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• BRIEF REPORTS •

Levels of plasma des-γ-carboxy protein C and prothrombin in patients with liver diseases

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Abstract

AIM: To study the plasma des-γ-carboxy protein C activity, antigen and prothrombin levels in patients with liver diseases and their clinical significance.

METHODS: Plasma protein C activity (PC:C) was detected by chromogenic assay and antigen (PC:Ag) and des-γ-carboxy protein C (DCPC) were detected by ELISA. Total prothrombin and unabsorbed prothrombin in plasma were detected by ecarin chromogenic assay.

RESULTS: Compared with the control, the levels of PC:C and PC:Ag in patients with hepatocellular carcinoma (HCC) and liver cirrhosis (LC) were lower (PC:C: 104.65±23.0%, 62.50±24.89%, 56.75±20.14%, PC:Ag: 5.31±1.63 μg/mL, 2.28±1.15 μg/mL, 2.43±0.79 μg/mL, $P<0.05$). The levels of PC:Ag in patients with acute viral hepatitis (AVH) also was lower (2.98±0.91 μg/mL, $P<0.01$), but PC:C was close to the control (93.76±30.49%, $P>0.05$). The levels of DCPC in patients with HCC were remarkably higher (0.69±0.29 μg/mL, 1.18±0.63 μg/mL, 0.45±0.21 μg/mL, $P<0.05$) and its average was up to 50% of total PC:Ag. But those of DCPC in patients with AVH were not significantly different from the control. The levels of total prothrombin were lower in patients with LC, but higher in patients with HCC. The levels of unabsorbed prothrombin were predominantly higher than those of other groups.

CONCLUSION: PC:C and PC:Ag in patients with liver diseases (except PC:C in AVH) were lower. The total prothrombin was lower in patients with LC. The higher level of unabsorbed prothrombin may be used as a scanning marker for HCC. DCPC may be used as a complementary marker in the diagnosis of HCC.

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INTRODUCTION

Protein C is a plasma glycoprotein of M_r 62 000 and is synthesized and degraded in the liver. There is 2-6 mg/L PC of plasma in healthy person, with about 72-139% biological activity. No difference in the content of protein C between males and females was found, but protein C shows an increased trend towards increasing age (with an average increase of 4% every 10 years). Thrombin formed during coagulation is responsible for conversion of protein C to activated protein C (APC). This activation takes place on the surface of endothelial cells and monocytes by compound thrombin with thrombomodulin[1]. Because protein C is a vitamin K-dependent plasma protein, it is highly homologous in structure to factors X, IX, VII and prothrombin. Many studies have demonstrated that there are changes of factors X, IX, VII and prothrombin in patients with liver diseases, and des-γ-carboxy (abnormal) prothrombin is a useful tumor marker in the diagnosis of hepatocellula carcinoma[2-4]. But up to now, few reports are available about the association between PC and liver diseases. Therefore, in the present study we not only reported the changes of PC and prothrombin in liver diseases, but also explored the relationship between des-γ-carboxy protein C (DCPC) and HCC.

MATERIALS AND METHODS

Clinical data

Fifty-three patients (33 males and 20 females, aged 20-81 years) were included in this study. Of them, 18 patients with hepatocellular carcinoma (HCC) , 20 with liver cirrhosis (LC) , 15 with acute viral hepatitis (AVH). They were from The Second Affiliated Hospital of Guangzhou Medical College and the Xiangya Hospital of Central South University. HCC and LC were diagnosed by clinical, pathological and ultrasonic examinations. AVH was diagnosed by clinical and immunological/RT-PCR examinations. Twenty healthy volunteers (10 males and 10 females, aged 25-65 years) were enrolled as the control group.

Materials

Blood sampling and preparation of plasma (for PC:C, PC:Ag assay) were as follows. Blood was drawn into 0.13 mol/L sodium citrate (9/1,v/v), plasma (for DCPC, prothrombin assay) was obtained by drawing blood (9vol) into 0.1 mol/L sodium oxalate (9/1, 1v/v) , and centrifugation at 4 000 r/min for 10 min. All were snap frozen and stored at -40 ℃.

PC activity (PC:C) and PC antigen (PC:Ag) kits were purchased from Shanghai Sun Biotech Company. Vials containing 50 U of ecarin were provided by Sigma Company. Chromogenic substance S2238 was obtained from American Diagnostica Inc. (ADI). $Bacl_2$, Tris, and others were of analytical grade and purchased from Shanghai Reagent Factory. ELX800 enzyme-linked immunosorbent detector was from America BIO-Tek Instruments Inc.

Methods

PC:C assay PC:C was detected by chromogenic assay (SH Sun Bio CO kits). Excessive actvator was put into the diluted human plasma, PC was activate and convert it into activated protein C

(APC). Then chromozym APC was hydrolyzed, and PNA was released. PNA levels are determined by measuring the sample solution absorbances at 405 nm and comparing against those of standard curves generated using a PC:C. The assay procedures performed according to the instructions of the manufacturer.

PC:Ag assay PC:Ag was determined by ELISA (SH Sun Bio CO. kits). Murine PC antibody was used as capture antibody and an enzyme-linked antibody fragment that specifically recognizes bound hPC as detection antibody. After plasma PC bound to capture antibody and detection antibody, the substrate was hydrolyzed by enzyme and chromagenic reaction occurred. The sample absorbance at 492 nm was proportional to the concentrations of plasma PC. The assay procedures were performed according to the manufacturer's instructions.

DCPC assay A total of 40 μL of 1 mol/L $BaCl_2$ was added into 500 μL of normal reference plasma or pending measured plasma. The mixture was surged for 30 min at 4 ℃. After centrifuged for 5 min,collect the supernatant was collected. Then the supernatant was absorbed by barium salt again and collected. The level of PC:Ag in the supernatant was measured by ELISA.

Prothrombin assay Ecarin could activate γ-carboxylated and des-γ-carboxylated prothrombin into thrombin and then thrombin amidolyse chromozym P. The absorbance at 405 nm was proportional to the concentration of γ-carboxylated and des-γ-carboxylated Prothrombin. Levels of prothrombin in plasma reflected the total prothrombin and those in plasma absorbed by $Bacl_2$ reflect the unabsorbed prothrombin.

Statistical analysis

Results were expressed as mean±SD. One way analysis of variance and Newman-keuls test were used for comparisons of the mean value in various groups. *P* values less than 0.05 was considered statistically significant.

RESULTS

PC:C and PC:Ag in liver diseases

Compared with the control, the levels of PC:C and PC:Ag in patients with hepatocellular carcinoma (HCC) and liver cirrhosis were lower ($P<0.05$). PC:Ag in acute viral hepatitis (AVH) also was lower, but PC:C was close to the control ($P>0.05$) (Table 1).

Table 1 The levels of PC:C and PC:Ag in patients with liver diseases (mean±SD)

	n	PC:C (%)	PC:Ag (μg/mL)
Control	20	104.65±23.0	5.31±1.63
Hepatocellular carcinoma	18	62.50±24.89[a]	2.28±1.15[b]
Liver cirrhosis	20	56.75±20.14[a]	2.43±0.79[b]
Acute viral hepatitis	15	93.76±30.49	2.98±0.91[b]

[a]$P<0.05$, [b]$P<0.01$ *vs* control group.

DCPC in liver diseases

As shown in Table 2, the difference of DCPC between acute viral hepatitis group and control group was not statistically significant ($P<0.01$). But DCPC in patients with HCC was remarkably higher than those in patients with acute viral hepatitis and the control ($P<0.01$).

Prothrombin in liver diseases

In contrast to the control, the levels of total prothrombin was lower in patients with liver cirrhosis ($P<0.05$). There was no significant difference in plasma prothrombin between acute viral hepatitis and control groups ($P>0.10$). But the level of total prothrombin in patients with HCC was markedly higher than that in other groups ($P<0.01$). The levels of unabsorbed prothrombin in patients with HCC were predominantly higher than those in the other groups ($P<0.01$).

Table 2 The levels of DCPC in patients with liver diseases (mean±SD)

	n	Total PC:Ag (μg/mL)	DCPC:Ag (μg/mL)	DCPC:Ag Total PC:Ag
Control	15	5.23±1.95	0.69±0.29	13.19%
Hepatocellular carcinoma	15	2.33±1.21	1.18±0.63[b]	50.64%[b]
Acute viral hepatitis	15	2.98±0.91	0.45±0.21	15.10%

[b]$P<0.01$ *vs* control and acute viral hepatitis groups.

Table 3 The levels of prothrombin in patients with liver diseases (mean±SD)

	n	Total prothrombin (%)	Unabsorbed prothrombin (%)
Control	20	101.99±12.29	0.30±0.18
Hepatocellular carcinoma	18	220.61±67.95[b]	2.87±0.89[b]
Liver cirrhosis	10	85.33±6.99[a]	0.95±0.45[a]
Acute viral hepatitis	15	99.05±14.97	1.09±0.36[a]

[a]$P<0.05$, [b]$P<0.01$ *vs* control groups.

DISCUSSION

Vitamin K-dependent zymogens, prothrombin, factor VII, IX , protein C and protein S are synthesized in the liver. It is understandable that the liver diseases are associated with thrombosis and/or hemorrhage. In the present investigation, we observed that the levels of total prothrombin decreased in patients with liver cirrhosis and increased in patients with HCC. The results are consistent with previous reports[5].

Prior to secretion into plasma, all the vitamin K-dependent proteins undergo post-transtional modifications by a vitamin K-dependent carboxylase that converts several specific glutamic acid residuces to γ-carboxyglutuminic acid (Gla). Gla residues are located in N-terminal of the mature proteins and contribute to the ability of these proteins to bind to Ca^{2+} and offer metal ions such as Ba^{2+}, *etc*. Ca^{2+} binding induces conformational changes leading to expression of membrane -binding phoepholipid, which is a key step to bring about biological activities. Therefore, des-γ-carboxylated proteins can not bind to divalent ions and lose their procoagulant or anticoagulant activities. Our data showed that after plasma was absorbed by barium salt, the levels of unabsorbed prothrombin from plasma of patients with HCC was very high and lower in patients with acute viral hepatitis and liver cirrhosis, and less in healthy volunteers. This fact further suggested that high levels of unabsorbed prothrombin in plasma could be used a scanning marker of HCC. It is due to unabsorbed prothrombin essentially reflecting des-γ-carboxy prothrombin(DCP). Recent studies demonstrated that DCP could not only differentiate HCC from nonmalignant chronic liver diseases, but also indicate prognosis for HCC[3,4]. Because the assay of unabsorbed prothrombin is more economical and simpler than the determination of DCP, it maybe spread in clinic, especially in the developing countries.

The activation of protein C is catalyzed by a compound of α-thrombin and the endothelial cell surface protein thrombomodulin. In contrast to other vitamin K- dependent coagulation factors, activated protein C (APC) functions as an anticoagulant by proteolytic inactivation of factor Va and VIIIa. APC also promotes

fibrinolytic response by forming compounds with plasminogen activator inhibitor-1 and by diminishing the activation of thrombin-activatable fibrinolysis inhibitor via inhibition of thrombin generation. Thus the deficiency of protein C is associated with thrombosis[6,7]. Previous reports demonstrated that the levels of protein C from plasma in patients with liver diseases were lower[8,9]. Our data showed that PC:C in patients with hepatocellular carcinoma and liver cirrhosis was lower than control group ,and the descendant degree was proportional to the damaged degree (unpublished data). Thus, the results provided further evidence for the association of protein C and liver diseases. However, PC:Ag in acute viral hepatitis (AVH) was also lower, but PC:C was close to the control , the reason is unknown. Protein C inhibitors synthesized in liver can specifically inactivate APC. Perhaps the decrease of protein C inhibitors in liver disease is one of the causes. In addition, the decrease of coagulation factors and some drugs may be partly responsible for it.

Because protein C is one of the vitamin K-dependent proteins synthesized in the liver, its biological functions are also dependent on complete Gla domain. Yashiikawa *et al*. reported that the impaired vitamin K-dependent γ-carboxylation in patients with HCC involved not only prothrombin, but also protein C[10]. Our data showed that the levels of DCPC in patients with HCC were surprisingly high, almost up to 50% of total protein C. This fact shows that the high level of DCPC from plasma can be used as a complemental tumor marker of HCC. As surgical removal or reduction in tumor was with chemotherapy is associated with reduction or elimination of the abnormal prothrombin (des-γ-carboxy prothrombin , DCP), the tumor itself is responsible for the production of DCP. We believe that DCPC may have the same production process like DCP. Up to date, it is still unknown why tumor cells of HCC make so many DCP and DCPC as well as des-γ-carboxy proteins. Because vitamin therapy can not reduce the concentration of DCP and DCPC, the production of des-γ-carboxy protein is not due to vitamin K deficiency. Its specific mechanisms remain unclear.

It is becoming increasingly clear that there is an interaction network between coagulation and inflammation and natural anticoagulants have strong anti-inflammatory effects[11,12]. Animal experiments indicated that protein C and activated protein C inhibits could inhibit DIC and improve survival rate[11-13]. Clinical trials could demonstrated that recombinant human activated protein C (rhAPC) could attenuate systemic inflammatory response syndrome and reduces mortality in patients with severe sepsis[14-16]. Therefore, it may be beneficial when an appropriate dose of protein C or rhAPC is given to the patients with liver diseases especially HCC to prevent and treat sepsis and DIC.

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