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Original Article

Short-term clinical evaluation of recombinant humanthrombopoietin treating on thrombocytopaenia in patients with liver cirrhosisassociated with viral hepatitis

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Abstract: Background: Patients with viral hepatitis related liver cirrhosis often have a complication of thrombocytopaenia, which limited the invasive manipulation and possibly resulted from reduction of thrombopoietin (TPO) production. The aim of this study is to evaluate the therapeutic effect of recombinant human thrombopoietin (rhTPO) onthrombocytopaenia in viral hepatitis cirrhosis patients. Methods: 24 patients with viral hepatitis related liver cirrhosis were enrolled, and they were subcutaneously injected rhTPO, 15000 unit/day. The platelet count (PLT) was recorded from 1 day before treatment to 14 days after treatment, and the effective rate was evaluated after treatment according to classification of liver function. Results: The baseline average of PLT was (29.75±10.49)×10^9/L, and the mean PLT were (41.43±11.62)×10^9/L, (56.11±23.86)×10^9/L and (79.55±41.23)×10^9/L at 3 days, 5 days and 7 days after rhTPO treatment, respectively. There were significant different between before and after rhTPO treatment (P<0.05). The effective rate at 3 days, 5 days and 7 days after rhTPO treatment were 12.50%, 45.83% and 70.83%, respectively. According to classification of liver function, the effective rate of patients with Child-Pugh class A reached 100% at 7 days after rhTPO treatment, and was obviously higher than that of patients with Child-Pugh class B and C (P<0.05). Conclusions: rhTPO treatment can quickly and effectively improve platelet count of patients with viral hepatitis related liver cirrhosis plusthrombocytopaenia, and the adverse drug reaction was mild and a low incidence.

Keywords: Viral hepatitis, liver cirrhosis, thrombocytopaenia, recombinant human thrombopoietin

Introduction

The common complication in patients with viral hepatitis related liver cirrhosis is thrombocytopenia. Severe thrombocytopenia (less than 50×10^9/L) can significantly enhance the risk of bleeding, which limited the invasive manipulation, such as liver biopsy, radiofrequency liver ablation, dental extraction, laparoscopic hernia repair, etc, even if patients had good liver function [1, 2]. For patients with hepatitis B and liver cirrhosis, severe thrombocytopenia restricted application of interferon. So, it is deemed that severe thrombocytopenia increased the mortality risk of cirrhosis patients, and an increase in platelet levels is necessary to reduce the risk of serious bleeding before invasive manipulation.

So far, the therapeutic method for enhancing platelet count (PLT), included platelet transfusion, surgical splenectomy and interventional partial splenic embolization (PSE) [3]. Complications of platelet transfusion include allergic and febrile nonhemolytic reactions, and the lifespan of red blood cells is approximately 120 days; post-splenectomy patients require immunizations (Hib vaccine, meningococcal vaccine, and pneumococcal conjugate vaccine) against overwhelming post-splenectomy infection (OPSI); the volume of embolized spleen is critical in PSE, but it is still difficult. Therefore, noninvasive method for viral hepatitis related liver cirrhosis is necessary to improve thrombocytopaenia. Interleukin (IL)-11 and leucogen were used to improve the platelet level, but the PLT increase level was limited, and therapeutic effects were poor in a short time [3].

TPO, which is a potent cytokine that regulates megakaryocyte and platelet production, is pri-

Table 1. Patient demographics, characteristics and adverse drug reaction after rhTPO treatment

Case	Sex	Age	Type of viral hepatitis	PLT of baseline ($\times10^9$/L)	PLT of peak ($\times10^9$/L)	Duration of treatment (day)	Child-Pugh score	Adverse drug reaction
1	F	38	B	19	70	2	B	
2	M	56	B	32	100	5	A	
3	M	70	B	20	56	3	C	
4	F	55	B	16	51	3	B	Headache
5	M	66	B	33	80	5	A	
6	F	46	C	45	102	7	A	
7	M	65	C	46	61	3	B	
8	M	47	C	38	81	3	A	
9	M	58	C	20	61	4	B	
10	M	57	B	17	27	6	C	
11	M	36	B	26	180	2	A	
12	F	65	B	17	55	2	B	
13	M	50	C	29	130	7	B	Low heat
14	M	60	B	25	43	6	A	
15	F	66	C	38	64	3	A	
16	F	26	B	38	175	5	A	
17	M	54	B	19	50	6	C	Low heat
18	F	48	C	25	77	5	A	
19	M	46	B	15	49	5	C	
20	M	43	C	43	71	5	B	
21	M	52	B	34	47	3	C	Low heat
22	F	56	C	47	76	5	A	Dizzy
23	M	63	C	32	51	3	C	
24	F	57	A	40	74	4	C	

marily produced in liver, and binds to the TPO receptor expressed on the stem cells, platelets, megakaryocytes and megakaryocyte progenitor cells [4]. Recently, TPO is often used in treatment of idiopathic thrombocytopenic purpura (ITP). In China, recombinant human thrombopoietin (rhTPO) is recommended for treatment of ITP and severe infection induced thrombocytopaenia [5]. The research about thrombocytopaenia in cirrhosis patients is rare. The study described here was designed to analyze the efficacy of rhTPO on treating thrombocytopaenia in patients with viral hepatitis related liver cirrhosis.

Patients and methods

Patients

The study was conducted in accordance with the Declaration of the Helsinki and approved by the Institutional Review Board of the Third Center Hospital of Tianjin in Tianjin, China. Written informed consent was obtained from each patient for the use of their blood and clinical information.

From March 2013 to November 2015, 24 patients (9 women, 15 men, mean age 53.3 years) confirmed having viral hepatitis related liver cirrhosis was recruited consecutively, including 14 hepatitis B and 10 hepatitis C. The hepatitis B and C virus related liver cirrhosis was diagnosed according to the American Association for the Study of Liver Disease practice guidelines [6]. The inclusion criteria including: (1) The PLT less than 50×10^9/L before treatment; (2) No platelet transfusion or drug therapy to improve platelets within 2 weeks before treatment, and no hormone, gamma globulin and interferon used within 2 weeks before treatment; (3) excluding patients with immunological hepatitis, alcoholic hepatitis, severe infection, immune thrombocytopenia and active bleeding.

Table 2. The condition of hepatic function and the result of blood routine examination for the patients before rhTPO treatment

Clinical features	All patients (n=24)	Child-Pugh class A (n=10)	Child-Pugh class B and C (n=14)
Age (years)	53.33±10.74	50.70±12.84	55.21±9.00
M/F	15/9	4/6	11/3
TPO time(days)	4.25±1.54	4.60±1.51	4.00±1.57
PLT ($\times10^9$/L)	29.75±10.49	34.70±7.91	20.21±10.91*
WBC ($\times10^{12}$/L)	2.97±1.67	3.68±2.41	2.47±0.51
HGB (g/L)	99.29±19.32	110.90±6.22	91.00±17.33*
ALT (IU/L)	73.41±80.87	107.10±116.01	49.36±27.56
AST (IU/L)	54.45±57.38	85.00±120.07	32.64±22.44
TBIL (μmol/L)	51.07±57.38	46.90±67.73	54.26±51.26
PTA (%)	65.92±16.86	77.00±18.60	58.00±10.07*

*P<0.05, compared with Child-Pugh class A.

Table 3. Platelet count after rhTPO treatment

Time	All patients (24)	Child-Pugh class A (n=10)	Child-Pugh class B and C (n=14)	Hepatitis B (14)	Hepatitis C (10)
Baseline	29.75±10.49	34.70±7.92	26.21±10.91	24.50±7.92	37.10±9.36
3 days	41.43±11.62*	45.67±14.40	38.25±8.30*	39.38±13.85*	44.75±6.00
5 days	56.11±23.86*	62.29±35.47*	52.18±12.99*	55.90±31.45*	56.38±10.43*
7 days	79.55±41.23*	102.89±44.90*	60.45±26.92*	80.70±55.65*	78.40±22.10*

*P<0.05, compared with before rhTPO treatment in same group.

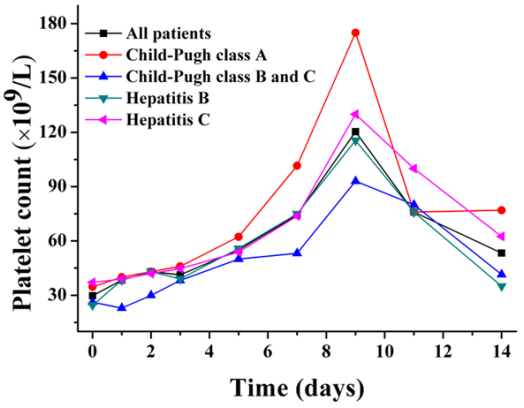

Figure 1. Changes of median platelet counts following the rhTPO treatment.

Therapeutic method

Recruited patients were subcutaneously injected recombinant human thrombopoietin (rhTPO) (TPIAO, Shenyang 3SBIOINC Co. Ltd., Shenyang, China), 15000 unit/day (2 to 7 days).

Blood sampling and laboratory data collecting

Laboratory parameters such as albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) were analyzed by an automatic biochemistry analyzer (PUZS-300, Perlong Medical, Nanjing, China) 1 day before treatment. At the same day, the prothrombin time (PT) was measured, and Child-Pugh classification was used as a prognostic indicator to evaluate the hepatic function index of patients.

Blood routine examination, including white blood cell count (WBC), heamoglobin (HGB) and PLT, was carried out by a hematology analyzer Sysmex XE-2100 (TOA Medical Electronics, Kobe, Japan) at 1 day before rhTPO treatment, 3 days, 5 days, 7 days and 14 days after rhTPO treatment. The PLT was collected just before surgical treatment and use of interferon, because of the treatment after thrombocytosis in patients.

Validity assessment: consulting idiopathic thrombocytopenic purpura (ITP) treatment standards [7], the PLT more than 50×10^9/L after rhTPO treatment was considered an effective treatment.

Statistical analysis

Measured data was recorded as mean ± standard deviation. Statistical analyses were per-

Table 4. Effective rate after rhTPO treatment

Time	All patients (24)	Child-Pugh class A (n=10)	Child-Pugh class B and C (n=14)
3 days	12.50% (3/24)	20.00% (2/10)	7.14% (1/14)
5 days	45.83% (11/24)	40.00% (4/10)	50.00% (7/14)
7 days	70.83% (17/24)	100.00% (10/10)	50.00%* (7/14)

*P<0.05, compared with Child-Pugh class A.

formed using SPSS-19.0 statistical software (IBM, Chicago, IL, USA). Differences between cases and controls regarding means were compared by using the *t*-test, and the validity was evaluated by analysis of variance (ANOVA). *p* less than 0.05 was considered as statistically significant.

Results

Demographic data before treatment

A total of 24 patients with viral hepatitis related liver cirrhosis plus thrombocytopaenia were recruited successfully. The mean value of baseline of PLT was (29.75±10.49)×10^9/L. Demographic data including gender distribution and age, PLTbefore treatment and peak value of platelet during the observation period were shown in **Table 1**. The condition of hepatic function and the result of blood routine examination were listed in **Table 2**. According to classification of liver function [8], 10 patients with Child-Pugh class A were divided into one group, and 7 patients with Child-Pugh class B and 7 patients with Child-Pugh class C were divided into another group. The patients with Child-Pugh class A have high level of baseline of PLT, prothrombin activity and hemoglobin than that of patients with Child-Pugh class B and C (P<0.05). There was no statistical difference in gender distribution, age, treatment time, ALT, AST and TBIL (P>0.05).

Variation of PLT after treatment

The mean PLT at 3 days, 5 days, and 7 days after rhTPO treatment was significant different from baseline before treatment (P<0.05), as shown in **Table 3**. According to Child-Pugh class and different causes of liver cirrhosis, platelets are improved obviously at 5 days, and 7 days after rhTPO treatment (P<0.05). Platelet change curve based on Child-Pugh class was shown in **Figure 1**.

Effective rate after treatment

The effective rate at 3 days, 5 days, and 7 days after rhTPO treatment was shown in **Table 4**. With rhTPO treatment time increasing, the effective rate was increasing. At 7 days after rhTPO treatment, the effective rate for patients with Child-Pugh class A reached 100%, and was significant higher than that of patients with Child-Pugh class B and C (P<0.05).

Adverse reaction after treatment

During the therapy, adverse reactions observed included low heat (3/24, 12.8%), dizzy (1/24, 1.2%) and headache (1/24, 1.2%), and full relieve within 3 days after treatment ended. 24 patients didn't terminate treatment because of adverse reactions, and 14 patients experienced invasive treatment after injection of rhTPO, including radiofrequency liver ablation (7 cases), dental extraction (1 case), laparoscopic hernia repair (2 cases), meningioma resection (1 case) and percutaneous transhepatic biliary drainage (3 cases). During and after the invasive treatment, no serious adverse reaction, such as major bleeding and thrombogenesis, was found.

Discussion

The primary reason for the thrombocytopenia on the cirrhosis patients was once thought to be hypersplenism secondary to portal hypertension [9]. However, portal hypertension didn't always improve thrombocytopaenia in the clinical trial. In addition, thrombocytopaenia has been observed in the cirrhosis patients with splenectomy or without splenomegaly [10], suggesting that other reasons were in relation to thrombocytopenia in liver cirrhosis patients.

In patients with viral hepatitis related liver cirrhosis, thrombocytopaenia possibly result from reduction of thrombopoietin (TPO)production, bone marrow inhibition and platelet destruction after hepatitis B and C virus infection, antiviral treatment with interferon, etc [11, 12]. The most important factor is reduction of TPO production [13]. Serum TPO level in cirrhosis patients was lesser than that in patients without cirrhosis and normal groups, and could be

back to normal after liver transplantation [14, 15]. These indicated that reduction of TPO production is a key factor, and suggested that TPO may promote platelet level in liver cirrhosis patients. Experimentally, after TPO or its receptor (c-Mpl) had been "knocked-out" from mice, the megakaryocyte and platelet values were reduced to approximately 10% of the normal level [16, 17].

Currently, TPO receptor agonists include romiplostim and eltrombopag, which have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [18]. Romiplostim [19], a non-peptide mimetics, is approved for treatment of chronic ITP in adults, and administered subcutaneously once weekly at a dose of 1-10 mg/kg. Eltrombopag [20] is a small non-peptide molecule oral platelet growth factor, and approved for treatment of chronic ITP at a dose of 25-75 mg/day. The two TPO receptor agonists have been investigated in clinical trials for treatment of thrombocytopaenia due to liver cirrhosis. PLT could increase from 40×10^9/L to 120×10^9/L, after one-week administration with a daily dose of 37.5 mg of eltrombopag [21].

However, TPO receptor agonists are not yet available and have not been approved by China's Food and Drug Administration. Compared with TPO receptor agonists, Recombinant human thrombopoietin (rhTPO) has the similar pharmacological activity and clinically curative effect. In China, rhTPO has been expressed by Chinese Hamster Ovary Cells through recombinant technology, and is a glycosylated form consisting of full-length human amino acid sequence.

All participants were viral hepatitis cirrhosis patients with severe thrombocytopaenia and 4.25 days of mean rhTPO treatment time. But, at 3 days, 5 days, and 7 days after rhTPO treatment, the mean value of PLT increased obviously with significant difference then baseline level. This was observed in patients not only with Child-Pugh class A, B and C, but also with hepatitis B and C. The mean value of PLT rose to above 50×10^9/L at 5 days after rhTPO treatment, and the effective rate reached 70.83% at 7 days after rhTPO treatment. These results indicated that rhTPO treatment could remarkable elevate PLT for viral hepatitis cirrhosis patients. As shown in **Figure 1**, after rhTPO treatment, PLT increased gradually at 3 to 5 days, reached a peak at 7 to 9 days, and down after 9 days. The variation trend of PLT was similar with previous report, in which TPO was used in treatment of ITP [22].

According to classification of liver function, the effective rate of patients with Child-Pugh class Areached 100% at 7 days after rhTPO treatment, and was obviously higher than that of patients with Child-Pugh class B and C. This result suggested that rhTPO is most suitable for patients with Child-Pugh class A. The baseline of PLT of patients with Child-Pugh class A was higher than that of patients with Child-Pugh class B and C. So, more time was need for PLT rising to more than 50×10^9/L in patients with Child-Pugh class B and C after rhTPO injection. The serum TPO level is positively associated with liver function and number of hepatocytes. The patients with Child-Pugh class A, have higher serum TPO level and more significant increase of PLT after same dose of TPO treatment than patients with Child-Pugh class B and C [11]. In addition, the reason of thrombocytopenia for patients with Child-Pugh class B and C was complicated, including portal hypertension, hypersplenism, platelet destruction caused by immunity or infection, dysfunction of platelet, etc. Therefore, increase of PLT might need extending the time of TPO treatment or uniting other therapeutic method for patients with Child-Pugh class B and C.

During the period of TPO treatment, adverse drug reactions with a low incidence, is mild including low heat, dizzy and headache. No patient discontinued TPO treatment because of adverse reactions, and 14 patients experienced invasive treatment after PLT rising to more than 50×10^9/L. All treatment was successfully accomplished and serious adverse reaction hadn't happened. These results indicated that TPO treatment of thrombocytopaenia is safe and well tolerated for viral hepatitis cirrhosis patients.

Conclusion

In our study, TPO treatment can effectively improve PLT in a short time, which reduce the risk of serious bleeding and improve the security of subsequent invasive treatment. In addition, the adverse drug reaction was mild and a

low incidence. There are some limits of our study, including retrospective study, small case number, and short follow-up time and TPO treatment time. Therefore, further studies over a longer period of time and in larger number of patients are necessary to examine the long-term effects of rhTPO treatment, and to define an optimal dosing schedule and a more durable PLT outcome.

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Disclosure of conflict of interest

None.

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