Study No. K920404

Kanglaite Injection Micronucleus Test

Author: Prof. Huang Zhengnan
Shanghai Institute of Pharmaceutical Industry (SIPI)

Sponsor: Zhejiang Kanglaite Pharmaceutical Co., Ltd.

Shanghai Institute of Pharmaceutical Industry (SIPI)

Personal Involved in This Study
Study Conductors: Huang Zhengnan, Cao Caiping, Shi Minghua, Yan Danpin
Animal Service Manager: Yan Yonggao
Quality Assurance: Qian Beili
Study Director: Prof. Huang Zhengnan
Director of the Department of Pharmacology: Prof. Li Bingsheng

Study Duration
Animal Arrived: 1992.8.5
Dosing Date: 1992.8.12
Dissection Date: 1992.8.13
Study Completion Date: 1992.10.20
Study Director Signature: Huang Zhengnan

Quality Assurance Statement

1992.11.10

Title: Anglesite Injection
Micronucleus Test

Study No. K920404

This study conforms to the principles of Good Laboratory Practice of China. The report has been reviewed and authorized by the Department of Pharmacology of Shanghai Institute of Pharmaceutical Industry (SIPI).

Qian Beili, Professor
Quality Assurance (GLP)
SIPI

Summary
The micronucleus test was used to study the mutagenic effect of Kanglaite Injection (KLT). The animals received KLT by the intravenous route. The dose levels of KLT were 25, 12.5 and 6.25 ml/kg body weight. The animals were killed at 24hr after the administration. Femoral bone marrow was aspirated and dispersed in fetal calf serum. Smears were prepared. The slides were stained in Giemsa solution, coded and examined by light microscopy. The incidence of micronucleated cell and the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NE) were calculated. The results indicated that KLT was negative in this test.

The micronucleus test appears to be a useful *in vivo* screening method for investigating genetic hazard of medicine. In this study the micronucleus test was used to assess cytogenetic effect of KLT.

1. Purpose
The purpose of this study was to determine the mutagenic effect of KLT by the micronucleus test.

2. Materials and Methods
2.1 Test Samples

KLT was obtained from Traditional Chinese Medicine Hospital of Zhejiang Province (Lot No. 920608).

Cyclophosphamide (CTX), supplied by Hualian Pharmaceutical Company, a well known carcinogen that causes chromosome damage was used as the positive control.

The corresponding vehicle served as negative control. Vehicle (suspension composed of soybean lecithin 1.5%, glycerine 2.5% and distilled water) was also obtained from TCM Hospital of Zhejiang Province (Lot No. 920605).

2.2 Animals
ICR mice weighting 20-22g were provided by BK Company.

2.3 Methods
ICR mice were randomly divided in to 5 groups. 5 males and 5 females each group. The dose levels of KLT were 25, 12.5 and 6.25 ml/kg body weight. The animals received test samples by the intravenous route. The animals were killed by cervical dislocation at 24hr after the administration. Since no difference was found in the preliminary toxicity test in which the mice were killed at 12, 18, 24, 48 and 72hr respectively (**Tab.1**). Femoral bone marrow was aspirated and dispersed in fetal calf serum. The suspension was centrifuged (1000rpm, 5min) and smears were prepared. The slides were stained in Giemsa solution for 10-12 min, coded and examined by light microscopy. Micronucleated polychromatic erythrocytes (MNPCE) were counted and the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was calculated. Altogether 1000 PCE with and without micronuclei (MN) were scored from each animal. NCE were also scored.

Animal arrived: 1992.8.5
Dosing date: 1992.8.12
Dissection date: 1992.8.13

3. Results and Discussion

The effects of KLT on micronuclei are shown in **Tab.2**. The incidence of MNPCE in negative control is 1.69‰. It was found that KLT did not cause any statistically significant MNPCE increase compared with the negative control ($p>0.05$).

The experiment with CTX demonstrated a significant increase MNPCE compared with the negative control ($p<0.01$). No significant decrease in the ratio of P/N was observed.

KLT was negative in this test and was considered to be no clastogenic. Nor did it interfere with normal mitotic cell division under the test condition employed or gave any indication of bone marrow inhibition.

Tab.1 KLT: Micronuclei at different sacrifice time

Sacrifice Time(h)	Dose ml/kg	Animal numbers	Number of PCE	MNPCE	
				Number of MN	□ ‰
12	25	2	2007	4	1.99
18	25	2	2000	3	1.50
24	25	2	2005	7	3.49
48	25	2	2007	4	1.99
72	25	2	2005	3	1.50

Tab.2 KLT: Effect of KLT on micronuclei in bone marrow poly chromatic erythrocytes

group	Dose ml/kg	Animal numbers	Number of PCE	Ratio of P/N*	MNPCE	
					Number of MN	$\bar{X}\pm SD$‰
Vehicle		10	10044	1.16	17	1.69±0.94
	6.25	10	10120	1.16	26	2.57±1.28
KLT	12.50	10	10040	1.19	18	1.78±1.30
	25.00	10	10030	1.10	24	2.39±1.79
CTX	40mg/kg	10	10123	1.61	151	14.93±6.11**

** P<0.01 Compared with vehicle control group
*P/N=polychromatic erythrocytes/normochromatic erythrocytes

References

1. Schmid. W. The micronucleus test. *Mutation Res.* 1975;31:9-15

2. Heddie, J.A; et al. The induction of micronuclei as a measure of genetoxycity. *Mutation Res.* 1983; 123(1):61-118

3. Huang Xingshu, Chen Xingzheng, Test method on mutagenesis, teratogenesis and carcinogenic of environmental chemical pollutants (ECPs), the first version. Hangzhou: Publishing House of Science and Technology, 1985, 218-235