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• VIRAL HEPATITIS •

Clinical and experimental study on therapeutic effect of umbilical cord blood transplantation on severe viral hepatitis

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Abstract

AIM: To investigate the therapeutic effect of umbilical cord blood transplantation (UCBT) on patients with severe viral hepatitis and on liver lesions in rats.

METHODS: One hundred and fifty three patients with severe viral hepatitis were included in the study between April 1990 and July 2002. The patients were treated with adult plasma transfusion (control), UCBT, plasma exchange (PE) and UCBT combined with PE (UCBT+PE) respectively. The therapeutic effectiveness was evaluated by serial determinations of liver function, lipids and immune function in all patients before and after the treatment. The model of experimental hepatic failure was constructed in SD rats by injecting carbon tetrachloride. Then, the rats were given normal saline, adult plasma or neonate cord blood intraperitoneally. After detection of liver function, the rats were killed and morphological changes of the liver were microscopically observed.

RESULTS: UCBT group and UCBT+PE group had much better improvement in liver and immune functions than control group and PE group. The patients in UCBT+PE group had the best clinical efficacy. UCBT was safe and had no side effects. The animal experiment showed significant improvements in liver function and survival rate in neonate cord blood group as compared with adult plasma group. The histopathology of rat's liver indicated that neonate cord blood application could decrease the liver injury and increase hepatocellular regeneration.

CONCLUSION: UCBT demonstrated a good therapeutic effect on severe viral hepatitis and no obvious side effects. Umbilical cord blood can attenuate the liver lesions and reproduce hepatocyte. The treatment of UCBT combined with PE was much better than that of single plasma exchange, thus UCBT can enhance the therapeutic effect of plasma exchange on severe viral hepatitis.

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INTRODUCTION

In 1987, a child with Fanconi' s anaemia received an allogeneic transplant using the cryopreserved umbilical cord blood (UCB). As a result, the potential of UCB as a source of haemopoietic stem cells for transplantation rapidly became a field of intense clinical and scientific interest. Because UCB is a relatively easily available starting material, it has been increasingly used as an alternative source of hematopoietic stem cells in allogeneic transplantation. Many umbilical cord blood banks have been established. The engrafting cells in UCB are found to be significantly higher than those in adult bone marrow or in mobilized peripheral blood from normal donors. It has been found that multipotent adult progenitor cells (MAPCs) can differentiate into hepatocyte-like cells *in vitro*[1]. Neurons, astrocytes and oligodendrocytes can be propagated *in vitro* from UCB cells[2,3]. The evidences indicate that stem cells can differentiate into hepatocytes[4,5]. This study evaluated the therapeutic effectiveness of UCBT on severe hepatitis, and tried to find a new therapy for severe viral hepatitis.

MATERIALS AND METHODS

Grouping and treatment

One hundred and fifty-three inpatients with severe viral hepatitis were included in the study from April 1990 to July 2002. These patients had a history of hepatitis for more than 8 weeks, with severe hepatic dysfunction, rapidly progressive jaundice, abdominal distention, asthenia, ascites, coagulopathy, encephalopathy and hepatorenal syndrome. The patients were randomly divided into control (adult plasma) group: 39 patients (36 male and 3 female) with a mean age of 39.4 years (range 17-56 years), UCBT group: 38 patients (37 male and 1 female) with a mean age of 41.2 years (range 13-62 years), PE group: 45 patients (43 male and 2 female) with a mean age of 37.5 years (range 21-58 years), and UCBT+PE group: 31 patients (all were male) with a mean age of 43.2 years (range 13-65 years). The patients were treated with 200 ml of fresh adult plasma/ blood transfusion (in control group and PE group) or 200 ml of umbilical cord blood transplantation (in UCBT group and UCBT+PE group) intravenously each time, one to three times a week for two to four weeks. Plasma exchange given was 1 500-3 000 ml each time, one to three times every week and totally two to five times. The rate of plasma separation and the flow rate of plasma exchange were controlled at the speed of 5-15 mL/min and 15-25 mL/min, respectively.

Collection and storage of umbilical cord blood

Immediately after the birth of the baby, the umbilical cord was cut off and the baby was separated from the placenta and mother. A sterile needle was inserted into the umbilical vein and the placental blood was drawn into a sterile blood collection bag containing ACD-B anticoagulant, provided by Shanghai Blood Center. Once the collection was complete, the specimen was packaged and sent to blood bank for processing and storage at low temperatures. The maternal blood sample was also collected for infectious disease analysis.

Equipment
PE was performed with COBE Spectra™ Auto PBSC version 6.0 apheresis system (COBE BCT, Inc. Colorado, USA).

Animals and experimental design
Male SD rats, weighing 150 to 180 g, were purchased from the Laboratory Animal Center, Xiangya Medical College, Zhongnan University. On the first and second day carbon tetrachloride was injected into each rat (1 ml/100 g) intraperitoneally to establish an acute liver failure model. On the third day several rats were killed as acute liver failure models and the livers were taken for histopathological examination, the other 81 survived rats were randomly assigned to control group (n=24), normal saline group (n=16), adult plasma group (n=22), and neonate cord blood group (n=19). Rats in groups 2-4 were intraperitoneally given 1 ml/100 g· d^{-1} normal saline, adult plasma or neonate cord blood respectively for five days and no treatment was given in group 1. On the eighth day, after detection of the liver function, all survived rats were killed and sections of liver tissues were taken immediately for histopathological examination. Excised liver tissues were fixed in 10 % neutralized formaldehyde, embedded in paraffin, and then routinely stained with hematoxylin and eosin.

Statistical analysis
The data were expressed as means ± standard deviation ($\bar{x}\pm s$), and the differences between the value of different groups were analysed by the t test or χ^2 test (SPSS version 9.0). The criterion of significance was set at P<0.05.

RESULTS

Changes of patient's liver function
There was no difference in liver function (Alb, ALT, serum total bilirubin and prothrombin activity) among all the groups before treatment. Alb and prothrombin activity (PTA) were much lower than normal but serum total bilirubin (TBil) was remarkably higher than normal in all groups before treatment. Alb and PTA increased in all groups after treatment. As compared with control group, the degrees of Alb and PTA increase were higher in UCBT group, PE group and much higher in UCBT+PE group (P<0.05). The change of ALT level was not significant after treatment in all groups. Serum TBil contents significantly decreased after treatment in UCBT group, PE group and UCBT+PE group (P<0.05), (Table 1).

Influence of UCBT and PE on immune functions of the patients
Immune functions of the patients were markedly improved after treatment in UCBT group and UCBT+PE group. The values of $CD4^+$, active T lymphocytes and IL2 were significantly increased after treatment in UCBT group and UCBT+PE group (P<0.05). However no significant change of the values was observed in control group and PE group. There was no significant difference of $CD8^+$ lymphocytes before and after treatment in all groups (Table 2).

Side effect and safety
No side effect or negative response was found in UCBT group. Three patients in control group, ten patients in PE group and eight patients in UCBT+PE group had rashes during the treatment. Four patients had fever in PE group. No fatal side effect was observed in all groups.

Survival of rats
On the eighth day, 89.5 % (17/19) rats survived in neonate cord blood group, 54.5 % (12/22) in adult plasma group, 31.3 % (5/16) in normal saline group and 25.0 % (6/24) in control group respectively. The survival rate of rats in neonate cord blood group was significantly higher than that in adult plasma group (P<0.05), normal saline group and control group (P<0.01).

Biochemical parameters of rats
The serum levels of ALT and TBil in neonate cord blood group were significantly lower than those in adult plasma group (P<0.05), normal saline group and control group (P<0.01). Meanwhile the serum level of AFP in neonate cord blood group was significantly higher than that in the other three groups (P<0.01), (Table 3).

Histopathology of rats
Histopathology of model rats' livers revealed lobular disarray, ballooning degeneration, fatty degeneration, inflammatory cell infiltration in the portal zone and areas of parenchyma, cholestasis and massive necrosis of hypatocytes (Figure 1). All indicated that there was an acute liver failure in model rats. After five-day treatment of neonate cord blood, hepatocellular necrosis and mononuclear cell infiltration were greatly decreased and only spotty hepatocellular necrosis occurred. Meanwhile

Table 1 Changes of biochemical parameters of patients before and after treatment ($\bar{x}\pm s$)

Group	ALT(u·L^{-1})		TBil (μmol·L^{-1})		Alb(g·L^{-1})		PTA(%)	
	Before	After	Before	After	Before	After	Before	After
Control	112.4±37.2	98.5±23.6	315.1±43.2	263.7±105.2	31.3±5.1	32.5±2.6	33.5±4.7	43.5±6.7
UCBT	95.6±31.9	84.3±35.7	376.3±55.1	203.1±85.4^{bc}	30.6±4.2	35.6±2.4^{a}	31.5±3.6	45.8±5.4^{a}
PE	116.3±44.1	81.9±42.5	356.6±48.5	195.6±103.7^{bc}	28.5±3.6	36.9±5.1^{bc}	28.8±7.2	48.3±7.5^{b}
UCBT+PE	104.5±38.6	85.3±36.2	405.7±59.4	173.2±125.6^{bc}	30.7±4.4	37.7±3.5^{bc}	29.7±6.5	52.3±10.2^{bc}

^{a}P<0.05 *vs* before treatment, ^{b}P<0.01 *vs* before treatment, ^{c}P<0.05 *vs* control group.

Table 2 Parameters of cellular and humoral immune of patients before and after treatment ($\bar{x}\pm s$)

Group	Active T lymphocyte (%)		$CD4^+$ lymphocyte (%)		$CD8^+$ lymphocyte (%)		IL2 (u· ml^{-1})	
	Before	After	Before	After	Before	After	Before	After
Control	35.6±7.5	44.1±14.3	32.7±5.3	35.6±8.2	22.5±3.6	23.7±5.2	41.3±10.5	48.6±21.5
UCBT	33.2±8.7	51.6±12.5^{ab}	30.5±6.9	44.3±11.6^{a}	23.2±4.7	25.6±9.5	44.5±9.6	62.7±28.6^{ab}
PE	36.1±6.3	42.7±16.9	29.5±8.2	34.7±7.4	20.4±6.9	26.7±8.4	48.6±11.3	51.3±31.9
UCBT+ PE	31.9±8.5	58.9±25.4^{ab}	27.1±7.3	48.5±21.3^{ab}	18.3±8.5	28.4±11.2	36.2±10.8	64.8±27.5^{ab}

^{a}P<0.05 *vs* before treatment, ^{b}P<0.05 *vs* control group.

obviously hepatocellular regeneration was observed in neonate cord blood group (Figure 2). There was no significant histopathological difference between control group and normal saline group. Multilobular necrosis and numerous lymphocytic infiltrations were noted, and no evident hepatocellular regeneration was demonstrated in control group and normal saline group (Figure 3). Local necrosis, moderate degree of lymphocytic infiltration and a few focal hepatocyte regenerations were observed in adult plasma group (Figure 4), (Table 4).

Table 3 Biochemical parameters of rats after treatment ($\bar{x}\pm s$)

Group	ALT($u \cdot L^{-1}$)	TBil ($\mu mol \cdot L^{-1}$)	AFP($\mu g \cdot L^{-1}$)
Control group	1025.3±36.9	78.6±3.4	21.5±13.8
Normal saline group	1016.8±318.2	65.6±18.3	23.7±15.9
Adult plasma group	365.6±118.4[a]	36.2±9.5[a]	28.9±18.7
Neonate cord blood group	186.7±39.5[bc]	14.3±6.0[bc]	686.4±186.5[bd]

[a]$P<0.05$ *vs* control group and normal saline group, [b]$P<0.01$ *vs* control group and normal saline group, [c]$P<0.05$ *vs* adult plasma group, [d]$P<0.01$ *vs* adult plasma group.

Table 4 Histological examinations of rats' liver after treatment ($\bar{x}\pm s$)

Groups	Ballooning degeneration	Fatty degeneration	Hepatocellular necrosis	Hepatocellular regeneration
Control group	+++	+++	+++	--
Normal saline group	+++	+++	+++	--
Adult plasma group	++	++	++	+
Neonate cord blood group	+	+	+	+++

+++: severe / extremely remarkable, ++: medium, +: mild, -: no / non-obvious.

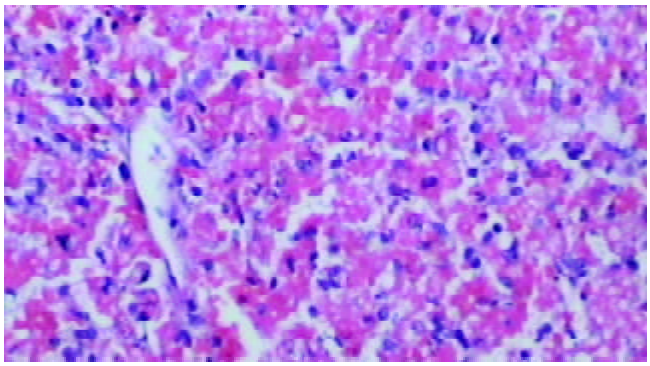

Figure 1 Liver tissue from an acute liver failure model rat (HE 200×).

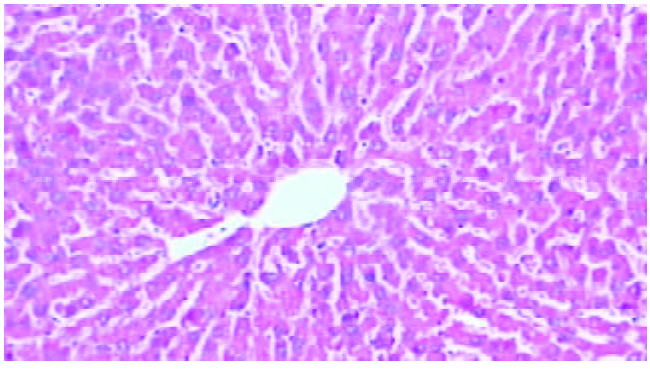

Figure 2 Liver tissue from a rat in neonate cord blood group (HE 100×).

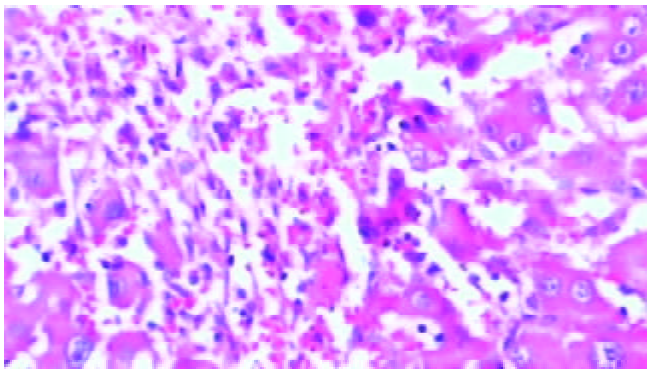

Figure 3 Liver tissue from a rat in control group and normal saline group (HE 400×).

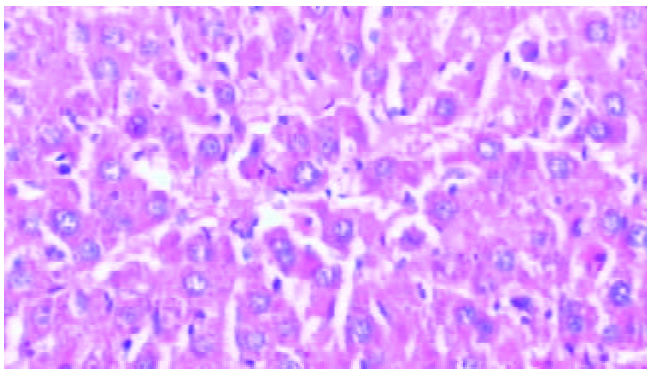

Figure 4 Liver tissue from a rat in adult plasma group (HE 200×).

DISCUSSION

Severe hepatitis is the most ominous manifestation of viral hepatitis. The overall mortality rate of patients with severe hepatitis in stage III to IV coma averages 70-80 %. The management of severe hepatitis includes conventional medical management, plasma exchange, charcoal hemoperfusion, bioartificial liver system and liver transplantation, but the therapeutic effectiveness of all these therapies is not satisfactory. Thus, it is an urgent challenge to find a new reliable therapy for severe hepatitis. Umbilical cord blood (UCB) is the blood that remains in the placenta and umbilical cord after birth. Until recently the placenta and umbilical cord blood were discarded after delivery as a medical waste. The presence of hematopoietic progenitor cells (HPC) in UCB was demonstrated in 1974. Experimental evidences have shown that UCB is a rich source of hematopoietic stem/progenitor cells (HSPC). However, it was not until 1989 that experimental and clinical studies were published, indicating that human UCB could be used in clinical settings. In same year, the first hematopoietic cell transplant in which UCB was used instead of bone marrow as the source of hematopoietic cells was reported. The hematopoietic system of a child with Fanconi's anemia was reconstituted by means of UCB from an HLA-identical sibling. Since then, there has been an expanding interest in the use of UCB as an alternate source of HSPC for transplantation[6]. Some data suggested that UCB was a better source of hematopoietic stem cells than bone marrow[7]. Recipients of cord-blood transplants from HLA-identical siblings have a lower incidence of acute and chronic GVHD than recipients of bone marrow transplants from HLA-identical siblings[8]. Frozen immature human UCB cells can be stored for more than 15 years, and efficiently retrieved, and remain effective for clinical transplantation[9]. After injected into developing neonatal mouse brains, immunodepleted mouse stem cells (MSCs) differentiated into astrocytes and neurons[10].

Intravenous administration of human UCB can reduce neurological deficiency in the rat after traumatic brain injury[11]. Transplantation of genetically marked bone marrow into immunodeficient mice revealed that marrow-derived cells migrated into areas of induced muscle degeneration, underwent myogenic differentiation, and participated in the regeneration of damaged fibers[12]. Experiments demonstrated the cardiomyogenic potential of hematopoietic stem cells[13] and the presence of circulating stem cells with osteogenic and adipogenic differentiation potential in human UCB. Hematopoiesis and the hepatic environment are known to have a close relationship at the time of liver development and systemic diseases. Transplanted cells isolated from bone marrow of rodents and humans have been shown to differentiate into oval cells, which are considered to be hepatic stem cells and hepatocytes in the liver. Purified hematopoietic stem cells have been shown to be able to replace original liver cells in mice with hereditary tyrosinemia. Intravenous injection of adult bone marrow cells in the $FAH^{-/-}$ mouse rescued the mouse and restored the biochemical function of its liver[14]. Hematopoietic stem cells can contribute to the hepatocyte lineage in humans and in rodent models of liver disease and regeneration[15] and adult human liver cells can derive from stem cells originating in the bone marrow or circulation outside the liver[16-18]. Hepatocytes can derive from bone marrow cells after irradiation in the absence of severe acute injury[19]. Adult bone marrow cells have tremendous differentiating capacity as they can also differentiate into epithelial cells of the liver, lung, GI tract, and skin[20, 21]. Human mesenchymal stem cells are thought to be multipotent cells, which are present in adult marrow, that can replicate as undifferentiated cells and have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma[22]. Transdetermination consequent to cell fusion could underlie many observations attributing to an intrinsic plasticity of tissue stem cells[23,24].

UCB can easily be collected and cryopreserved and has a number of significant advantages over bone marrow such as no risk to the donor, no donor attrition, minimal risk of viral transmission, immediate availability, high rate of engraftment, more tolerant of tissue mismatches and lower rate of rejection.

In the present study, we observed that TBil content reduced and Alb, PTA increased after treatment in all groups, especially in UCBT group, PE group and UCBT+PE group. The best therapeutic effectiveness was shown in UCBT+PE group. The effects of reducing Tbil content and increasing Alb and PTA in UCBT+PE group were more significant than those in control group ($P<0.01$) and PE group ($P<0.05$). This indicates that UCBT can enhance the therapeutic effectiveness of PE on severe hepatitis. Although the effect in UCBT+PE group was also better than that in UCBT group, the difference was not significant ($P<0.05$). The therapeutic effectiveness in UCBT group was significantly better than that in control group ($P<0.05$), indicating that UCB can more effectively improve liver function than adult plasma. The reason may be that stem cells derived from UCB can differentiate into hepatocytes and cholangiocytes in a culture system simulating liver regeneration and containing cholestatic serum, which is similar to the condition of patients with severe hepatitis[25-28].

We also found that active T lymphocytes, $CD4^+$ lymphocytes and IL2 were evidently increased after treatment in UCBT group and UCBT+PE group, but no significant improvement of immune function was found in control group and PE group. The results indicate that UCB can improve the immune function of patients with severe hepatitis. It may be because stem cells derived from UCB can differentiate into lymphocytes and reconstitute thymic function[29-32].

The animal experiment demonstrated that the survival rate of rats in neonate cord blood group was significantly higher than that in adult plasma group ($P<0.05$), control group and normal saline group ($P<0.01$), and the ALT and TBil values in neonate cord blood group were obviously lower than those in control groups, saline group ($P<0.01$) and adult plasma group ($P<0.05$). The histopathological examination of rats' livers showed that only mild hepatocyte necrosis and mononuclear cell infiltration were present in the rats of neonate cord blood group after treatment, but massive hepatocyte necrosis and numerous lymphocytic infiltrations were found in other groups. There was obvious hepatocellular regeneration in neonate cord blood group after treatment. However, there was only a few local hepatocellular regenerations in adult plasma group and no hepatocellular regeneration in normal saline group and control group after treatment. All of these indicate that neonate cord blood can protect liver, decrease hepatic injury and promote hepatocellular regeneration. It implies that there are some liver regeneration stimulating factors in neonate cord plasma and some stem cells in neonate cord blood can differentiate into hepatocytes.

Moreover, no side effect or negative response was found in the patients of UCBT group during whole treatment. It showed a high safety in UCB administration. Transient fever, rash and allergic shock were noticed in some patients in control group, PE group and UCBT+PE group. The results indicate that UCBT is much safer than adult fresh plasma transfusion.

In conclusion, this study shows that UCBT can improve liver function and immune function of patients with severe hepatitis, enhance the therapeutic effectiveness of PE, alleviate hepatic injury and promote hepatocellular regeneration. The study indicates that UCBT is a new reliable and safe therapy for severe hepatitis.

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