Experimental study on the protective effect of embryonic stem cell transplantation on immune and hematopoietic function in tumor chemotherapy

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Abstract: Objective: To study the effects of embryonic stem cell transplantation (EST) on bone marrow hematopoietic function inhibition, cell immune function inhibition and organ toxicity in mice induced by cyclophosphamide. **Method:** 40 Wistar rats; Subcutaneous inoculation of W256 tumor sarcoma cells in the right armpit and divided for four groups, with 10 rats in each group; (1) Physiological saline control group (NS); (2) Cyclophosphamide treatment group (CTX); (3) Embryonic stem cell transplantation group (EST); (4) After cyclophosphamide therapy with the use of embryonic stem cell transplantation group (CTX+EST). **Results:** (1) With cyclophosphamide (i.p.30mg / kg/day x 5 day) chemotherapy, animal peripheral hemogram with chemotherapy compared to before, the white blood cell count and net woven red blood cell count and hemoglobin content decreased significantly respectively ($P < 0.001$, $P < 0.01$, $P < 0.001$); The total positive rate of lymphocyte ANAE and the percentage of ANAE were decreased respectively ($P<0.001$ and $P<0.05$); Peripheral blood lymphocytes to PHA stimulation induced proliferation response reduction ($P<0.05$) and thymus weight loss ($P<0.001$); (2) After 4 days of embryonic stem cell migration (i.v. 4x107/200g/), white blood cell count and red blood cell count were significantly increased in EST CTX group ($P<0.001$ and $P<0.01$) respectively; The amount of hemoglobin has a rising trend; EST group in peripheral blood had no obvious change; CTX EST group the thymus weight was restored ($P<0.01$). The effect of embryonic stem cell transplantation on phagocytic function of neutrophil and the proliferation of lymphocytes stimulated by PHA was not obvious. After the transplantation of embryonic stem cells, the ^{3}H-TdR content of bone marrow hematopoietic cells of EST group and CTX+EST group, the total positive rate of lymphocyte ANAE and the percentage of ANAE were higher than that of NS group CTX. There was no significant difference in average tumor weight between the CTX group and the E CTX +EST group ($P>0.05$), and the tumor inhibition rate was 81.5% and 82%, respectively. There was no significant difference in the average tumor weight between the EST group and the NS group too. **Conclusion**: (1) EST promote cyclophosphamide chemotherapy after peripheral hemogram and recovery of function, but the normal blood but no obvious effect; (2) EST has stimulated the proliferation of bone marrow hematopoietic cells; (3) the effect of EST on the percentage of ANAE positive lymphocytes in peripheral blood and the percentage of point like ANAE lymphocytes induced by cyclophosphamide chemotherapy; (4) EST can promote the recovery of thymus weight after cyclophosphamide chemotherapy; (5) EST does not affect the anti-tumor effect of cyclophosphamide on W250 sarcoma of rats, and it does not inhibit the tumor itself.
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1. Introduction:

Chemical drugs in the treatment of cancer in the comprehensive treatment of the patients with malignant tumor accounted for an important position, but the in killing tumor cells at the same time, the side effect on the body to produce the clinical application is limited. People are still searching for an effective way to overcome this problem. In recent years, the research on embryonic stem cell transplantation and its biological characteristics and function has proved that embryonic stem cells are important hematopoietic stem cells [1-8]. Embryonic stem cell transplantation (EST) for the treatment of leukemia, plastic anemia and combined immunodeficiency disease has achieved remarkable effect [9-14]. Due to the low antigenicity of fetal liver cells, after transplantation in vivo generally do not produce immune rejection phenomenon [15-17]. Therefore, the application of embryonic stem cell transplantation has been paid more and more attention in clinical practice. Laifeng Xue, Kochupillai and Hu Bin, et al. [18, 19] have reported that in malignant tumor chemotherapy the process of application of embryonic stem cell transplantation significantly improve the peripheral

blood [20, 21, 28]. But due to the limitation of clinical observation and study of the conditions to clear the exact curative effect and its mechanism still need to be confirmed by further studies.

The study selected W-256 sarcoma (W256) as a tumor animal model, experimental study of embryonic stem cell transplantation (EST) on tumor chemotherapy induced immune and hematopoietic function, embryonic stem cell transplantation in the application of chemical drugs in the treatment of cancer to provide effective clinical data and function mechanism.

2. Experimental materials and methods:

2.1. Experimental materials:

2.1.1. Experimental animal;

2.1.1.1. Wistar rats, female, body weight 180-220 grams, provided by the experimental animal center of Zhengzhou University;

2.1.1.2. W256 transplanted tumor in Rat Species, provided by the Institute of medicine by the Chinese Academy of Sciences;

2.1.2. Main reagents and drugs:

2.1.2.1. Cyclophosphamide (CTX), Made in Shanghai twelfth pharmaceutical factory, China;

2.1.2.2. RPMI1640 culture media, made in the United States- GIBCO;

2.1.2.3. N- hydroxyethyl piperazine N1-2 ethyl sulfonate, POPOP, made in West Germany-Merck;

2.1.2.4. Calf serum CS), made in Tianjin biochemical products factory, China;

2.1.2.5. Phytohemagglutinin (PHA), made in Guangzhou Institute of pharmaceutical industry, China;

2.1.2.6. Thymidine -^{3}H (^{3}H-TdR), Specific activity of 25Ci/mM (1mCi/ml), made in Institute of atomic energy, Chinese Academy of Sciences, China;

2.1.2.7. 2, 5-diphenylo-xazole (ppo) Flicker pure, toluene and Analysis of ethanol, that all were made in Shanghai reagent factory products, China;

2.1.2.8. 49 type glass fiber membrane: cut with =25mm standby, made in Shanghai red paper factory, China;

2.1.2.9. 40 hole plastic micro plate: after 30 minutes of dry ethanol immersion, drying, ultraviolet radiation disinfection 2 hours after the use, made in Shanghai plastic three plant products, China;

2.1.2.10. Acetate 1-Naphthyl, chemical purity, made in Shanghai Reagent Factory, China;

2.1.2.11. Nitro tetrazolium, made in Shanghai Qianjin Reagent Factory, China;

2.1.2.12. Safranine, methyl green and brilliant cresyl blue that were made in Shanghai reagent factory the third branch, China.

2.1.3. Instruments:

2.1.3.1. CO_2 incubator: made in Japan Kanazawa products;

2.1.3.2. MM-1 type micro oscillator, made in Jiangsu Tai county medical equipment factory, China;

2.1.3.3. FJ-21020 double channel liquid scintillation counter, made in Xi'an state 262 factory, China.

2.2. Experimental method:

2.2.1. W256 tumor cells of rats were cultured for 7 days; W256 tumor cells were grown for seventh days after 1-2ml, according to the 1:3 of the tumor cells, 0.2ml was inoculated into the abdominal cavity of rats (100 grams or so /each Rat), passage once every 7 days;

2.2.2. Preparation of embryonic stem cell suspension; Under sterile conditions from gestational age 17-20 days after allogeneic fetal rat 9-12, physiological saline flushing the surface blood, caesarean section in the liver, set 200 mesh nylon net ophthalmic bending shear repeatedly cut into pieces, plus 10-20 ml saline flush filter, filtrate is collected from the fetal liver cell suspension, with 4 needle suction head for single cell suspension, with saline to adjust a nuclear cell concentration to $4x10^7$/ml. approved by the trypan blue exclusion test [21], cell activity in more than 85%. Preparation to be used within 1 hour.

2.2.3. Experimental grouping and processing; The ascites of W256 tumor cells was seventh days and days, the tumor cell concentration was adjusted to $2.5x10^7$/ml with normal saline; 40 rats were injected with W256 tumor cell suspension 0.2ml (including $5x10^6$ tumor cells) at right axillary subcutaneous injection, and were randomly divided into four groups (10 in each group); (1) normal saline control group (NS); (2) cyclophosphamide treatment group (CTX); (3) embryonic stem cell transplantation group (EST); (4) cyclophosphamide group + embryonic stem cell transplantation group (CTX+EST); After second days, CTX group and EST+CTX group were injected intraperitoneally (i.p.) CTX30mg/kg/day (CTX with normal saline solution, concentration of 20mg/ml) for 5 consecutive days. NS group and EST group with saline (i.p.) as control. 7 days EST group and CTX EST vein group slow injection (i.v.) of fetal liver cell suspension liquid 1ml/200 grams (4x107/ml), 1 times a day, for 4 consecutive days; NS group and CTX group with 1ml physiological saline (i.v.) as control. The 14 day bleed the animals were sacrificed.

2.3. Acquisition of experimental data:

2.3.1. Dynamic observation of peripheral hemogram； all animals from the experimental to the animals were sacrificed and every 2 - 3 days blood samples were collected from tail vein, according to conventional methods of checking white blood cell count (WBC), leukocyte differential count (DC) and.

Red blood cell count (RET): Sanis method is used to measure hemoglobin (HB);

2.3.2. Proliferation ability of bone marrow hematopoietic cells measured by ^{3}H-TdR incorporation method [22];

2.3.2.1. The animals were killed 5 days after the end of the EST, aseptic removal by the side of the femoral and removal of the soft tissue around the femur, with RPMI1640 medium (containing 20% CS, 25mm HEPES, 100u / ml penicillin and 0.1 mg / ml streptomycin) 2ml out of bone marrow cells, sterile vial (including 25 units of heparin), with 4 needle aspiration once, then, after the cultured liquid adjustment nucleated cell concentration to $2.5x10^{6}$/ml, 40 hole plastic microtiter plate in each hole with 200ul cell suspension, each sample repeated three hole injection;

2.3.2.2. With physiological saline and diluted 3H TDR liquid 25 times, and add 5 UL/hole (final con. is 0.2 g ci/hole). Through micro oscillator and uniformly mixing, at 37 degrees, 5% CO_2 saturated concentration of CO_2 culture box culture for 4 hours the cells were harvested;

2.3.2.3. After the termination of the culture, with a dropper absorb holes in the cell suspension and drop in on a glass fiber filter, vacuum filtering, respectively, with 5 ml of distilled water for dissolving the filter membranes of cells, 5 ml of 5% trichloroacetic acid precipitated protein, finally add 3 ml of anhydrous ethanol enable dehydration deposit, decolorization, label post 37 degrees oven drying;

2.3.2.4. Take 5g of PPO, 0.3g of POPOP, toluene 1000ml configuration scintillation liquid. Determination and the dried membrane immersed in containing 5ml scintillation liquid flash in the liquid bottle and heads, with a liquid scintillation counter to measure pulses per minute (CPM), calculated three hole average CPM value, in order to determine ability of the added value of bone marrow hematopoietic cells;

2.3.3. Effect of EST on PHA induced lymphocyte proliferation (^{3}H-TdR incorporation) **[23]:**

2.3.3.1. Before the animals were killed, the femoral artery blood 2ml, heparin anticoagulation;

2.3.3.2. 6 bottles of penicillin with each blood samples and each plus 100 microliters of whole blood, PHA stimulation group and control group, each of the three bottles, were added to 100 ug / ml PHA and does not contain the PHA RPMI1640 1 ml of liquid, with 5% $NaHCO_3$ to adjust the pH to 8.0;

2.3.3.3. Mixed it and the rear temperature at 37 degrees in the training of 72 hours, 16 hours before the termination of training, plus a bottle of 3H-TdR 1 ci/5 ul;

2.3.3.4. In the termination of culture, the ^{3}H-TdR content was measured by the filter membrane method, and the stimulation index (SI) indicated the ability of lymphocyte proliferation after PHA stimulation, The stimulation index (SI) value was calculated by the following formula [22];

SI= Plus CPM average PHA tube value / Do not plus CPM average PHA tube value.

2.3.4. Effect of EST on the percentage of positive lymphocytes in peripheral blood of each type of alpha - acetate (ANAE): Animals in each group before chemotherapy, after chemotherapy and embryonic stem cell transplantation, respectively, the tail vein blood, according to Wu Jinglan and other reported methods [24], observation and counting ANAE lymphocytes and sub type count;

2.3.4.1. Will phosphate buffer solution (PBS, pH 7.2) and heparin anticoagulant by 3:1 mix, smear, dry it and then put it at 4 degrees formalin acetone buffer PH6.6 fixation for 1 min, water washing and dry it;

2.3.4.2. In the incubation solution (alpha - acetate final concentration of 0.5mg/ml, PH6.4, at 37 degrees) for 90 minutes, remove the water after washing, 1% methyl green staining for a few seconds, water rinse, dry it in air;

2.3.4.3. 100 cells were observed under oil microscope, according to the distribution characteristics of ANAE reaction product in the cytoplasm, ANAE positive lymphocytes divided sample type (a 1-3 thick red particles) and diffuse type (a scattered in small particles or pink sheet) respectively. The calculation of point type, diffuse and total ANAE lymphocyte percentage;

2.3.5. Effect of EST on phagocytosis of neutrophils, the phagocytic activity of neutrophils was determined by the four nitrogen blue reduction test (NBT) [25]; Measuring time was same the ANAE test;

2.3.5.1. Will trace the anticoagulant blood, PBS and 0.2% tetrazole nitrogen blue physiological saline by 21:1 mix, added to the reaction plate hole of the organic glass, 37 degrees water bath for 20 minutes, absorb upper cell smear, statically at room temperature for 10 minutes, dry it in air;

2.3.5.2. Fixed with methanol for 1 minutes, washed, dried. Put it at the 1% Safranine dye for 1 minutes, washed and dried in air;

2.3.5.3. Under oil microscope observation of 100 neutrophils containing black armour to precipitate NBT positive cells, calculated the percentage of NBT positive cells;

2.3.6. Effect of EST on the weight of immune organs; after the animal was sacrificed by spleen and thymus, with a torsion balance, calculate the spleen index and thymus index according to the following formula [26];

Spleen (Thymus) index = Spleen (Thymus) weight (g)/ weight (g).

2.3.7. Effect of EST on the weight of growth of W256 tumor cells in rats; after the animals were killed, the tumor size and texture were observed, the tumor was weighed and the tumor inhibition rate (TIR) [27] was calculated;

TIR= (1- average tumor weight in the experimental group/average tumor weight in NY group) x 100%.

2.4. Statistical analysis:

Statistical analysis of all data uses SPSS 21.0. The normal distribution of the data described by means of representation (X±S); before and after treatment data were compared with paired measurement data of t test, the data between groups were compared with two samples t test.

3. Results:

3.1. Effect of EST on peripheral blood; After CTX chemotherapy, WBC, Ret and HB were significantly decreased (P<0.001, P<0.01, P<0.001) before and after chemotherapy (Table 1). After the transplantation of embryonic stem cells, EST group and NS group compared blood no significant change (P > 0.05); CTX+EST group and the CTX group compared WBC and RET were increased, the difference was significant (respectively, P < 0.001, P < 0.01), hemoglobin (HB) increased, but no statistical significance (Table 2).

Fifth days after the end of chemotherapy, the white blood cell count began to change, compared with the NS group, CTX group and EST CTX group, the proportion of granulocyte increased, the proportion of lymphocytes decreased (P<0.001), After the two groups or between NS and EST group comparison, no significant difference in the classification count of EST+CTX group after embryonic stem cell transplantation, compared with the CTX group, WBC recovery showed a rapid upward trend (Tables 2, 3).

Table 1. Effects of cyclophosphamide on WBC, Ret and HB in W256 rats

	Before cyclophosphamide	After cyclophosphamide	P Value *
WBC /μl	15965±3046	1315±616	P<0.001
Ret (%)	1.14±0.77	0.16±0.17	P<0.001
Hb (g)	15.0±1.3	12.4±1.2	P<0.001

* Results of t test for paired data before and after chemotherapy in group

Table 2. Effects of EST on WBC, Ret and HB in W256 Rats

Groups	Number of animals	WBC /Ul	Ret (%)	Hb (g)
NS	10	19485±4028	1.02±0.70	13.9±0.9
CTX	10	4550±1633 *	1.24±0.98 *	12.1±2.0 *
EST	10	18490±3686	1.64±0.64	14.1±1.3
CTX+EST	10	8680±1492 * *	3.39±1.92 * *	13.0±1.7

* compared with NS having a statistical significance; WBC: P<0.001; Hb: P<0.05; Ret: P<0.05

** compared with CTX having a statistical significance: WBC: P<0.001; Ret: P<0.05

Table 3. Effects of cyclophosphamide on the classification of white blood cells in W256 rats

Group	Number of animals	Granulocyte （%）	lymphocyte （%）	Monocyte （%）
NS	10	22.70±7.96	74.50±7.17	2.80±2.39
CTX	10	45.50±4.58 *	52.60±5.32 *	1.90±1.73
EST	10	21.80±6.92	75.30±6.72	1.90±2.08
CTX+EST	10	44.80±10.18 *	52.20±10.06 *	2.40±2.01

* compared with NS, P<0.001; Comparison of CTX group and CTX+EST; P>0.05

3.2. Effect of EST on the amount of ^{3}H-TdR in bone marrow hematopoietic cells: EST group and EST+CTX group compared with NS group, the 3H-TdR content increased, the difference was significant (P<0.01 and P<0.001 respectively); EST+CTX group 3H-TdR content was higher than the CTX group (P<0.05) (Table 4).

Table 4. Effects of embryonic stem cell transplantation on ^{3}H-TdR content in bone marrow hematopoietic cells

Group	Number of Animals	^{3}H-TdR content (Cpm/5x10^{5} Cells)
NS	10	19376.1±3345.5
CTX	10	22489.3±5051.1
EST	10	24578.6±4134.2*
CTX+EST	10	27674.2±4563.7**

*Compared with NS Group, P<0.01; **Compared with CTX Group, P<0.05.

3.3. Effect of EST on PHA induced lymphocyte proliferation; after injection of CTX, the SI of CTX group was lower than that of NS group (P<0.05); the embryonic stem cell transplantation had no significant effect on the lymphocyte proliferation response in EST group and EST CTX group (Table 5）.

Table 5. Effects of embryonic stem cell transplantation on PHA induced lymphocyte proliferation

Group	Number of Animals	Control (Cpm)	PHA (Cpm)	stimulation index
NS	10	857.8±121.7	18880.2±3316.6	22.1±2.9
CTX	10	722.0±128.7	13265.5±2824.2	18.5±2.9*
EST	10	835.0±140.6	19446.7±5167.5	23.0±2.4
CTX+EST	10	808.2±111.6	16760.8±3666.2	20.7±2.8

*Compared with NS Group; P<0.05.

3.4. The effect of EST on the percentage of ANAE lymphocytes：after chemotherapy, the total positive rate and the percentage of ANAE lymphocytes in CTX and CTX+EST groups were lower than those in NS group, and the difference was significant (P<0.05 and P<0.001) (Table 6). After embryonic stem cell transplantation, the total positive rate and the point type lymphocytes in EST group were higher than those in the NS group (P<0.05); The percentage of the dot pattern lymphocytes in EST CTX group was higher than that in group CTX (P<0.05) (Table 7).

Table 6. Effect of cyclophosphamide on the percentage of ANAE lymphocytes in different types of lymphocytes

Group	Number of Animals	Dot pattern (%)	Diffuse pattern (%)	Total Positive (%)
NS	10	57.70±8.96	10.50±3.47	68.20±7.52
CTX	10	42.30±7.53*	14.60±7.62	56.90±9.29**
EST	10	49.40±13.05	12.30±5.60	61.60±9.87
CTX+EST	10	40.30±6.85*	11.30±3.92	51.60±8.13**

* Compared with NS Group; P<0.001; * * Compared with NS Group; P<0.05.

Table 7. Effect of EST on the percentage of ANAE lymphocytes in different types

Group	Number of Animals	Dot pattern (%)	Diffuse pattern (%)	Total Positive (%)
NS	10	59.60±6.08	9.80±2.49	69.40±7.00
CTX	10	53.80±13.07	11.90±4.82	64.70±12.21
EST	10	69.10±12.91*	10.50±8.25	79.60±10.15*
CTX+EST	10	60.80±11.43**	12.10±3.21	72.90±11.36

*Compared with NS Group; P<0.05; **Compared with NS Group; P<0.05.

3.5. Effect of EST on phagocytosis of neutrophils: Cyclophosphamide and embryonic cell transplantation had no effect on the positive rate of neutrophil BNT dry. There were the effect on the number of NBT positive cells, compared with the NS group, the number of NBT positive cells in CTX group and EST+CTX group were less (P<0.001 and P<0.01) respectively, The degree of reduction of the EST+CTX group was lighter than that of the CTX group, and the difference between the two groups was very significant (P<0.001) (Table 8).

Table 8. Effects of CTX and EST on the phagocytic function of neutrophils

Group	Number of Animals	NBT Positive rate (%)	NBT Positive Cells (/Ul)
NS	10	14.30±5.21	598.6±167.6
CTX	10	15.50±9.57	269.5±161.1*
FSCI	10	18.20±6.86	704.6±296.7
CTX+FSCI	10	18.60±5.56	754.3±162.8**

*Compared with NS Group; P<0.001; **Compared with CTX Group; P<0.001.

3.6. Effect of EST on the weight of immune organs: The effect of CTX and EST on the spleen index was not obvious (P>0.05), but the thymus index was significantly affected; CTX compared with NS group, the thymus index in CTX group was significantly decreased (P>0.001); CTX+EST compared with CTX group, thymus index in EST+CTX group were significantly increased (P<0.01); EST compared with the NS group, EST had no significant effect on the thymus index (Table 9).

Table 9. Effects of CTX and EST on the weight of immune organs in W256 rats

Group	Number of Animals	Spleen Index	Thymus Index
NS	10	3.25±0.84	1.43±0.61
CTX	10	4.51±2.70	0.58±0.37*
EST	10	3.34±1.26	1.24±0.54
CTX+EST	10	3.95±1.74	1.03±1.50**

*Compared with NS group; P<0.001; **Compared with CTX Group; P<0.001

3.7. Effect of EST on the growth of W256 tumor cells in rats: CTX significantly inhibited the growth of W256 tumor cells, compared with the NS group, the average tumor weight of CTX group and CTX+EST group was significantly lower than that of NS group (P<0.001). There was no significant difference in the mean tumor weight between the two groups of CTX and CTX+EST. EST group did not inhibit tumor growth, compared with the NS group, the average tumor weight of the two groups was no significant difference (P>0.05).

Table 10. Effects of CTX and EST on the growth of W256 tumor cells in rats

Group	Number of Animals		Weight (%)		Tumor Weight (g)	Inhibition rate (%)
	Tumor inoculated	Tumor Growth	Tumor Before	Tumor After		
NS	10	10	218.6	231.2	20.5±8.1	—
CTX	10	7	211.4	197.0	3.8±3.0 *	81.5
EST	10	10	210.6	221.4	21.3±10.6	-3.9
CTX+EST	10	8	221.2	207.3	3.7±3.1 *	82.0

* Compared with NS Group; P<0.001.

4. Discussion;

4.1. Effect of EST on hematopoietic function after chemotherapy; CTX is a kind of alkylating anticancer drug in the treatment of tumors at the same time, has an obvious inhibitory effect on bone marrow. The clinical data showed that eighth days after the application of CTX, peripheral blood dropped to the lowest point, returned to normal levels in 17~20 days [28,29]. The number of bone marrow cells decreased 3 days after treatment, and the fourth days after stopping the medicine began to recovery [30]. Our experimental results show that after 5 days of continuous 30mg / kg CTX injection and peripheral hemogram in the six days fell to the lowest point, the 14 day has rebounded significantly, but still below the level before the medication. After chemotherapy, the transplantation of embryonic stem cells, the peripheral hemogram recovery significantly faster (table 1, 2); CTX EST group of peripheral blood white cell count and net woven red blood cell count group was higher than that of CTX (P < 0.001 and P < 0.05), indicating that EST has the functions on improvement of peripheral hemogram decreased after chemotherapy.

The proliferation of bone marrow hematopoietic cells was faster than that of lymphocytes [30] in the course of recovery of CTX, therefore, peripheral blood white blood cell classification count can be changed. The results of this study also showed that the proportion of lymphocytes in peripheral blood decreased with the decrease of lymphocyte proportion after ninth days. It is worth mentioning that the characteristics of the blood cell transplantation does not alter the chemotherapy of embryonic stem (Table 3), so that, Hematopoietic stem cells into the blood

circulation occurs less likely to proliferate and differentiate, EST to promote the improvement of blood after chemotherapy mainly with the hematopoietic function of bone marrow.

After fifth days of embryonic stem cell transplantation, the amount of ^{3}H-TdR in bone marrow hematopoietic cells was compared. The EST group was higher than that in the NS group, and the CTX+EST group was higher than that in the CTX group, and the difference was significant; that is to explain the role of EST in promoting the proliferation of hematopoietic cells in bone marrow. At the same time, the peripheral blood examination results show that EST on peripheral blood and normal saline (NS) group no significant difference, the EST of normal peripheral blood had no significant effect, which may and normal hematopoietic bone marrow and blood between balance [31] about. That is to say, although the EST of normal bone marrow hematopoietic cells also stimulated the proliferation, but the body may be releasing to the circulating pool through regulation of bone marrow hematopoietic cell in mature blood cell velocity, thus maintaining peripheral hemogram stable.

In the past, it was generally believed that the mechanism of EST promoting the recovery of hematopoietic function was mainly the replacement of embryonic stem cell transplantation [12,32], but so far there is lack of evidence for long-term implantation of embryonic stem cells into the host. In recent years [33], it is gradually recognized that the role of stimulating hematopoiesis may be related to some humoral factors [34-36] produced by embryonic stem cell transplantation. It is now known that these factors exist in the supernatant of embryonic stem cell lysis and in vitro culture of embryonic stem cell transplantation, and stimulate the formation of red blood cells and granulocyte progenitor cells [37, 38]. Patients with malignant tumor in chemotherapy often because of peripheral hemogram jealousy and reduced interrupt chemotherapy or a secondary infection, and even the loss of life. Thus, EST as an adjunctive therapy to the recovery of hematopoietic function after chemotherapy, has very important value in clinical application.

4.2. Effect of EST on cellular immune function after chemotherapy: Animal and human experimental results show that ANAE can be used as a marker of mature T lymphocytes [39-43] in the quiescent stage of peripheral blood. The positive rate of ANAE in lymphocytes represents the level of cellular immunity, the significance of E was similar to that of the experiment[44-46]. In addition, the ANAE dot pattern was thought to be characteristic of the helper T cell [44, 47, 48], whereas the ANAE dispersion was mainly the inhibitory T cell [44, 49]. The experimental results show that after injection of CTX, the total positive rate of ANAE and percentage of sample type were decreased, lymphocyte to PHA stimulation caused the value of the reaction solution was significantly decreased, that of CTX on the immune function of T cells have obvious inhibition may mainly inhibits T helper cell. After the transplantation of embryonic stem cells, the percentage of ANAE and the total positive rate were recovered to the normal level (Table 7), that showed that EST could promote the recovery of thymus weight after chemotherapy. It is well known that the thymus is the site of T cell maturation, and the ANAE activity in lymphocytes of lymphocytes is mature and gradually increased [50]. Through our study that EST possible by promoting the repair of thymus structure and function after chemotherapy, to further promote the differentiation and maturation of T cells, to increase the number of T cells and the adjustment of T cell subsets proportion, so as to enhance the cellular immune function. But we cannot rule out the multiple embryonic stem cell transplantation from different individual embryonic stem cells in the lymphocyte or lymphocytes and host lymphocytes between the mutual stimulation effect on cellular immune function of organism [51, 52]. Chemotherapeutic drugs kill tumor cells, on the other hand suppress immune function and the relationship between how to adjust good chemotherapeutic drugs in tumor immune function of host three to maximize the elimination of tumor cells, protect the body's immune function are becoming increasingly the attention of people. As Hayhoe [53] reported, ANAE is the release of lysosomal enzymes, the activity and T lymphocyte killing target cells on. Thus, EST improve lymphocyte ANAE activity in the treatment of tumor cells is worthy of further study.

Have been reported in the literature, and after chemotherapy in patients with malignant tumor, embryonic stem cell transplantation can enhance the patient's ability to fight off infection [54], this effect is considered possible and embryonic stem hematopoietic cells in the presence of stimulating factor enhances neutrophil response to bacteria and activation of macrophages about [55, 56, 14]. According to the effect of EST on the phagocytic function of neutrophils, the results showed that NBT had no significant effect on the positive rate of EST in neutrophils; However, the number of NBT positive cells in CTX+EST group was significantly higher than that in CTX group ($P<0.001$), so we believe that the increased resistance to infection after embryonic stem cell transplantation was related to the increase of the total number of neutrophils.

4.3. Effects of EST CTX and EST on the growth of W256 tumor cells in rats; Wu Zuze et al. [58], in vitro experimental study showed that there was a kind of low molecular weight active component of human fetal liver tissue in inhibiting proliferation of human

leukemia cell line (HL-60) cells, However, the relationship between embryonic stem cell transplantation and solid tumors has not been reported at home and abroad. Our research results show that single application of EST and chemotherapy after application of EST had no effect on rat W256 tumor cells growth and EST group the average tumor weight was not decreased, however, does not affect the CTX on entity type W256 tumor inhibitory effect (Table 10).

According to literature reports [59-62], embryonic and juvenile tissues contain more vascular factors, which play an important role in the growth and development of normal embryos and immature animals, The solid tumor tissue restored the nature of the cells of the embryo and immature cells, producing a large number of tumor angiogenesis factors (TAF), To make the blood vessels grow into the tumor and supply the nutrition of the tumor, so it can promote the growth of the tumor. Whether embryonic stem cells can affect the production of TAF in W256 tumor cells by vascular or embryonic stem cell transplantation is worthy of attention and need to be further studied.

5. Conclusion:

5.1. EST is to promote the recovery of peripheral blood after cyclophosphamide chemotherapy effect, but the normal peripheral blood had no significant effect;

5.2. The role of EST in stimulating the proliferation of bone marrow hematopoietic cells;

5.3. EST has a positive effect on the percentage of ANAE positive lymphocytes in peripheral blood and the percentage of dot pattern of ANAE lymphocytes induced by cyclophosphamide chemotherapy.

5.4. EST has the effect of promoting the recovery of thymus weight after chemotherapy.

5.5. EST does not affect the inhibitory effect of cyclophosphamide on W256 tumor sarcoma in rats. It also does not inhibit the growth of solid W256**.**

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Reference:

1. Syman M et al. Fetal hemopoiesis in diffusion chamber culture. I. The pattern of pluripotent stem cell growth. Cell Tissue Kinet 1976;9:41-44.
2. Mandal A, Raju S, Viswanathan C. Cryopreserved hepatic progenitor cells derived from human embryonic stem cells can arrest progression of liver fibrosis in rats.
3. Steven D Schwartz et al: Embryonic stem cell trials for macular degeneration: a preliminary report. The Lancet 2012:379(9817):713-720.
4. Hann IM, et al. Development of pluripotent hematopoietic progenitor cells in the human fetus. Blood 1983;62:118-121.
5. Balashova VA, et al. Cellular composition of hemopoietic tissue of the liver and spleen in the human fetus. Arkh Anat Gistol Embriol 1984;86(4):80-83.
6. Prummer O, et al. The fetal liver as an alternative stem cell source for hemolymphopoietic reconstitution. Intern J Cell Clon 1986;4(4):237-249.
7. Nahid Bijeh, Amir Rashid lamir, Samaneh Sadeghynia, Keyvan Hejazi: Evaluation of the enzymes of liver and Hematological values in life saving women. Biomedicine and Nursing 2016:2(1):85-90.
8. Wu Zuze, et al. Study on the dynamics of human hematopoietic progenitor cells (CFU-C) and T lymphocytes in the liver of human embryonic development. Chinese Science 1981; 9:1160-1164.
9. Basma G Moussa, Ashraf Helal. Surgical management of Brown Tumor of the Mandible Associated with Ectopic Primary Hyperparathyroidism. Biomedicine and Nursing 2015 1(1):80-87.
10. Lucarelli G, et al. Fetal liver transplantation in aplastic anemia and acute leukemia. Amsterdam: Excerpta Medica 1980:284-299.
11. Kansal V, et al. Fetal liver transplantation in aplastic anemia. Acta Haemat 1979;62:128-134.
12. Toutaine JL, et al. Treatment of severe combined immunodeficiency with bone marrow or fetal liver treatment. Nouv Presse Med 1980;9:2215-2219.
13. Tan Hanjun, et al. Primary efficacy of fetal liver cell infusion in the treatment of aplastic anemia and acute leukemia. The people's Liberation Army Medical Journal 1984, 9:118-119.
14. Qian Fengwei, et al. Report of some blood diseases in fetal liver cells. Chinese Journal of Hematology 1985；6(6)：355-358.
15. Wu Zuze, et al. Study on the mechanism of fetal liver cell infusion and whole embryo injection in

the treatment of aplastic anemia. Journal of Applied Physiology, 1985;1(1):9-16.
16. Uphoff DE. Preclusion of secondary phase of irradiation syndrome by inocutation of fetal hematopoietic tissue following lethal total body X irradiation. JICI 1958;20:625-632.
17. Cainn G, et al. Transplantation of DLA-compatible and incompatible and fetal liver hematopoitic cells in dogs. Transplantation 1987;44(6)742-747.
18. Champlin R, et al. Fetal liver transplantation in dogs: results with DLA-compatible and incompatible grafts. Prog Clin Boil Res 1985;193:195-204.
19. Xue Laifeng, et al. Application of embryonic cell infusion in the treatment of malignant tumor with chemotherapy and partial hematologic diseases. Chinese Journal of Oncology 1986;8(5):367-369.
20. Kochupillai V, et al. Fetal liver infusion in acute myelogenous leukemia. Thymus 1987;10:117-123.
21. Hu Bin, et al. Application of fetal hematopoietic cell infusion in chemotherapy of malignant tumors. Journal of Practical Oncology 1989; 4(1): 31-33.
22. Editor in chief of the dragon and the chief editor. Immunology experimental technology. Beijing: Department of immunology, Beijing Medical University, 1988:97-106.
23. Jiang Yitao, et al. The regulation of cell proliferation and differentiation of mouse hematopoietic stem 850 gamma ray irradiation, Journal of Nanjing Medical College, 1986;6(4):245-247.
24. Wang Jinyun, et al. Huangqi, effect of Cordyceps sinensis on rat blood lymphocyte transformation function ^{3}H-TdR incorporation method. Journal of traditional Chinese medicine and Western medicine 1987; 7 (9):543.
25. Wu Jinglan, et al. Method for ANAE labeling of human peripheral blood lymphocytes and cell typing. Chinese Journal of Anatomy 1985; 16 (2):202-207.
26. Examination room, Beijing children's Hospital: the reduction reaction of nitrogen and blue and the activity of alkaline phosphatase were used in the identification of infectious diseases. Chinese Journal of Medicine 1981; 4 (1): 49-52.
27. Liu Geng, et al. The immune pharmacological action of Cordyceps sinensis. Journal of Traditional Chinese Medicine and Western Medicine 1985; 5 (10):622-624.
28. Wu Dequan, et al. Experimental study on transplanted lung adenocarcinoma (P615) in mice with 615 inbred strain mice. Chinese Journal of Oncology 1984; 6 (1) 1-3.
29. Sun Yan, et al. Toxicity of anticancer drugs, complications and treatment, Zhang Zhiyi ed. malignant tumor chemotherapy. Shanghai: Science and Technology Press, 1981:118-123.
30. Wang Zhaoyan. The toxic and side effects and prevention of cancer chemotherapy. Journal of Practical Cancer 1986; 1 (3):1-6.
31. William D. Dewys, et al. Hematopoietic recovery after large dose of cyclophosphamide: correlation of proliferative state with sensitivity. Cancer Res 1970;30(6):1692-1697.
32. Wu Zuze. The relationship between bone marrow hematopoietic cells and peripheral blood leukocytes. Wu Zuze. Introduction to hematopoietic cell dynamics. Beijing: Science Press 1978:54-57.
33. Wu Zuze. The progress of research on fetal liver cell transplantation. Chinese Journal of Hematology; 1987; 8 (2):120-122.
34. Gale RP, et al. Fetal liver transplantation. New York: Alan R Liss 1985;267-295.
35. Cork MJ, et al. A stimulator of murine haemopoietic stem cell proliferation produced by human fetal liver cells. Br J Haematol 1986;63:775-779.
36. Ru Xiujing, et al. In vitro study on the therapeutic mechanism of fetal liver infusion in the treatment of leukemia. Chinese Journal of Hematology 1988; 9 (10):599-601.
37. Ji Shuquan, et al. Analysis of clinical therapeutic effect of fetal liver cell infusion. Chinese Journal of Hematology 1989; 10 (4):216-217.
38. Ji Shuquan, et al. Experimental study and clinical application of fetal liver cell suspension, Journal of General Hospital of the Air Force PLA 1989; 5 (2):77-79.
39. Feng Yongqing, et al. A preliminary study on the costimulatory factors of GM-CSF secretion from fetal liver cells, Journal of First Military Medical University; 1990; 10 (1):21-23.
40. Mueller J, et al. Nonspecific acid esterase activity: a criterion for differentiation of T and B lymphocytes in mouse lymph nodes. Eur J Immunol 1975;5:270-276.
41. Ranki A, et al. Nonspecific esterase activity in human lymphocytes histochemical characterization and distribution among major lymphocyte subclasses. Clin Immunol Immunopath 1978;10:47-49.
42. Ranki A, et al. Identification of resting human T and B lymphocytes by acid α-naphthyl acetate esterase staining combined with rosette formation with staphylococcus aureus astrain cowan. Scand J Immunol 1976;5:1129-1131
43. Yang Yinsen, et al. Study on the immune status

of benzene poisoned rats by using T cell esterase staining method, Communication of medical research 1980; 11:26-27.
44. Sun Guoxian, et al. Lymphocyte acid alpha naphthol acetate esterase assay to investigate the method and its application. Chinese Journal of Medicine 1981; 4:76-77.
45. Knowles DM, et al. The demonstration of acid α-naphthyl acetate esterase activity in human lymphocytes: usefluness as a T-cell marker. Cellular Immunol 1978;3:112-123.
46. Manconi PE, et al. Alphanaphthyl acetate esterase activity in human lymphocytes: distribution in lymphocyte populations and in mitogen activiated cells. Scand J Immunol 1979;9:99-102.
47. Ren Jinrong, et al. The level of cellular immunity in patients with esophageal and cardiac cancer and normal persons were observed by esterase staining. Shanghai Journal of Immunology 1983; 3 (3):133.
48. Grossi CE, et al. Morphological and histochemical analysis of two human T-cell subpopulations bearing receptors for IgM or IgG. J Exp Med 1978;147:1405-1412.
49. Peter CL, et al. Distinctive functional characteristics of human "T" lymphocytes defined by E-rosetting or a monoclonal anti T cell antibody. Enr J Immunol 1981;11:329-334.
50. Ferrari FA, et al. Reliability of α-naphthyl acetate esterase staining of blood smears for the enumeration of circulating human T lymphocytes. Clin Exp Immunol 1980;41:358-362.
51. Han Jingshu, et al. The changes of acid nonspecific esterase in peripheral blood lymphocytes and thymus cells in normal human. Chinese Journal of Hematology 1982; 3 (4):206-20.
52. Li Chunmei, et al. The changes of T lymphocyte in the treatment of aplastic anemia with fetal liver cell suspension. Military Medical Science Academy of the PLA Journal 1984; 5:609-612.
53. Xiao Peng, et al. Effects of peripheral blood lymphocyte transformation in patients with aplastic anemia after transfusion of fetal liver cells. Military Medical Science Academy of the PLA Journal 1984; 2:157-159.
54. Hayhoe F.G.J and Quaglino D. Haematological cytochemitry. London: Longman Group Limited, 1980;147-171.
55. Xi rainman, et al. Application of fetal liver cell suspension infusion in the treatment of leukemia. Chinese Journal of Hematology; 1987; 8 (9):536-538.
56. Wu wei et al. Specific hematopoiesis regulation factors of clone stimulating factor, zhanggusheng editor. Research progress of cell immunology. Beijing: People's Medical Publishing House, 1983; 249-250.
57. Chen Zhang. Basic and clinical study of hematopoietic stimulating factors. Chinese Journal of Hematology 1989; 10 (1):48-51.
58. Har, Tuanzhu et al. Study on liver cytosol in experimental fulminant hepatic failure curative effect. Chinese Journal of Medicine 19879; 69 (5):267-270.
59. Wu CT, et al. Effects of human fetal liver extract on the growth of HL-60 cells. Exp Hematol 1989;17:304-307.
60. Wu Kefu. Cell growth regulator. Beijing: Science Press, 1988:96-97.
61. Xiu Ruijuan, et al. HeLa cell nuclear. SP2/0 cells and the development of vascular growth. Chinese Journal of Oncology 1987; 9:95-97.
62. Folkman J, et al. Tumor angiogenesis. Adv Cancer Res 1985;43:175.

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