tr>
<td>ANH (n=15)</td>
<td>12/3</td>
<td>65.7±8.3</td>
<td>56.8±8.3</td>
<td>710.9±75.9</td>
<td>350.5±70.7*</td>
</tr>
</tbody>
</table>

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

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., 2007). But the coagulation status after ANH is generally found significantly decreased (p<0.05, when 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))

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFMC (mg/mL)</td>
<td>Control</td>
<td>52.60±10.10</td>
<td>54.60±11.70</td>
<td>56.60±12.80</td>
<td>57.70±9.20</td>
<td>54.90±10.10</td>
</tr>
<tr>
<td></td>
<td>ANH</td>
<td>48.40±6.20</td>
<td>50.60±10.80</td>
<td>53.90±9.10</td>
<td>55.00±11.90</td>
<td>53.60±11.40</td>
</tr>
<tr>
<td>F1+2 (nmol/L)</td>
<td>Control</td>
<td>0.73±0.28</td>
<td>0.78±0.24</td>
<td>0.86±0.30</td>
<td>0.90±0.26</td>
<td>0.85±0.30</td>
</tr>
<tr>
<td></td>
<td>ANH</td>
<td>0.70±0.20</td>
<td>0.77±0.33</td>
<td>0.86±0.32</td>
<td>0.91±0.38</td>
<td>0.87±0.41</td>
</tr>
</tbody>
</table>

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 above time points; E. Dimer concentration measured at time points. Values are (x±s). Asterisks indicate significant difference (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 A indicates significant differences (p<0.05) between control and ANH patients; B. Expression of PAC-1. Bars are (x±s)
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 Ib/IIIa complex and CD62P. GP Ib/IIIa complex is formed on the surface of platelet membrane during platelet activation. The activated GP Ib/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 in both groups, but there seemed to be an increasing trend, indicating the activation of coagulation pathway. Elevation of the circulating levels of SFMC and F1+2 could indicate the activity 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 could indicate the activation of coagulation pathway.

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


