Thrombopoietin-Producing Hepatocellular Carcinoma


Abstract

A 66-year-old man with hepatocellular carcinoma (HCC) showed marked thrombocytosis (110.7x10^4/μl). Bone marrow (BM) aspirates demonstrated an increase of mature megakaryocytes (MgK). The serum thrombopoietin (TPO) level was increased to about 100-fold that of the normal level in the terminal stage. However, the platelet count gradually decreased to 13.5x10^4/μl. The autopsy specimen revealed normoplastic BM with decreased MgK, mainly consisting of the immature type, and it was negative for tumor cells. Liver specimen showed markedly fatty metamorphosis. Immunohistochemical staining of TPO demonstrated that hepatocytes were weakly stained and HCC cells strongly stained, suggesting TPO-producing HCC.

(Internal Medicine 42: 730-734, 2003)

Key words: thrombopoietin, thrombopoietin-producing tumor, hepatocellular carcinoma, thrombocytopoiesis

Introduction

Thrombopoietin (TPO), the primary hematopoietic growth factor involved in the regulation of megakaryocyte (MgK) growth and development as well as platelet production has recently been isolated and cloned (1-4). Although the complexities of TPO regulation are not fully understood, two possible mechanisms have been postulated. One mechanism is that TPO plasma levels are dependent on the rate of platelet/MgK TPO receptor (Mpl)-mediated uptake and catabolism (5-7). An elevated platelet level would lead to increased binding and catabolism of TPO to platelet/MgK receptors and consequently a decreased plasma TPO level, thereby limiting MgK production which would lead to a decreased production of platelets. The other mechanism is that plasma TPO concentrations are probably regulated by feedback control at the level of gene expression. McCarty et al (8) showed that TPO mRNA expression in the bone marrow and spleen is increased in thrombocytopenic animal models. Sungaran et al (9) demonstrated that in thrombocytopenic human subjects with aplastic anemia, postchemotherapy marrow aplasia, and immune thrombocytopenia, the stromal cells show marked TPO mRNA expression, suggesting that TPO mRNA expression in human bone marrow might be modulated by platelet mass. However, these two mechanisms may not be mutually exclusive or operate in concert.

To date hepatoblastoma and ovarian carcinoma have been reported to produce TPO. Nickerson et al (10) described two children with hepatoblastoma, marked thrombocytosis, and extremely elevated alpha fetoproteins. They suggested that a specific cytokine like TPO affecting megakaryocytopoiesis may be associated with excessive MgK seen in the bone marrow and consequent platelet production. Furuhashi et al (11) reported a case of TPO-producing ovarian carcinoma confirmed by immunohistochemistry. They attempted to study the serum TPO levels because of marked thrombocytosis.

Here, we describe a case of TPO-producing hepatocellular carcinoma (HCC) with thrombocytosis, and discuss the causes of thrombocytopenia observed with progression of HCC in the terminal stage.

Case Report

A 66-year-old man had symptoms of abdominal distension and appetite loss two months before admission in April 2000. He had a drinking habit. Physical examination showed a huge hard mass in the abdomen. Laboratory data showed leuko- and thrombocytosis, that is, the white blood cell (WBC) count was 12,800/μl, the differential showed...
Computed tomography of the abdomen demonstrated liver tumors and the largest one measured 15×11×18 cm in the left lobe.

For examination of thrombocytosis, bone marrow aspirates were obtained. The bone marrow specimen showed a marked increase in mature, platelet-producing megakaryocytes, negative for tumor cells (HE stain, ×200).

With the progression of hepatocellular carcinoma, the serum thrombopoietin (TPO) level continuously increased. Despite the increased TPO level, the platelet count was inversely proportional to the serum TPO level and gradually decreased to 13.5×10^4/μl. Anti-TPO antibody neutralizing TPO activity was negative.

55% neutrophils and 38% lymphocytes and the platelet count was 67.4×10^4/μl. Aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were 63 and 19 IU/l, respectively. Prothrombin time and activated partial thromboplastin time were in the normal range. Alpha fetoprotein was 1.7 ng/ml. Hepatitis B surface antigen, and anti-hepatitis C antibody were negative. Ultrasonography (US) and computed tomography (CT) (Fig. 1) of the abdomen demonstrated multiple liver tumors and the largest one measured 15×11×18 cm in the left lobe. Hepatocellular carcinoma (HCC) was diagnosed by needle biopsy and the background liver demonstrated chronic active hepatitis (non-B, non-C). After he was treated by transcatheter arterial embolization (TAE) of the left hepatic artery, the platelet count was decreased from 67.4×10^4/μl to 48.0×10^4/μl. As an enlargement of HCC was not seen, he was discharged in August 2000. He was readmitted because of abdominal pain in January 2001. As the platelet count increased to 86.7×10^4/μl, bone marrow aspiration was performed. All nucleated bone marrow cells were 12.0×10^5/μl and megakaryocyte (MgK) count was 125/μl. Bone marrow specimen showed a marked increase in mature, platelet-producing MgK, negative for tumor cells (Fig. 2). Platelet aggregation studies to ADP were normal. Among the several kinds of cytokines affecting megakaryocytopoiesis, the serum levels of thrombopoietin (TPO) (12), erythropoietin (EPO), interleukin (IL)-6, transforming growth factor (TGF)-β1 and hepatocyte growth factor (HGF) were increased. In particular, the serum TPO level was extremely high, 14.73 fmol/ml (normal range 0.40±0.29). With the progression of HCC, the serum TPO level continuously increased. However, the platelet count was inversely proportional to serum TPO level. The platelet count gradually decreased to 13.5×10^4/μl, despite the increased TPO level, of 38.45 fmol/ml in August (Fig. 3). Thrombocytopenia caused by disseminated intravascular coagulation (DIC) was denied. Like TPO, the serum levels of EPO, IL-3 and IL-6 were increased in August, compared with those in March (Table 1). The patient died in September 2001. Immunohistochemical staining of TPO was performed. A 4-μm section was cut from the paraffin blocks of liver. Each section was mounted on a glass slide and deparaffinized. Rabbit anti-human TPO polyclonal antibody, diluted 200 times (a gift from Kirin Brewery Co., Ltd., Tokyo, Japan) was applied for 2 hours at 37°C. The primary antibody was visualized using the Simple Stain MAX-PO (Nichirei, Tokyo, Japan) according to the instruction manual (13). Although hepatocytes with fatty

Internal Medicine Vol. 42, No. 8 (August 2003)
Table 1. cytokines affecting thrombopoiesis

Cytokines stimulating megakaryocytopoiesis

<table>
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<tbody>
<tr>
<td>TPO (0.40±0.29 fmol/ml)</td>
<td>14.73</td>
<td>38.45†</td>
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<tr>
<td>EPO (8–36 μ/ml)</td>
<td>45.6</td>
<td>172†</td>
</tr>
<tr>
<td>G-CSF (4.7–18.1 μg/ml)</td>
<td>20</td>
<td>21</td>
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<tr>
<td>GM-CSF (&lt;8μg/ml)</td>
<td>&lt;8</td>
<td>&lt;8</td>
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<tr>
<td>IL-3 (&lt;3μg/ml)</td>
<td>&lt;31</td>
<td>155†</td>
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<tr>
<td>IL-6 (&lt;4 μg/ml)</td>
<td>9.2</td>
<td>34.6†</td>
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Cytokines inhibiting megakaryocytopoiesis

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<tr>
<td>TGF-β1 (&lt;2.26 ng/ml)</td>
<td>19.1</td>
<td>27.7</td>
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<tr>
<td>PF4 (&lt;20 ng/ml)</td>
<td>&gt;100</td>
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<td>β-TG (&lt;50 ng/ml)</td>
<td>&gt;200</td>
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Others

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<td>TNF-α (&lt;5 pg/ml)</td>
<td>&lt;5</td>
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<tr>
<td>HGF (&lt;0.4 ng/ml)</td>
<td>1.12</td>
<td>1.36</td>
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The serum level of cytokines inhibiting megakaryocytopoiesis, such as TGF-β1, PF4, β-TG increased, however, there was no change in the serum cytokine level in March and August. In contrast, the serum level of EPO, IL-3 and IL-6 stimulating megakaryocytopoiesis was enhanced in August compared with that in March. EPO: erythropoietin, G-CSF: granulocyte colony-stimulating factor, GM-CSF: granulocyte macrophage colony-stimulating factor, IL: interleukin, TGF-β1: transforming growth factor-β1, PF4: platelet factor 4, β-TG: β-thromboglobulin, TNF-α: tumor necrosis factor-α, HGF: hepatocyte growth factor.

Figure 4. Autopsy liver tissues showed fatty metamorphosis and hepatocellular carcinoma (HCC) (HE stain, ×400).

Figure 5. To demonstrate thrombopoietin (TPO)-producing HCC, immunohistochemical staining of TPO was performed in 20% formalin-fixed paraffin-embedded sections of autopsy HCC tissues using rabbit anti-human TPO polyclonal antibody. HCC cells were strongly stained by anti-TPO antibody (×400).

Figure 6. Autopsy liver tissues of another patient with HCC and cirrhosis (HE stain, ×200).
metamorphosis were weakly stained, HCC cells were clearly stained by anti-TPO antibody, suggesting TPO-producing HCC (Figs. 4–6). As a control, immunohistochemical staining of TPO was performed using liver tissues of another patient with HCC and liver cirrhosis. HCC cells were negative and hepatocytes were weakly positive (Figs. 7, 8). BM metastasis, apparent splenomegaly and thrombosis due to DIC were not observed.

Discussion

We described a 66-year-old man with HCC and marked thrombocytosis, which is very rare among HCC patients. After he was treated by TAE of the left hepatic artery, the platelet count decreased from 67.4x10^4/µl to 48.0x10^4/µl, and then recovered. Although the serum TPO level was not measured, the decreased platelet count could be related to the decreased TPO level based on the reduction in mass volume. For examination of thrombocytosis (platelet count 1 10.7x10^4/µl), bone marrow aspirates were obtained. The bone marrow specimen showed a marked increase in mature, platelet-producing MgK, negative for tumor cells. Several kinds of cytokines affecting megakaryocyto- and thrombocytopoiesis were examined, and a high serum TPO level was noted. With the progression of HCC, the serum TPO level continuously increased. Immunohistochemical staining of TPO was performed using an autopsy liver specimen. Although hepatocytes with fatty metamorphosis were weakly positive, HCC cells were clearly stained by anti-TPO antibody, suggesting TPO-producing HCC (Figs. 4–6). In situ hybridization of the tumor material to complimentary DNA of TPO was not examined.

Table 2 shows platelet counts and plasma levels of endogenous TPO in thrombocytopenic patients (14). The encoded polypeptides of TPO have a predicted molecular mass of approximately 35,000 kDa. If TPO consists of a full-length form, it is possible to convert fmol/ml to pg/ml (2 fmol/ml×35,000=70 pg/ml). However, if the TPO molecule was truncated by proteolysis, the conversion was not correct.

With the progression of HCC, the platelet count was inversely proportional to the serum TPO level. The autopsy specimen showed normoplastic BM with decreased MgK, mainly consisting of immature type (Fig. 9). The causes of thrombocytopenia in the terminal stage could be attributed to: 1) TPO dysfunction, 2) an increase of cytokines affecting thrombocytopoiesis, or 3) disappearance of platelet TPO receptor (Mpl) via catabolism.

Anti-TPO antibody neutralizing TPO activity was measured with a radioimmunoassay using glycosylated recombinant full-length human TPO as the antigen, as previously described (15) and the result was negative. The erythropoietin (EPO)-like domain of TPO contains the receptor-binding portion which is biologically active. Even though the C-terminal domain was detached from the full-length, 70-kDa form by proteolytic conversion, it is still possible to detect TPO protein by TPO/ELISA, even with the loss of biological activity. 2) Among cytokines affecting thrombocytopoiesis, the serum levels of cytokines inhibiting mega-
karyocytopenia (16, 17), such as TGF-β1, platelet factor 4 (PF4), β-thromboglobulin (β-TG) were increased against megakaryocytopenia accelerated by TPO. However, there was no change in serum cytokine levels in March and September. In contrast, the serum levels of EPO, IL-3 and IL-6 stimulating megakaryocytopenia (18, 19) were enhanced in August, compared with that in March. Suppressed megakaryocytopenia may have induced these cytokines, or there is a possibility that HCC cells produced multicytokines, although immunohistochemical staining, other than TPO, was not performed. A physiological regulator enhancing TPO gene expression, and hepatocyte growth factor (HGF) (20) showed a rather high level (Table 1). Cohen-Solal K et al (21) examined whether or not, the expression of Mpl is regulated by its ligand, TPO. The levels of Mpl transcripts and Western blot analysis of MgK produced in the presence or absence of TPO, showed no difference in Mpl levels. These results indicated that TPO does not have a major effect on the transcription or translation of Mpl. However, they also suggested that an excess of circulating TPO could lead to the disappearance of Mpl from platelets via catabolism. As TPO production was self-supported by HCC in this case, the serum TPO level was extremely high. Consequently, it may mimic down-regulation of Mpl.

Acknowledgments: We would like to thank Asako Takematsu, Kazuko Mochizuki, Kazuhiko Kobayashi, and Chiemi Kurita for their excellent technical assistance.

References


