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Establishment of a Mouse Thrombocytopenia Model Induced by Cyclophosphamide

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Abstract: An experiment was conducted to compare the effects of two mouse thrombocytopenia models induced by cyclophosphamide at two different administration routes to determine a proper cyclophosphamide administration route that could cause stable thrombocytopenia. A suitable drug dosage that could induce thrombocytopenia in mouse efficiently with the definite administration route was then investigated. BALB/c mice were randomly divided into Normal, Model A and Model B groups. To Model A, 200 mg/kg of cyclophosphamide was given by vena caudalis injection as first dose and 30 mg/kg as maintenance dose by intraperitoneal injection at the following 6 days. To Model B, 150 mg/kg of cyclophosphamide was given by subcutaneous injection once a day for consecutive 3 days. All groups were under investigation for 15 days. The result suggested that a decrease in the number of blood platelets of Model B at the 7th day were significantly than that of Normal. Other platelet related indices like platelet distribution width, mean platelet volume and platelet-large cell ratio of Model B increased significantly in comparison with those of Normal group. The platelets count was reduced but fluctuated greatly, and more than half of the mice died in Model A. Therefore, subcutaneous injection of cyclophosphamide for 3 days was used for the cyclophosphamide dosage test. BALB/c mice were randomly divided into Normal, cyclophosphamide low dose (100 mg/kg), medium dose (120 mg/kg) and high dose (140 mg/kg) groups. All groups were under investigation for 11 days. Though all 3 dosages successfully initiated thrombocytopenia as the platelets number dropped at the 7th day, the low dose was considered to be a suitable one that was of high efficacy and low toxicity. Thus, BALB/c mice challenged by subcutaneous injection of cyclophosphamide 100 mg/kg per day for 3 consecutive day is one simple, feasible and stable mouse thrombocytopenia model that could be used for pharmacodynamic test of the drugs which are supposed to have platelets increasing effect.

Key words: Thrombocytopenia; Cyclophosphamide; Model

环磷酰胺诱导小鼠血小板减少症模型的建立

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摘要：比较由环磷酰胺两种不同给药方式诱导小鼠血小板减少症模型的效果，并对效果较稳定的一种给药方式进行最佳造模剂量摸索，以期确定一个造模效果较好，毒副作用较低，利于观察治疗药物疗效的血小板减少症模型。模型 A 组，第 1 天尾静脉注射环磷酰胺 200 mg/kg，然后连续 6 d，每天 1 次以维持剂量 30 mg/kg 腹腔注射环磷酰胺。模型 B 组，按 150 mg/kg 皮下注射环磷酰胺，每天 1 次，连续 3 d。结果显示模型 B 组造模效果较好，故以模型 B 组给药方法进行剂量摸索实验。由第 7 天的血小板计数可知环磷酰胺低（100 mg/kg）、中（120 mg/kg）、高（140 mg/kg）剂量均可引起血小板减少症，而低剂量组与其他组比较有高效低毒的特点，更有利于观

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察治疗药物的作用，可用于具有升血小板作用药物的药效学研究。

关键词：血小板减少症；环磷酰胺；模型
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Cyclophosphamide is a synthetic alkylating agent chemically related to the nitrogen mustards with antineoplastic and immunosuppressive activities, and was introduced as an antitumour agent in 1958. The important factor for therapeutic and toxic effects of cyclophosphamide is the requirement of metabolic activation by hepatic microsomal cytochrome P_{450} mixed functional oxidase system. In the liver, cyclophosphamide is converted to the active metabolites aldophosphamide and phosphoramide mustard, which bind to DNA, thereby inhibiting DNA replication and initiating cell death. The renal cleavage of inactive circulating metabolites will bring about toxic byproducts like acrolein, which is the causative agent in cyclophosphamide cystitis (Cox et al, 1979).

Cyclophosphamide, as a cell cycle-independent DNA and protein alkylating agent, which has a wide spectrum of anti-tumor activity, is one of the common chemical drugs for the therapy of malignant lymphomas, leukaemias, neuroblastoma, retinoblastoma and carcinomas of the ovary, breast, endometrium and lung, usually in combination with other chemotherapeutic drugs (de Jonge et al, 2005). Fulminant cardiac toxicity is the most severe dose-limited toxicity of cyclophosphamide whose other side effects are hematopoietic depression, hemorrhagic cystitis, gonadal dysfunction, alopecia, nausea, gastrointestinal toxicity, renal toxicity, antidiuretic effect and vomiting. Also, it was reported that cyclophosphamide could induce chromosome aberration of bone marrow and liver cells (He et al, 1984). In conventional chemotherapy, cyclophosphamide is one of the most commonly employed drugs which are applied in high dose regimen to treat metastatic breast cancer (Legha et al, 1979). In the setting of this, host toxicity will lead to the limitation of the therapeutic efficacy of this drug and the systemic distribution of activated drug metabolites that have significant cytotoxic effects including cardiac and renal toxicity (Chen et al, 1996).

We chose two candidate models reported as thrombocytopenia model induced by cyclophosphamide to verify their feasibility. In New Drug Pharmacological Research Methods (Lü, 2007), it notes that cyclophosphamide induced leucopenia animal model can also be the model of thrombocytopenia. A few original articles illustrated platelets count reduced significantly in the cause of leucopenia of mice (Shou et al, 2008). Therefore, we focus on two mice models of thrombopenia induced by cyclophosphamide at two different administration routes to verify a definite one. Then the assured administration method was used in the following experiment to find out a suitable cyclophosphamide dose.

1 Materials and Method

1.1 Animals and materials

1.1.1 Animals Special pathogen free BALB/c mice, weighed (19±2)g, half male and half female, were purchased from medical science experimental animal center of Guangdong Province in China (Certificate NO.SCXK (Guangdong) 2003-0002). The mice were kept in the cages at 24℃, 65% humidity, with alternating 12h light/12h dark (Certificate NO.2008-0085). Animals were given free access to standard laboratory mice chow and tap water. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals and the related ethical regulations of Jinan University and Guangzhou University of Chinese Medicine according to the internationally accepted principles.

1.1.2 Materials Cyclophosphamide was purchased from HengRui Pharmaceutical limited company, Jiangsu (Batch number: 07112221). Calcium chloride (500 g, Guangzhou Chemical Agent Factory, 20070801-1), acetic acid (500 mL, Tianjing No.1 Chemical Agent Factory, 060707) and perchloric acid (500 mL) were analytically pure.

Sysmex KX-21 hematology analyzer (TOA Medical Electronics, Inc, Kobe, Japan) and cell packs diluents (TOA Medical Electronics, Inc, Kobe, Japan) was provided by Huarui Hospital affiliated to Southern Medical University. TU-1810S UV-VIS spectrophotometer (Beijing Puxi General Equipment Co.Ltd.), Anke TDL80-2B table centrifuge (Shanghai Anting Scientific Instrument Plant), DK-8D electrothermal thermostatic water tank (Shanghai Yiheng Technical Co. Ltd.), blood cell counting chamber (Shanghai Qiujing Biochemical Reagent and Equipment Co.Ltd.), Nikon ECLIPSE TS100 fluorescence inverted microscope (Nikon Co. Ltd.), were provided by

Pharmacy College of Jinan University.

1.2 Experimental protocol

1.2.1 Comparison of two thrombopenia models

1.2.1.1 Animal model Twenty-six BALB/c mice, half male and half female, were divided into three groups randomly due to their weight and gender, namely Normal, Model A and Model B with 8, 10 and 8 mice, respectively. The Model A had two extra mice to account for its high death rate in the preliminary experiment. Model A received 200 mg/kg of cyclophosphamide by vena caudalis injection as first dose and 30 mg/kg as maintenance dose by intraperitoneal injection in the following 6 days (Zhang et al, 2003). Model B received 150 mg/kg of cyclophosphamide by subcutaneous injection for 3 days (He et al, 2003). The capacity of dosage of the drug is 0.1 mL/10 g.

1.2.1.2 Observation indexes

1.2.1.2.1 General condition The usual activities, fur luster, amount of diet and drinking, conditions of urination and defecation, and the change of weight and mortality of the mice were observed closely. The animals were weighed everyday. This process lasted for 15 days.

1.2.1.2.2 Blood cell analyses A 10 μL blood sample was collected by tail cut before administration at the 1st, 4th, 7th, 11th, and 15th day mornings and transferred into a 1.5 mL EP tube containing 0.5 mL cell pack diluents. Blood cell analyses were conducted soon after the collection. The day interval was selected based on previous reports (Zhang et al, 2003. He et al, 2003) and changed slightly according to preliminary experiment.

1.2.1.2.3 Measurement of clotting time On the 16th day, a glass capillary (Inner diameter=0.9~1.1 mm, length=10 cm) was inserted into one eye of mice. The capillary was removed and placed on the desk horizontally when the blood full filled it. Every 30 s the capillary was broke and stretched gently to see if there was fibrin threads of blood at the breakage point. The time from bleeding to the fibrin threads occurred was recorded. These were performed at a room temperature of 16℃.

1.2.1.2.4 Bone marrow nucleated cells count Mice were sacrificed by cervical spine dislocation, then dissected to remove the right femur. The clingy muscle of femur was eliminated and the ends were then snipped off. The medullary cavity was washed by 10 mL 3% glacial acetic acid in 10mL syringe and the collected wash was scattered through type 4 syringe needles. Bone marrow nucleated cells were counted by dropping one drop of washing liquid on the globulimeter and observed under microscope (10×40). Cells were calculated as the total amount of cells from four large grid multiples 2.5×10^4/femur.

1.2.2 Modification of thrombopenia model

1.2.2.1 Animal model 48 BALB/c mice, half male and half female, were randomly divided into Normal, Cyclophosphamide low, medium and high dose groups with each of 12 mice due to their weight and gender. The three dosage groups were given 100, 120, 140 mg/kg cyclophosphamide by subcutaneous injection respectively. The volume of dosage is 0.1 mL/10 g. Mice in Normal group received the same volume of normal saline.

1.2.2.2 Observation indexes

1.2.2.2.1 General condition The same as described in 1.2.1.2.1. This process lasted for 11 days.

1.2.2.2.2 Blood cell analyses A 10 μL blood sample was collected by tail cut before administration at the 1st, 7th and 11th day mornings and transferred into a 1.5 mL EP tube with 0.5 mL cell pack diluents in it. Blood cell analyses were analyzed soon after the collection.

1.2.2.2.3 Measurement of clotting time Clotting time measurement was performed at room temperature 16℃ 6 hours after taking blood for blood cells test on the 11th day. Two methods were used for the assay.

Capillary tube method: The same as described in 1.2.1.2.3.

Slide method: After the removal of one eye of each mouse, two drops of blood was dropped onto the slide, one drop for one slide end. One of the blood drops was teased every 30 s with a needle until fibrin threads of blood appeared. The time from bleeding to the appearance of fibrin threads was recorded. The other drop of blood was used for recheck.

1.2.2.2.4 Bone marrow nucleated cells count The left femur of mice was harvested for bone marrow nucleated cells count. For detailed procedure see 1.2.1.2.4.

1.2.2.2.5 Bone marrow DNA contents Mice were sacrificed by cervical spine dislocation, then dissected to remove the right femur, eliminate the clingy muscle and then snip off the ends. The right femur bone marrow was harvested by washing the cavity with 10 mL 5 mmol/L $CaCl_2$ in a 10 mL syringe with type 6 syringe needle. The washings were then placed into 4℃ refrigerator for 30 min, centrifuged at 2 500 r/min for 15 min, and the supernatant was discarded. 5 mL 0.2 mol/L of $HClO_4$ was then added into the precipitant, mixed thoroughly, and heated at 90℃ by thermostatic water bath for 15 min. After cooling down, it was centrifuged at 3 500 r/min for

10 min. The absorption value of the supernatant was measured under UV-VI spectrophotometer at 268 nm.

1.3 Statistical analysis

Data are presented as ($\overline{X} \pm s$). Statistical analysis was performed with the independent-samples *t* test by SPSS 13.0 software. Differences were considered significant when $P<0.05$.

2 Results

2.1 Comparison of two thrombopenia models

2.1.1 General condition At the 1st day, the hair of the mice in the two model groups became erect as soon as the cyclophosphamide was injected. Moreover, the hair color was not as bright as the ones of the Normal group. They also developed bowed backs and dysphoria. Mice of Model A group were much worse compared with those of Model B group. All these appearances disappeared one day after the last administration. Mice in Model A died from the 6th day, the death rate was 40% on the 7th day and came to final 60% on the 15th day. As to Model B, there was no death within 13 days (Fig.1) Autopsy of dead mice in both model groups showed hepatomegaly and the livers were grainy, even some hearts were covered with white particles.

The body weights of female mice in Model B group first decreased after injecting cyclophosphamide. However, on the 10th day they began to rise to almost the Normal level by the 15th day where the males continued to decrease. The weight of the Normal group mice increased gradually and tended to be stable at later periods. However, the weight of the Model groups dropped rapidly after injection of cyclophosphamide but began to rise 3 days after ceasing injections to the original level on the 15th day (Fig2).

2.1.2 Blood cell analyses In comparison to the Normal group, Model A platelets count decreased on day 7 and day 15 to about 59% and 74% of the Normal level respectively (Fig.3); platelet distribution width (Fig.4), mean platelet volume (Tab.1) and platelet-large cell ratio (Tab.2) did not change; white blood cells count dropped sharply on day 4 and day 7 (Fig.5). When Model B was compared with the Normal group, its platelets count decreased on day 7 and day 11 to about 33% and 75% of the Normal level, respectively (Fig.3); platelet distribution width increased on day 7 and day 11 (Fig.4); mean platelet volume increased on day 4, day 7 and day 11 (Tab. 1); platelet-large cell ratio increased on day 4

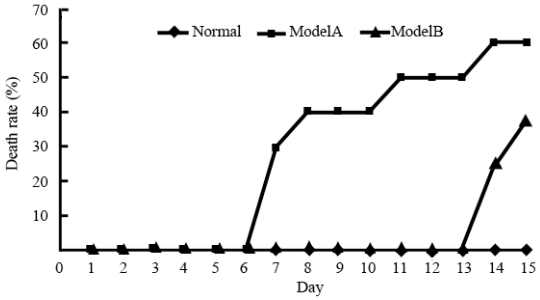

Fig. 1 Death rate of BALB/c mice affected by cyclophosphamide

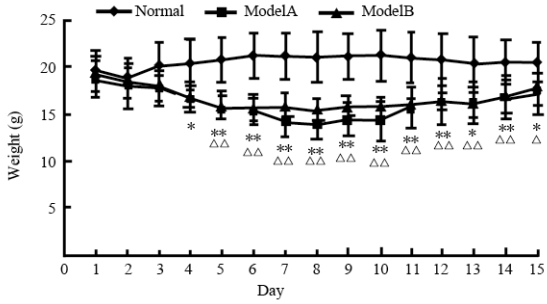

Fig. 2 Weight changes of BALB/c mice affected by cyclophosphamide

Data were expressed as ($\overline{X} \pm s$). Model A vs Normal: $*P<0.05$, $**P<0.01$; Model B vs Normal: $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$.

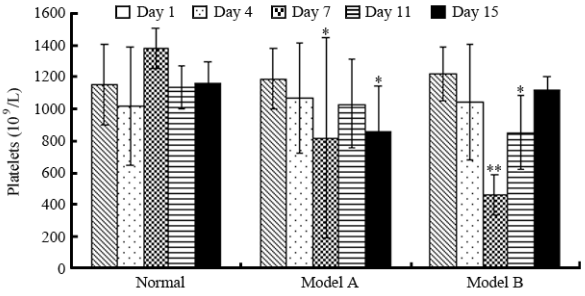

Fig. 3 Platelets of mice at different phases induced by cyclophosphamide

Data were expressed as ($\overline{X} \pm s$). Compared to Normal on the same day: $*P<0.05$, $**P<0.01$.

Tab. 1 Mean platelet volume of mice at different phases induced by cyclophosphamide ($\overline{X} \pm s$)

Group	Dose(mg/kg)	Mean platelet volume (fl)									
		N	Day 1	N	Day 4	N	Day 7	N	Day 11	N	Day 15
Normal	—	8	5.92±0.19	8	5.92±0.13	8	5.93±0.20	8	5.77±0.13	8	6.10±0.26
Model A	30(1st dose:200)	10	5.87±0.10	10	5.96±0.11	6	6.06±0.36	5	5.74±0.21	4	6.11±0.25
Model B	150	8	5.86±0.15	8	6.18±0.13**	8	6.24±0.25*	8	6.23±0.16**	5	6.01±0.36

Compared to Normal on the same day, $*P<0.05$, $**P<0.01$.

Tab. 2 Platelet-large cell ratio of mice at different phases induced by cyclophosphamide ($\overline{X} \pm s$)

Group	Dose (mg/kg)	Platelet-large cell ratio (%)									
		N	Day 1	N	Day 4	N	Day 7	N	Day 11	N	Day 15
Normal	—	8	2.63±1.89	8	2.75±1.04	8	2.88±1.25	8	2.50±0.76	8	3.75±1.28
Model A	30(1st dose:200)	10	2.00±0.94	10	2.90±0.88	6	3.33±1.37	5	2.60±0.55	4	4.25±0.50
Model B	150	8	2.50±1.31	8	4.38±1.77*	8	4.00±1.77	8	4.00±0.76**	5	3.60±1.14

Compared to Normal on the same day, *P<0.05, **P<0.01.

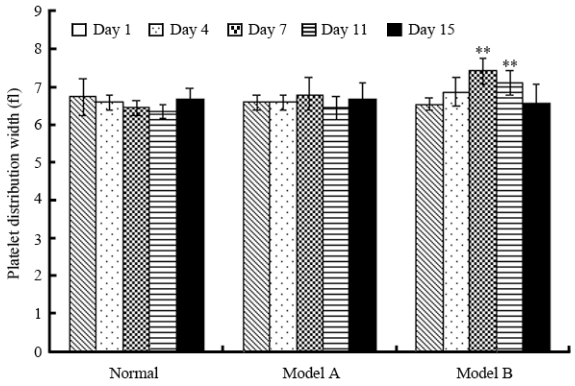

Fig. 4 Platelet distribution width of mice at different phases induced by cyclophosphamide

Data were expressed as ($\overline{X} \pm s$). Compared to Normal on the same day: **P<0.01.

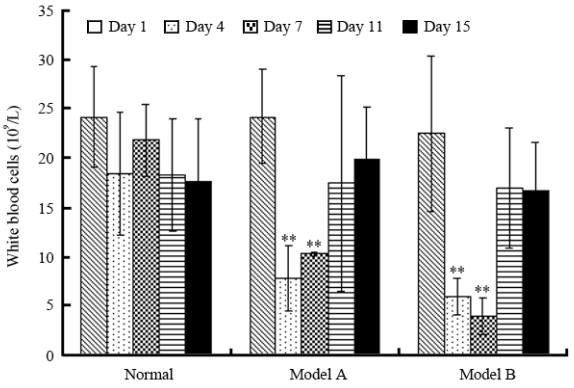

Fig. 5 White blood cells of mice at different phases induced by cyclophosphamide

Data were expressed as ($\overline{X} \pm s$). Compared to Normal on the same day: **P<0.01.

and day 11 (Tab.2); white blood cells count dropped sharply on day 4 and day 7 as Model A did (Fig.5). All differences between Normal group and model groups were of significance unless otherwise mentioned.

2.1.3 Clotting time and bone marrow nucleated cells count The rest of mice in two model groups were less than 6. There was no significant difference of clotting time and bone marrow nucleated cell count between Normal and model groups (data not shown).

2.2 Modification of thrombopenia model

2.2.1 General condition The hair of the mice in the 3 models became erect as soon as cyclophosphamide was injected, and the hair color lacked the brightness of the Normal group. All these appearances disappeared one day after the last dosage. Mice in model groups began to die from the 6th day (Fig.6). One mouse in Normal group died on the 7th day without abnormal pathology. Autopsy of the dead mice of model groups showed hepatomegaly, with the livers grainy, some hearts were covered with white particles, and some even contracted pneumonia or renal atrophy.

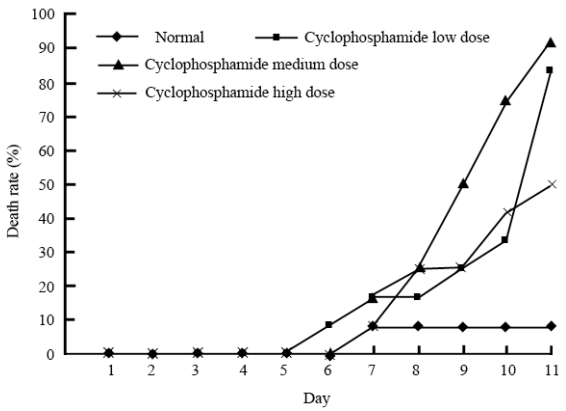

Fig. 6 Death rate of BALB/c mice affected by cyclophosphamide

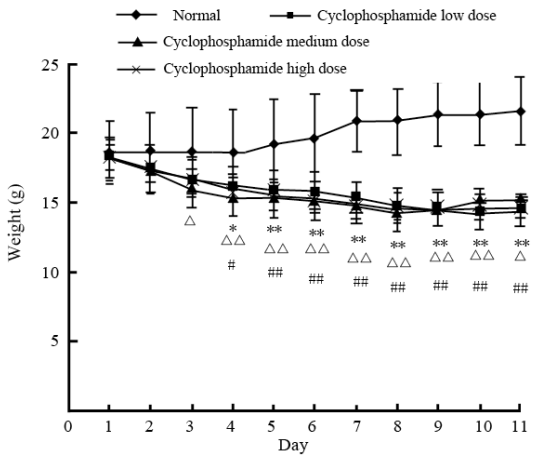

Fig. 7 Effect of cyclophosphamide on weight of BALB/c mice

Data were expressed as ($\overline{X} \pm s$). Cyclophosphamide low dose vs Normal: *P<0.05, **P<0.01; Cyclophosphamide medium dose vs Normal: $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01; Cyclophosphamide high dose vs Normal: $^{\#}P$<0.05, $^{\#\#}P$<0.01.

Tab. 3 Platelet distribution width of BALB/c mice influenced by cyclophosphamide on day 1, day 7 and day 11 ($\overline{X} \pm s$)

Group	Dose (mg/kg)	Platelet distribution width (fl)					
		N	Day 1	N	Day 7	N	Day 11
Normal	—	12	7.58±0.88	11	6.55±0.46	11	6.49±0.21
Cyclophosphamide low dose	100	12	7.06±0.89	10	6.63±3.51	2	8.22±0.14**
Cyclophosphamide medium dose	120	12	6.50±2.07	10	5.79±4.12	0	—
Cyclophosphamide high dose	140	12	5.81±2.74	11	4.99±3.99	6	9.91±2.08*

Compared to Normal on the same day, *P<0.05, **P<0.01.

Tab. 4 Mean platelet volume of BALB/c mice influenced by cyclophosphamide on day 1, day 7 and day 11 ($\overline{X} \pm s$)

Group	Dose (mg/kg)	Mean platelet volume (fl)					
		N	Day 1	N	Day 7	N	Day 11
Normal	—	12	6.33±0.28	11	5.96±0.21	11	5.97±0.13
Cyclophosphamide low dose	100	12	6.15±0.36	10	6.27±2.51	2	6.92±0.14**
Cyclophosphamide medium dose	120	12	5.74±1.82	10	4.57±3.16	0	–
Cyclophosphamide high dose	140	12	5.14±2.41	11	4.11±3.27	6	7.39±0.63**

Compared to Normal on the same day, **P<0.01.

Tab. 5 Platelet-large cell ratio of BALB/c mice influenced by cyclophosphamide on day 1, day 7 and day 11 ($\overline{X} \pm s$)

Group	Dose (mg/kg)	Platelet-large cell ratio (%)					
		N	Day 1	N	Day 7	N	Day 11
Normal	-	12	0.04±0.02	11	0.03±0.01	11	0.03±0.01
Cyclophosphamide low dose	100	12	0.04±0.02	10	0.04±0.03	2	0.06±0.00**
Cyclophosphamide medium dose	120	12	0.04±0.01	10	0.03±0.03	0	—
Cyclophosphamide high dose	140	12	0.03±0.02	11	0.03±0.04	6	0.10±0.07

Compared to Normal on the same day, **P<0.01.

The weight of the Normal group mice increased gradually and tended to be stable at the end stage while the Model ones dropped rapidly after administrating cyclophosphamide, especially in the medium dose group (Fig7).

2.2.2 Blood cell analyses In comparison to the Normal group, the following blood cell analytic results had significant differences. Platelet decreased on day 7 of 3 dosage groups and on day 11 of high dose group (Fig.8). The platelets count of mice in cyclophosphamide low dose group on day 7 was 30% of the Normal ones while the medium and high dose groups were 18% and 21%, respectively. Platelet distribution width increased on day 11 of low and high dose groups (Tab.3). Mean platelet volume increased on day 11 of low and high dose groups (Tab.4). Platelet-large cell ratio increased of low dose group on day 11 (Tab.5). White blood cells counts were dropped greatly on day 7 of 3 dosage groups (Fig.9).

2.2.3 Clotting time, bone marrow nucleated cells count and bone marrow DNA content The clotting time of cyclophosphamide low and high dose groups detected by capillary tube method were longer than that of Normal group, and the difference between cyclophosphamide low dose group and Normal was significant (Tab. 6). The bone marrow nucleated cells and bone marrow DNA content (Tab. 7) of cyclophosphamide low and high dose

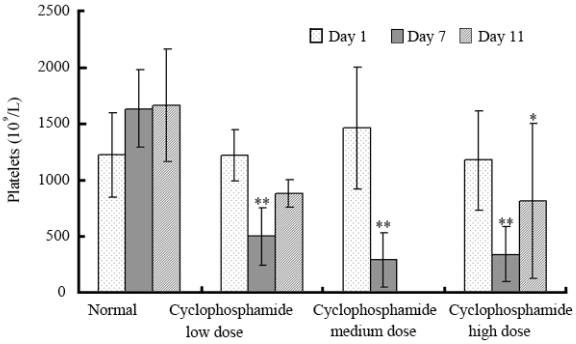

Fig. 8 Platelets of BALB/c mice influenced by cyclophosphamide on day 1, day 7 and day 11

Data were expressed as ($\overline{X} \pm s$). Compared to Normal on the same day: *P<0.05, **P<0.01.

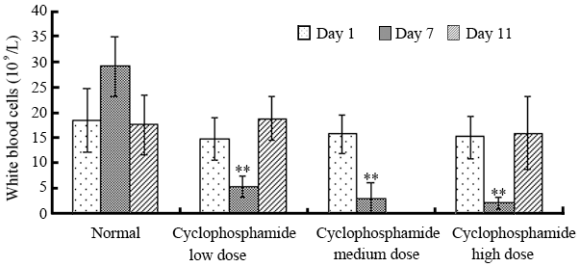

Fig. 9 White blood cells of BALB/c mice influenced by cyclophosphamide on day 1, day 7 and day 11

Data were expressed as ($\overline{X} \pm s$). Compared to Normal on the same day: **P<0.01.

Tab. 6 Effect of cyclophosphamide on clotting time of BALB/c mice ($\bar{X} \pm s$)

Group	Dose (mg/kg)	N	Clotting time (s)	
			Capillary tube method	Slide method
Normal	-	11	179.36±74.29	114.09±71.91
Cyclophosphamide low dose	100	2	374.50±12.02**	220.50±135.06
Cyclophosphamide high dose	140	6	248.00±113.60	143.33±121.15

** $P<0.01$ vs Normal using the same method.

Tab. 7 Effect of cyclophosphamide on bone marrow nucleated cells count and bone marrow DNA content detection of BALB/c mice ($\bar{X} \pm s$)

Group	Dose (mg/kg)	N	Bone marrow nucleated cells count (10^7/femur)	Bone marrow DNA content (Absorption value)
Normal	—	11	1.137±0.347	0.90±0.46
Cyclophosphamide low dose	100	2	0.913±0.633	0.62±0.46
Cyclophosphamide high dose	140	6	0.905±0.467	0.66±0.30

groups were lesser than those of Normal group, but their differences were of no significance because their sample number varied too much and their standard deviation was too great.

3 Discussion

The suppressive effect of cyclophosphamide on haematogenesis is well known. The aim of the comparison experiment was to find out a feasible model of thrombopenia of mice induced by cyclophosphamide at two different administration routes. Mice in Model A tended to be more affected by cyclophosphamide, as its body weight decreased more and died sooner compared with those of Model B. The effect of thrombocytopenia was greater in mice of Model B, which was much more stable than that of Model A, as the platelets count significantly decreased on day 7 and day 11 and with no death within 13 days. The platelets of Normal increased slightly at the 7th day, which may be correlated with the stimulation and compensation of blood lost. Through blood cell analyses and weight changes, it was clear that self-healing was induced towards the toxicity created by cyclophosphamide. This accorded with the clinical marrow inhibition adverse response of cyclophosphamide, where white blood cells decreased and the lowest amount of platelet occurred after administration of 1−2 weeks and self healing occurs 2−3 weeks later. We tested the clotting time and counted bone marrow nucleated cells at the end of this experiment. There was no significant difference of those two indices between Normal and model groups, which may be due to the haematogenesis recovering to almost normal level at that time interval. But these phenomena did not mean the toxic effects of cyclophosphamide were eliminated, but actually increased the number of deaths day by day.

In Model B, platelet distribution width, mean platelet volume and platelet-large cell ratio increased and white blood cell decreased sharply. Platelet distribution width is an indication of variation in platelet size which can be a sign of active platelet release. As for mean platelet volume, its elevated level is an indication of increased megakaryocytic shedding of platelets and abnormally high values correlate primarily with thrombocytopenia. Platelet large cell ratio increases in thrombocytopenia, and is inversely related to platelet count and directly related to platelet distribution width and mean platelet volume (Babu & Basu, 2004). Cyclophosphamide, a chemotherapy drug, also well known as a potent immunosuppressive drug in humans and experimental animals, can reduce white blood cell. The white blood cells count data was coordinated with that of one article previously reported, which was first dropped sharply and then raised (Masahiro et al, 1999).

It was clear that Model A could reduce platelet to some extent and last for a longer time, however, mice of the group died rapidly and the platelets count was not stable. This differed from the descriptions of Zhang et al. (2003). We used mice of both sexes, at the body weight range of 17−21 g, whereas Zhang et al(2003) only used males at the age of 6-8 weeks. Whether the aspects of sex, age and/or body weight variation can lead to those differences needs further exploration.

The administration route of Model B, subcutaneous injection, was used to carry out the following cyclophosphamide dosage modification experiment to seek a suitable dosage which is most effective for inducing thrombopenia with less toxic effect.

From the results of blood cell analyses, platelets and

white blood cells of the three dosage groups dropped sharply in comparison with the Normal group. Cyclophosphamide high dose affected the mice much more than low and medium dosages. Day 7 was suitable for inducing thrombocytopenia. Furthermore, the low dose (100 mg/kg) could be a suitable dosage as the platelets count did not decrease as much as the other two dosages, allowing the treatment drug to ameliorate it. Platelets' function is to take part in the clotting process. Thrombocytopenia happens when platelets are lost from the circulation faster than they can be replaced from the bone marrow where they are made. Our experimental results showed mice in the model groups having the trend of prolonged clotting time and decreased bone marrow nucleated cells and bone marrow DNA contents, however, those parameters had no statistical significant difference when comparing with Normal group's. Increasing the sample size may produce a significant result.

In conclusion, BALB/c mice challenged by subcutaneous injection of 100 mg/kg cyclophosphamide once a day for 3 days consecutively could be a feasible mouse model of thrombocytopenia induced by cyclophosphamide. The 7th day is the most suitable test point. The platelets count of mice in this thrombocytopenia model was definitely reduced and stable. This model is easy to mimic, and is suitable for pharmacodynamic test of the drugs that are designed to increase platelets counts.

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