Acute Toxicity and Influence on Micronucleus of Mice Bone Marrow of TDI

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Abstract: Oral acute toxicity of 2, 4-tolyene diisocyanate (TDI in short) on mice and its influence on micronucleus frequency of mice bone marrow were studied. And the results showed that the LD_{50} of TDI on mice orally was 1995mg/kg, and at the dosage of 1/2 LC_{50}, 1/4 LC_{50}, the difference was significant compared with the negative group.

Key words: TDI; acute toxicity; micronucleus

1 Introductions

Toluene diisocyanate (TDI in short) is a kind of important raw material in the industrial production of polyurethane, which is widely used in daily life. The molecular weight of TDI is 174.2, and it will easily pollute the environment in the form of steam, dust and others during the production and transportation process. Lung injury induced by TDI has already been reported [1-4]. In this article, it is mainly studied the oral acute toxicity on mice and the impact on the micronucleus.

2 Materials and Methods

2.1 Materials

2.1.1 Tested Compounds and Positive Control

2,4 - toluene diisocyanate was purchased from Tianjin Chemical Reagent Sixth Branch Factory, cyclophosphamide was the injection from Shanghai Hualian Pharmaceutical Co. Ltd.

2.1.2 Animal

Healthy Kunming mice, provided by the Animal Center of Harbin Medical University

2.2 Methods

2.2.1 Oral Acute toxicity Test

60 Kunming mice (18 ~ 22g, male and female stay half) were divided randomly into 6 groups. And the exposure dose were 1216, 1512, 1900, 2375, 2968.75 and 3710.94 mg/kg respectively. Mice death within 7 days after the once gavage was observed.

2.2.2 Mice Bone Marrow Micronucleus Test [5-7]

50 Kunming mice (18 ~ 22g, male and female stay half) were divided randomly into 5 groups. And then mice were treated with TDI via respiratory tract with the help of 50 L static exposure tank. It was reported[6] that the lethal concentration (LC_{50}) of TDI inhaled by the respiratory tract was 9.7×10-6/4 h. Four dose groups were invited: 1/2 LC_{50}, 1/4 LC_{50}, 1/8 LC50 and1/16LC_{50}. Meanwhile, a negative control group (normally raised) and a positive control group (cyclophosphamide, 40mg/kg intraperitoneally) were set up. Each dose group was treated for 14 days, 4h a day, and the cyclophosphamide group was treated by 30h exposure method, which was that the exposure interval of the two treatment was 24h, 6h after the second exposure the mice would be killed vertebraly, taking the marrow, stained with Gimsa, observed with double-blind method. Each animal was counted 1000 polychromatic cells, recorded the number of cells that contained micronuclei. And then micronucleus rate was calculated. All the data were processed through one-way ANOVA.

3 Results

3.1 Oral Acute Toxicity Test

After seven days of observation, there was mice death. And the death and the dose were showed in Table 1.

Table 1 Acute Toxicity Experiment of TDI on Mice Orally

	animal (n)	dose(d) (mg/kg)	Lgd (x)	death (n)	death rate(p)	P^2
1	10	1216	3.08(d1)	2	0.2	0.04
2	10	1512	3.18(d2)	3	0.3	0.09
3	10	1900	3.28(d3)	4	0.4	0.16
4	10	2375	3.38(d4)	6	0.6	0.36
5	10	2968.75	3.47(d5)	8	0.8	0.64
6	10	3710.94	3.57(d6 x_m)	9	0.9	0.81

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According to Kou's improved method[8] $LD_{50}=lg\text{-}1[x_m\text{-}i(\sum p\text{-}0.5)]$, the 95% credibility range was $lg\text{-}1(x_{50}\pm 1.96Sx_{50})$ that it could be gained that the LD_{50} of TDI on mice orally was 1995mg/kg, and the credibility was 1703-2336mg/kg. It showed that the oral toxicity of TDI was low, which belonged to low toxic organic chemicals.

3.2 Bone Marrow Micronucleus Test

It showed that at the dosage of 1/2 LC_{50}, 1/4 LC_{50}, the difference was significant compared with the negative group, as was shown in Table 2. The regression analysis showed there was a good dose-effect relationship between micronucleus rate and the exposure dose. For female r=0.9648, P<0.001 and for male, it was r = 0.9765, P<0.001.

Table 2 Micronucleus Frequency of Mice Bone Marrow

	dose	animal (n)		PCE (n)		Micro-nucleus (n)		frequency(‰)	
		F	M	F	M	F	M	F	M
1	0	7	7	7000	7000	21	22	3	3.14
2	1/16 LC_{50}	7	7	7000	7000	28	26	4	3.85
3	1/8 LC_{50}	7	7	7000	7000	38	38	5.4	5.4
4	1/4 LC_{50}	7	7	7000	7000	53	57	7.6^{**}	8.14^{*}
5	1/2 LC_{50}	7	7	7000	7000	82	92	11.7^{***}	13.1^{***}
6	40mg/kg cp	7	7	7000	7000	335	486	47.8^{***}	69.4^{***}

compared with the negative $^{***}P<0.001$ $^{**}P<0.005$ $^{*}P<0.05$

4. Discuss

At the anaphase of karyomitosis, when chromosomes entered regularly into the daughter cells to form the nucleus, in the cytoplasm there are still some chromatids or chromosomes without centromeric fragment or ring, and those are called micronuclei. In the end, it would form one or more separate sub-nucleus, which is included in the cytoplasm of the daughter cells. It is called micronucleus because it is much smaller than nucleus. Micronucleus is completely separated in cells slurry, and the stain and the structure are the same as the nucleus, while the volume is smaller than 1/3 of the nucleus. Its arrival usually comes from the effect of chromosome breakage agent. Besides when attacked by the pituitary poison, the nucleus fails to form and it will be replaced by a group of small nuclei. However, the small nuclei at this time are often larger than the typical micronuclei. In this, the effect of TDI on mice bone marrow micronucleus were observe, which showed that at the dosage of 1/2 LC_{50}, 1/4 LC_{50}, the difference was significant compared with the negative group, and that was to say TDI had some certain chromosomal damage to mice under a certain dosage.

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