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

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INTRODUCTION
Protein C is a plasma glycoprotein of M, 62,000 and is synthesized and degraded in the liver. There is 2-6 mg/L PC in plasma of 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[11]. 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, Hunan, China. The patients met the criteria of the National Natural Science Foundation of China. Diagnostica Inc. (ADI). Bacl2, Tris, and others were of analytical reagent grade. Chromogenic substance S2238 was obtained from American Diagnostica Inc. (ADI). Bacl2, Tris, and others were of analytical grade and purchased from Shanghai Reagent Factory. ELX800 enzyme-linked immunosorbent detector was from America Biotek Instruments Inc.

METHODS

Blood sampling and preparation of plasma (for PC:C, PC:Ag assay) was 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 (9 vol) into 0.1 mol/L sodium oxalate (9/1, v/v), and centrifugation at 4,000 rpm for 10 min. All were snap frozen and stored at -40 °C. 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). Bacl2, Tris, and others were of analytical grade and purchased from Shanghai Reagent Factory. ELX800 enzyme-linked immunosorbent detector was from America Biotek Instruments Inc.

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

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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:Ag: 5.31±1.63 µg/mL, 2.43±0.79 µg/mL, P<0.05). The levels of PC:C and 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.
Hepatocellular carcinoma 18 62.50±24.89 a 2.28±1.15 b
Control 20 104.65±23.0 5.31±1.63

Viral hepatitis and control groups. Scores were significantly higher than those in patients with acute viral hepatitis and the control (P <0.05). The results are consistent with previous reports[5].

**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 | 2.28±1.15     |
| Liver cirrhosis | 20  | 56.75±20.14 | 2.43±0.79     |
| Acute viral hepatitis | 15  | 93.76±30.49 | 2.98±0.91     |

**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±0.63b      | 50.64%b             |
| Acute viral hepatitis | 15  | 2.98±0.91           | 0.45±0.21       | 15.10%              |

**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±67.95b         | 2.87±0.89b               |
| Liver cirrhosis | 10  | 85.33±6.99           | 0.95±0.45               |
| Acute viral hepatitis | 15  | 99.05±14.97         | 1.09±0.36               |

**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[3].

Prior to secretion into plasma, all the vitamin K-dependent proteins undergo post-translational modifications by a vitamin K-dependent carboxylase that converts several specific glutamic acid residues to γ-carboxyglutaminic acid (Gla). Gla residues are located in N-terminal of the mature proteins and contribute to the ability of these proteins to bind to Ca2+ and offer metal ions such as Ba2+, etc. Ca2+ binding induces conformational changes leading to expression of membrane -binding phosopholipid, 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 may be widely used in clinics, in especially in developing countries.
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