Observation on the stability of rat myelosuppression model established by different doses of cyclophosphamide solution

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Abstract Objective to investigate the stability of different doses of cyclophosphamide solution in establishing rat myelosuppression model. Methods In this study, 30 SD rats were randomly divided into control group (Group C) and model group (M1, M2, M3, M4). The rats in the model groups were injected with cyclophosphamide solution by intraperitoneal injection at doses of 30、40、(30+15) and (40+20) mg/kg respectively to establish a bone marrow suppression rat model. The changes of WBC, lym, Mon, GRA, RBC, Hb and PLT in peripheral blood were observed on the 1st, 5th and 13th day after modeling. Results （1）After modeling, the weight of rats in the model group was lower than that in the control group ($P < 0.05$). （2）On the 5th day, the levels of WBC, lym, mon and gra in the serum of each model group were lower than those before modeling($P < 0.05$). The content of RBC in M1 and M2 groups was lower than that before modeling ($P < 0.05$), the content of Hb in M1, M2 and M3 groups was lower than that before modeling ($P < 0.05$), and the content of PLT in M1, M2, m3 and M4 groups was lower than that before modeling ($P < 0.05$). （3）On the 13th day, the levels of WBC, lym, mon and gra in M1 and M2 groups were higher than those on the 5th day, except for the GRA in M2 group.The levels of WBC and lym in m3 and M4 were lower than those in M1 and M2 ($P < 0.05$); The contents of RBC and Hb in M1 and M2 groups were lower than those before modeling ($P < 0.05$); Compared with M1 and M2, the content of Hb in m3 and M4 groups was higher ($P < 0.05$); The PLT content in the peripheral blood of M3 and M4 rats was

higher than that of the 5th day, but lower than that of M1 and M2 groups ($P < 0.05$). Conclusion The improved method can maintain the stability of rat myelosuppression model.

Key words Cyclophosphamide;rat; myelosuppression model

Myelosuppression is a common side effect after tumor chemotherapy. According to statistics[1], 80% of patients will have myelosuppression in the course of tumor chemotherapy, which has the most obvious impact on the immune system and hematopoiesis. A large number of basic studies have confirmed that cyclophosphamide(CTX) solution can be used to establish rat myelosuppression model. However, at present, the dosage of established rat myelosuppression model is varied, including one-time large dose (100 or 150mg/kg) CTX injection and multiple small dose (30, 40mg/kg or 80mg/kg) administration[2-6], which makes the model inconsistent. Some scholars observed that CTX solution at a dose of 50-150mg/kg could produce severe immunosuppression and death, while that at a dose of 10-50mg /kg could produce moderate myelosuppression. Many scholars are still controversial about the stability of the current rat myelosuppression model. They believe that the hematopoietic function of bone marrow in rats can gradually return to normal after the modeling, and the therapeutic effect cannot be fully reflected. In this study, two doses (30 and 40mg/kg) were selected based on the current commonly used small dose [7-8] and the improved modeling method of "Pharmacodynamics Compelation of Guidelines for Preclinical Study of New Drugs (Western Drugs)". In order to ensure the stability of the model, the experiment designed a half-dose injection after modeling to maintain the model. Therefore, the author mainly observed the stability of CTX solution with traditional dose and modified maintenance dose to establish rat myelosuppression model in this paper.

1. Materials and methods

1.1 Laboratory Animals

Thirty male rats (SPF, SD) (purchased from Animal Experimental Center of Southern Medical University, license number: SCXK(Guangdong) 2016-0041) with body weight of 200±20g.

1.2 Drugs and Instruments

Drugs: Cyclophosphamide: 5g/ bottle (H20160467, BaxteroncologyGmbH);Sterilization of water for injection.

Preparation of solution: weigh 500mg CTX, dissolve in 50ml sterilized water for injection, and completely dissolve after shaking.It was prepared into 10mg/mL cyclophosphamide solution for ready use.

Instrument: Mindray Animal Automatic Blood Cell Analyzer Model: BC-2800VET.

1.3 Grouping and modeling

Grouping:Thirty male rats were fed in SPF animal room (room temperature was 20℃ ~ 25℃, relative humidity was 50%-70%, the feeding environment was clean and quiet) in Guangdong Branch of Academy of Sciences of Chinese Hospital of Traditional Chinese Medicine. After feeding for 7 days, the rats were randomly divided into 5 groups with 6 rats in each group by using the random number generator of SPSS software. The control group and the model groups (M1 group, M2 group, M3 group and M4 group).The control group was fed normally without intervention.

Building the model:From the modeling, CTX solution was intraperitoneally injected into rats in each model groups at the same time every day, once a day, for consecutive 5 days, in which rats in M1 and M2 groups were injected at a dose of 30 and 40mg/kg, respectively.According to the current study [8], drug injection was stopped after the myelosuppression model was established on the 5th day of modeling. The M3 and M4 groups were modeled using the improved modeling method, that is, 30 and 40mg/kg doses were modeled from the beginning of the modeling. After the model was built, the original dose was halved and injected every other day (the dose of M3 was expressed as (30+15) mg/kg and the dose of M4 as (40+20) mg/kg).This was maintained until the 13th day of modeling, and the drug injection was stopped.

1.4 Collection and detection of indicators

The rat tails in the model group were disinfected with 75% alcohol before modeling, on the 5th day and on the 13th day respectively.Then tail vein blood, rejected the first drop of blood, then add 5% EDTA drops of anticoagulant EP tubes, collecting 1.5 ml, blending using automatic blood cell analyzer for each group after rat caudal vein in the peripheral blood white blood cells(WBC), lymphocytes(Lym), monocytes(Mon), granulocyte(Gra), Red blood cell(RBC) , hemoglobin(Hb) and platelet(Plt) levels tested.Peripheral blood images were observed to test whether the model was successful.

1.5 Statistical methods

SPSS19.0 software was used for statistical analysis, and the measurement data were expressed as mean ± standard deviation ($\bar{X}$±SD). One-way variance (ANOVA) was used for comparison between multiple groups. LSD method was used for pair comparison when variance was homogeneous, and Kruskal-Wallis test method was used for comparison when variance was not homogeneous.

The difference was statistically significant when $P<0.05$.

2. Results

2.1 Changes in general condition of rats in each group after modeling

The rats in the control group were flexible, had normal appetite, bright hair color, and their

body weight increased daily. Compared with the control group, the model group showed signs of paleness in the eyeball and auricle, bleeding from mouth and nose and other symptoms at the later stage of modeling. No death occurred in each group. Before modeling, there was no significant difference in the body weight of each group. From the 3rd day of modeling, the average body weight of rats in control group was higher than that in model groups, and there was no difference in body weight among all groups ($P > 0.05$). On the fifth day of modeling, the body weight of rats in model groups were lower than that in control group, with a difference ($P < 0.05$). On the 13th day after modeling, the body weight of rats in the model group was still lower than that in the control group ($P < 0.05$), but there was no difference in body weight among all groups in the model groups(Figure 1).

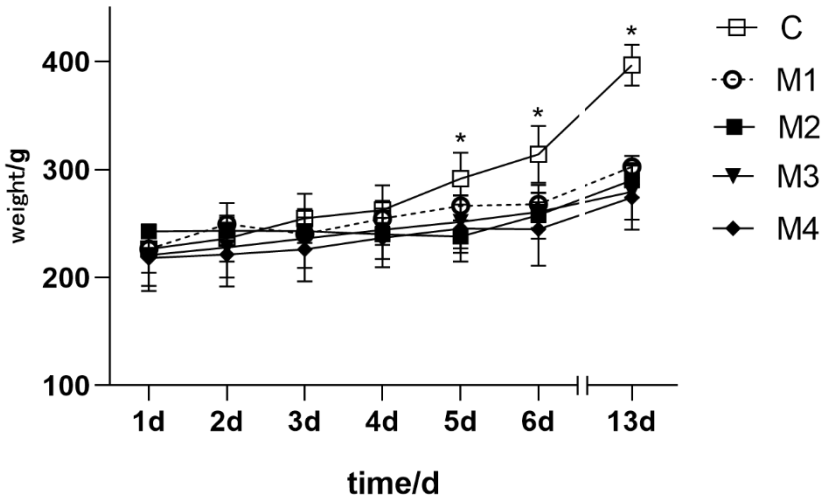

Figure 1 Changes in body weight of rats in each group before and after modeling

2.2 Changes of WBC, LYM, MON and GRA contents in peripheral blood after modeling

Before modeling, there were no significant differences in WBC, LYM, MON and GRA in peripheral blood of rats. On the 5th day of modeling, WBC, LYM, MON and GRA in peripheral blood of rats in each model group were lower than before modeling, the differences were statistically significant ($P < 0.05$), and the nucleated cell count was 20% lower than the normal value (i.e., successful modeling), and there was no difference between groups. On the 13th day of modeling, intra-group comparison: WBC, LYM, MON and GRA in peripheral blood of M1 and M2 groups were all increased compared with those on the 5th day of modeling, with statistical significance ($P < 0.05$), and GRA in peripheral blood of M2 group showed no difference. Peripheral blood WBC and LYM in M2 group were significantly lower than those before modeling ($P < 0.05$). Peripheral blood WBC in M1 group was significantly lower than that before modeling ($P < 0.05$).The WBC and LYM of peripheral blood in M3 group were

significantly lower than those before modeling ($P < 0.05$), and had no significant difference compared with those on the 5th day of modeling. The GRA of peripheral blood in M3 group was not significantly different from that before and after modeling.The levels of WBC, LYM and GRA in peripheral blood of M4 group were significantly lower than those before modeling ($P < 0.05$).Comparison between groups: WBC, LYM and GRA in peripheral blood of M3 and M4 were decreased compared with those of M1 ($P < 0.05$).The ratio of WBC, LYM, MON and M2 in peripheral blood of M3 and M4 were decreased, and there was a difference ($P < 0.05$).M3 peripheral blood GRA was significantly lower than that of M1 ($P < 0.05$), M4 peripheral blood MON was significantly lower than that of M2 ($P < 0.05$). Figure 2, Table 1.

2.3 Changes of RBC and Hb contents in peripheral blood after modeling

Before modeling, there was no significant difference in RBC and Hb in peripheral blood of rats.On day 5 of modeling, intra-group comparison: RBC in peripheral blood of rats in M1 and M2 groups was lower than before modeling, the difference was statistically significant ($P < 0.05$);Peripheral blood Hb of rats in M1, M2 and M3 groups was lower than that before modeling, with statistical significance ($P < 0.05$).Comparison between groups: Hb in peripheral blood of M3 and M4 rats was higher than that of M1 rats, with difference ($P < 0.05$);Peripheral blood Hb of M4 rats was higher than that of M2, and there was a difference ($P < 0.05$).On the 13th day of modeling, intra-group comparison: RBC and Hb in peripheral blood of rats in M1 and M2 groups were lower than those before modeling, with a difference ($P < 0.05$), but had no difference compared with that on the 5th day of modeling;RBC and Hb in peripheral blood of rats in M3 and M4 groups were not significantly decreased compared with before modeling ($P < 0.05$).The Hb in peripheral blood of rats in M4 group was significantly lower than that on the 5th day of modeling ($P < 0.05$).Comparison between groups: Hb in peripheral blood of rats in M3 and M4 groups was higher than that in M1 and M2 groups, and the difference was significant ($P < 0.05$). Figure 2, Table 1.

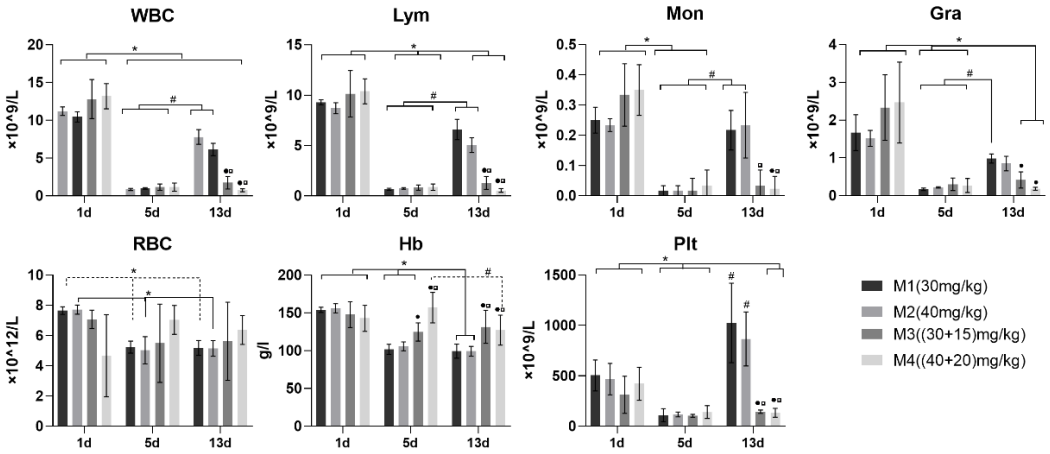

Figure 2 Comparison of peripheral blood cell content of rats in each group after modeling

2.4 Changes of PLT content in peripheral blood after modeling

Before modeling, there was no significant difference in PLT in peripheral blood of rats.On day 5 of modeling, intra-group comparison: PLT in peripheral blood of rats in M1, M2, M3 and M4 groups was lower than before modeling, with statistical significance ($P < 0.05$);On day 13 of modeling, PLT in peripheral blood of rats in M1 and M2 groups was higher than that on day 5 of modeling, the difference was statistically significant ($P < 0.05$).M3 and M4 were still lower than before modeling, and the difference was significant ($P < 0.05$).Comparison between groups: On the 5th day of modeling, there was no difference in the content of PLT in peripheral blood among all groups;On the 13th day of modeling, PLT in peripheral blood of rats in M3 and M4 groups was lower than that in M1 and M2 groups, with statistical significance ($P < 0.05$). Figure 2, Table 1.

Table 1 Blood routine difference of each group before and after modeling（ $\bar{X}$±SD）

Time(day)	Groups	WBC（×10^9/L）	Lym（×10^9/L）	Mon（×10^9/L）	Gra（×10^9/L）	RBC（×10^12/L）	Hb（g/L）	PLT（×10^9/L）
1d	M1	11.20±1.38	9.28±0.62	0.25±0.10	1.67±1.16	7.64±0.61	154.00±8.46	504.00±153.75
	M2	10.45±1.65	8.7±1.34	0.23±0.05	1.51±0.52	7.71±0.75	156.00±15.38	467.83±155.52
	M3	12.80±2.59	10.13±2.31	0.33±0.10	2.33±0.87	7.07±0.60	147.83±17.15	311.83±184.59
	M4	13.18±1.66	10.37±1.24	0.35±0.08	2.47±1.07	4.67±2.72	142.83±17.20	421.33±162.36
5d	M1	0.83±0.36*	0.65±0.27*	0.02±0.04*	0.17±0.08*	5.23±0.98*	101.67±16.52*	108.83±64.61*
	M2	0.96±0.23*	0.73±0.198*	0.02±0.04*	0.22±0.04*	5.02±2.23*	105.67±14.51*	117.50±23.86*
	M3	1.13±0.42*	0.82±0.26*	0.02±0.04*	0.3±0.17*	5.49±2.59	124.67±12.08*•	102.5±13.81*
	M4	1.15±0.54*	0.85±0.32*	0.03±0.05*	0.27±0.19*	7.04±0.96	157.00±20.32•▫	141.00±63.68*
13d	M1	7.78±2.38*#	6.58±2.47#	0.22±0.16#	0.98±0.30#	5.18±1.19*	99.33±23.17*	1024.83±394.72#
	M2	6.11±1.99*#	5.03±1.78*#	0.23±0.27#	0.85±0.47	5.14±1.24*	99.33±15.60*	865.33±267.87#
	M3	1.71±0.81*•▫	1.27±0.64*•▫	0.03±0.05▫	0.42±0.21*•	5.61±2.59	131.5±21.74•▫	142.83±16.85*•▫
	M4	0.73±0.19*•▫	0.53±0.18*•▫	0.02±0.04•▫	0.19±0.04*•	6.37±0.96	127.17±19.82#•▫	133.00±44.71*•▫

*P < 0.05, the comparison within the group was different from that 1 day before modeling;#P < 0.05, compared with 5 days after modeling, there was a difference within the group;•P < 0.05, the comparison between groups was different from that of M1;▫P < 0.05, there was a difference between the two groups compared with M2;•P < 0.05, there was a difference between groups compared with M3.

3. Discuss

According to the decrease degree of WBC, RBC and PLT in peripheral blood after chemotherapy, the World Health Organization classified myelosuppression into five grades of 0 ~ IV.Can be obtained from cyclophosphamide because its effect is stable, at the same time the cost is not high, as is commonly used in clinical alkylating agent, effective its bone marrow suppression caused mainly by direct DNA damage of bone marrow hematopoietic stem cells, cause the irregular necrosis and apoptosis of hematopoietic cells, cause the activity decline of hematopoietic progenitor cells, bone marrow suppression [9-11]. Cyclophosphamide is often

used to establish immunosuppression models and has been widely accepted and used in pharmacodynamics evaluation and safety evaluation of drugs.CTX inhibitory effect on organism, comprehensive performance is mainly for individual growth is restrained, in a number of reports [12 to 15], CTX group animals compared with normal group weight loss rate of 11-26%, and the rats in the late delivery is mainly characterized by severe anemia (eye and pale in areas such as the pinna), the symptom such as bleeding, mouth and nose bleeding and nixie.

The experimental study showed that CTX could complete the establishment of myelosuppression model. After the successful modeling, the peripheral blood white blood cells, red blood cells and platelets were reduced compared with those before the modeling, and anemia symptoms and oronasal hemorrhage occurred in the eyeball, ear and other parts, indicating that the clinical symptoms of the rats were basically consistent with the laboratory indicators. However, the model established by traditional low-dose CTX solution (30, 40mg/kg) had poor stability. On the 7th day after the successful modeling, the peripheral blood white blood cells and platelets of rats basically returned to normal, while the recovery of red blood cells and hemoglobin was slow, which was considered to be related to the longer average survival time of red blood cells. It was observed from the experimental results that the improved modeling method could maintain the stability of the model. Although there was no significant difference in the effect of the two maintenance doses, the comprehensive analysis of the average index and the body weight of the rats was that the dose (40+20) mg/kg was more stable than the other doses, and there was no death of rats during the modeling process. Therefore, the maintenance dose modeling method is worthy of being used as a new method to study the myelosuppression model.

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