STUDY REPORT

LI Bingsheng, CHEN Xiuhua

Department of Pharmacology
Shanghai Institute of Pharmaceutical Industry
1320 Beijing Road (W), Shanghai, 200040, China
Telephone: 86-21-62897218, Fax: 86-21-62897218

1 The Effect of Kanglaite Injection on SW1990 Human Pancreatic Cancer Xenograft in Nude Mice

1.1 Purposes

To investigate the effect of Kanglaite Injection on inhibiting the growth of SW1990 human pancreatic cancer xenograft in nude mice.

1.2 Investigational Drug

Name: Kanglaite Injection
Provided by: Zhejiang Kanglaite Pharmaceutical Co., Ltd.
Batch Number: 0512151-2
Active Pharmaceutical Ingredient: Coix Seed Oil for Injection
Shape and Properties: Emulsion
Specifications: 100ml: 10g

1.3 Positive Control Drug

Name: Gemcitabine (Gemcitabine Hydrochloride, Gemzar)
Produced by: Lilly France S.A.
Active Pharmaceutical Ingredients: Gemcitabine
Specifications: 200mg/vial
Batch Number: FF5C98L
Produced on: 20050224
Preparation Methods: Prepared with physiological saline to required concentrations

1.4 Animals

Strain: BALB/C nude mice (SPF class)
Provided by: Shanghai Experimental Animal Center of the Chinese Academy of Sciences
Quality Certificate NO: SCXK (Shanghai) 2002-0010
Sex: Male
Body Weight: 18-20g
Number of Animal in Each Group: 6, and 10 for the control group

1.5 Transplantation Tumor

SW1990 human pancreatic cancer cell line was preserved by this department.

1.6. Methods

Well grown SW1990 tumor mass was cut into even-sized small lumps of 2-3 mm a piece under sterile conditions. Each nude mouse was subcutaneously inoculated one small tumor lump to the right axillary fossa with a trocar. The mice were randomized into 5 groups, which were as follows:

(1) Negative control group (physiological saline, 25ml/kg, ivX10);

(2) Gemcitabine group (100mg/kg, ipX1, day 10 after inoculation);

(3) Kanglaite Injection low dose group (6.25ml/kg, ivX10);

(4) Kanglaite Injection medium dose group (12.5ml/kg, ivX10);

(5) Kanglaite Injection high dose group (25ml/kg, ivX10);

A small lump could be seen at the inoculation location in all animals 7 days after the inoculation. Administration of the drugs was then begun according to the above scheme. The drug volume for the high, medium and low dose Kanglaite Injection groups was respectively 0.5, 0.25 and 0.125ml/20g body weight. The long diameter (a) and the short diameter (b) of the tumor lump were measured with a vernier every 4 days and the tumor volume ($v=ab^2/2$) was calculated. 22 days after the inoculation, the mice were sacrificed by neck dislocation. After the body weights were taken, the animals were dissected for the tumor lump. The tumors were then weighed and the tumor inhibition rates were calculated.

$$\text{Tumor Inhibition Rate \%} = \frac{\text{Average tumor weight in control group - Average tumor weight in treatment group}}{\text{Average tumor weight in control group}} \times 100\%$$

1.7 Results

When Kanglaite Injection at the dose of 25, 12.5 and 6.25 ml/kg was injected through the caudal vein of the nude mice for 10 consecutive days when the tumors were visible 7 days after their inoculations, the tumor inhibition rates of Kanglaite Injection for SW1990 human pancreatic cancer xenograft in nude mice were respectively 52.42%, 43.62% and 22.72%. An analysis of the data demonstrates that the differences between the tumor inhibition effects of Kanglaite Injection high and medium dose groups and that of the blank control group are statistically significant ($p<0.01$ and $p<0.05$). For the results, please see tables 1-1 and 1-2 and Figure 1.

1.8 Conclusions

Kanglaite Injection, when administered intravenously, is markedly effective in inhibiting the growth of SW1990 human pancreatic cancer xenograft in nude mice.

Table1-1: The Effect of Kanglaite Injection on SW1990 Human Pancreatic Cancer Xenograft in Nude Mice

Group	Dose (ml/kg)	Scheme	Number of Animals		Animal Body Weight (g)		Tumor Weight (g) $\bar{x}$ ±SD	Tumor Inhibition Rate %
			Beginning	End	Beginning	End (Tumor Removed)		
Negative Control (NS)	25	iv x 10	10	10	19.02	21.92±3.60	0.61±0.19	
Gemzar	100mg/kg	ip x 1	6	6	19.25	17.89±0.67*	0.31±0.11**	48.84
Kanglaite Injection	6.25	iv x 10	6	6	19.72	23.34±0.74	0.47±0.16	22.72
	12.5	iv x 10	6	6	18.82	21.08±1.61	0.34±0.14*	43.62
	25	iv x 10	6	6	18.63	21.07±2.03	0.29±0.07**	52.42

Compared with the Negative Control Group: * $p<0.05$, ** $p<0.01$

Table1-2: The Tumor Inhibition Effect of Kanglaite Injection on SW1990 Human Pancreatic Cancer Xenograft in Nude Mice: Change of Tumor Size

Group	Dose (ml/kg)	Scheme	Number of Animals		Number of Days after Inoculation, Tumor Size (mm^3), $\bar{x}$ ± SD				
			Beginning	End	7d	11d	15d	19d	22d
Negative Control (NS)	25	iv x 10	10	10	66.00 ±14.83	152.17 ±36.51	347.50 ±90.00	644.25 ±231.51	994.67 ±377.89
Gemzar	100 mg/kg	ip x 1	6	6	63.58 ±25.08	130.58 ±77.00 (14.18%)	227.25 ±148.90* (34.60%)	393.33 ±261.55* (38.95%)	574.42 ±420.36* (42.25%)
Kanglaite Injection	6.25	iv x 10	6	6	63.58 ±18.38	139.33 ±62.12 (8.43%)	284.67 ±121.76 (18.08%)	506.50 ±216.50 (21.38%)	747.50 ±306.86 (24.85%)
	12.5	iv x 10	6	6	69.17 ±14.29	120.08 ±31.13* (21.08%)	229.25 ±83.34* (34.03%)	419.58 ±123.08* (34.87%)	625.67 ±209.33* (37.10%)
	25	iv x 10	6	6	67.08 ±14.18	114.08 ±28.99* (25.03%)	220.00 ±80.88** (36.69%)	393.08 ±172.35* (38.99%)	537.08 ±297.28* (46.00%)

Compared with the Negative Control Group: * $p<0.05$, ** $p<0.01$. Number in () is tumor inhibition Rate.

2 The Effect of Kanglaite Injection in Combination with Gemcitabine on SW1990 Human Pancreatic Cancer Xenograft in Nude Mice

2.1 Purposes

To investigate the effect of Kanglaite Injection in Combination with Gemcitabine on inhibiting the growth of SW1990 human pancreatic cancer xenograft in nude mice.

2.2 Investigational Drug

Name: Kanglaite Injection
Provided by: Zhejiang Kanglaite Pharmaceutical Co., Ltd.
Batch Number: 0512151-2
Active Pharmaceutical Ingredient: Coix Seed Oil for Injection
Shape and Properties: Emulsion
Specifications: 100ml: 10g

2.3 Positive Control Drug

Name: Gemcitabine (Gemcitabine Hydrochloride, Gemzar)
Produced by: Lilly France S.A.
Active Pharmaceutical Ingredients: Gemcitabine
Specifications: 200mg/vial
Batch Number: FF5C98L
Produced on: 20050224
Preparation Methods: Prepared with physiological saline to required concentrations

2.4 Animals

Strain: BALB/C nude mice (SPF class)
Provided by: Shanghai Experimental Animal Center of the Chinese Academy of Sciences
Quality Certificate NO: SCXK (Shanghai) 2002-0010
Sex: Male

Body Weight: 18-20g
Number of Animal in Each Group: 6, and 10 for the control group

2.5 Transplantation Tumor

SW1990 human pancreatic cancer cell line was preserved by this department.

2.6. Methods

Well grown SW1990 tumor mass was cut into even-sized small lumps of 2-3 mm a piece under sterile conditions. Each nude mouse was subcutaneously inoculated one small tumor lump to the right axillary fossa with a trocar. The mice were randomized into 8 groups, which were as follows:

(1) Negative control group (physiological saline, 25ml/kg, ivX10);

(2) Gemcitabine group (100mg/kg, ipX1, day 10 after inoculation);

(3) Kanglaite Injection low dose group (6.25ml/kg, ivX10);

(4) Kanglaite Injection medium dose group (12.5ml/kg, ivX10);

(5) Kanglaite Injection high dose group (25ml/kg, ivX10);

(6) Kanglaite Injection low dose (6.25ml/kg, ivX10) + Gemzar (100mg/kg, ipX1, day 10 after inoculation) group;

(7) Kanglaite Injection medium dose (12.5ml/kg, ivX10) + Gemzar (100mg/kg, ipX1, day 10 after inoculation) group;

(8) Kanglaite Injection high dose (25ml/kg, ivX10) + Gemzar (100mg/kg, ipX1, day 10 after inoculation) group;

A small lump could be seen at the inoculation location in all animals 7 days after the inoculation. Administration of the drugs was then begun according to the above scheme. The drug volume for the high, medium and low dose Kanglaite Injection groups was respectively 0.5, 0.25 and 0.125ml/20g body weight. 100mg/kg Gemzar was injected ip day 10 after the inoculation in the Gemcitabine group and the high, medium and low dose Kanglaite Injection + Gemcitabine groups. The long diameter (a) and the short diameter (b) of the tumor lump were measured with a vernier every 4 days and the tumor volume ($v=ab^2/2$) was calculated. 22 days after the inoculation, the mice were sacrificed by neck dislocation. After the body weights were taken, the animals were dissected for the tumor lump. The tumors were then weighed and the tumor inhibition rates were calculated. The evaluation of the existence of a synergistic effect or an antagonistic effect when the two drugs were used together was performed using the Jin Zheng Jun formula ($q=Ea+b/Ea+Eb-EaxEb$). In the formula, Ea+b indicates the effect of the drug combination, Ea and Eb represents respectively the effect of drug A and drug B when the drugs are used alone. A q value between 0.85 and 1.15 indicates an additive effect, a q value above 1.15 indicates an enhanced effect and a q value below 0.85 indicts an antagonistic effect.

2.7 Results

When Kanglaite Injection at the dose of 25, 12.5 and 6.25 ml/kg was injected through the caudal vein of the nude mice for 10 consecutive days when the tumors were visible 7 days after their inoculations, the tumor inhibition rates of Kanglaite Injection for SW1990 human pancreatic cancer xenograft in nude mice were respectively 52.42%, 43.62% and 22.72%. The tumor inhibition rate for Gemzar at the dose of 100mg/kg is 48.84%. When Kanglaite Injection at the dose of 25, 12.5 and 6.25 ml/kg was administered in combination with Gemzar at the dose of 100mg/kg, the tumor inhibition rates were respectively 74.70%,

60.95% and 52.70% and the q values were respectively 0.9873, 0.8565 and 0.8715. The q values for the high, medium and low dose Kanglaite Injection in combination with Gemzar were all above 0.85, indicating that there is an additive effect for all three Kanglaite Injection doses when used in combination with Gemzar. For the results, please see tables 2-1 and 2-2 and Figure 2.

2.8 Conclusions

When Kanglaite Injection at the dose of 25, 12.5 and 6.25 ml/kg was used in combination with Gemzar at the dose of 100mg/kg in SW1990 human pancreatic cancer xenograft in nude mice, their tumor inhibition effects are markedly stronger than those when Kanglaite Injection or Gemzar is used alone. There is an apparent additive effect when the two drugs are used together.

Table2-1: The Effect of Kanglaite Injection in Combination with Gemzar on SW1990 Human Pancreatic Cancer Xenograft in Nude Mice

Group	Dose (ml/kg)	Scheme	Number of Animals		Animal Body Weight (g)		Tumor Weight (g) $\bar{x}$ ±SD	Tumor Inhibition Rate %	q
			Beginning	End	Beginning	End (Tumor Removed)			
Negative Control (NS)	25	iv x 10	10	10	19.02	21.92 ±3.60	0.61 ±0.19		
Gemzar	100 mg/kg	ip x 1	6	6	19.25	17.89 ±0.67*	0.31 ±0.11**	48.84	
Kanglaite Injection	6.25	iv x 10	6	6	19.72	23.34 ±0.74	0.47 ±0.16	22.72	
	12.5	iv x 10	6	6	18.82	21.08 ±1.61	0.34 ±0.14*	43.62	
	25	iv x 10	6	6	18.63	21.07 ±2.03	0.29 ±0.07**	52.42	
Kanglaite Injection Low dose + Gemzar	6.25 ml/kg +100 mg/kg	iv x 10 ip x 1	6	6	18.82	18.06 ±0.88*	0.29 ±0.08**	52.70	0.8715
Kanglaite Injection Medium dose + Gemzar	12.5 ml/kg +100 mg/kg	iv x 10 ip x 1	6	6	19.77	19.23 ±1.45	0.27 ±0.13**	60.95	0.8565
Kanglaite Injection High dose + Gemzar	25 ml/kg +100 mg/kg	iv x 10 ip x 1	6	6	19.30	20.25 ±2.45	0.15 ±0.05**	74.70	0.9873

Compared with the Negative Control Group: * $p<0.05$, ** $p<0.01$

Table2-2: The Effect of Kanglaite Injection in Combination with Gemzar on SW1990 Human Pancreatic Cancer Xenograft in Nude Mice: Change of Tumor Size

Group	Dose (ml/kg)	Scheme	Number of Animals		Number of Days after Inoculation, Tumor Size (mm^3), $\bar{x}$ ± SD				
			Beginning	End	7d	11d	15d	19d	22d
Negative Control (NS)	25	Iv X10	10	10	66.00 ±14.83	152.17 ±36.51	347.50 ±90.00	644.25 ±231.51	994.67 ±377.89
Gemzar	100 mg/kg	ip X 1	6	6	63.58 ±25.08	130.58 ±77.00 (14.18%)	227.25 ±148.90* (34.60%)	393.33 ±261.55* (38.95%)	574.42 ±420.36* (42.25%)
Kanglaite Injection	6.25	iv X 10	6	6	63.58 ±18.38	139.33 ±62.12 (8.43%)	284.67 ±121.76 (18.08%)	506.50 ±216.50 (21.38%)	747.50 ±306.86 (24.85%)
	12.5	iv X 10	6	6	69.17 ±14.29	120.08 ±31.13* (21.08%)	229.25 ±83.34* (34.03%)	419.58 ±123.08* (34.87%)	625.67 ±209.33* (37.10%)
	25	iv X 10	6	6	67.08 ±14.18	114.08 ±28.99* (25.03%)	220.00 ±80.88** (36.69%)	393.08 ±172.35* (38.99%)	537.08 ±297.28* (46.00%)
Kanglaite Injection Low dose + Gemzar	6.25 ml/kg +100 mg/kg	iv x 10 ip x 1	6	6	64.25 ±10.04	112.67 ±10.78* (25.96%)	220.08 ±36.66** (36.67%)	369.33 ±106.15** (42.67%)	522.75 ±127.87** (47.44%)
Kanglaite Injection Medium dose + Gemzar	12.5 ml/kg +100 mg/kg	iv x 10 ip x 1	6	6	67.08 ±14.18	103.58 ±31.23* (31.93%)	179.92 ±70.26** (48.23%)	306.75 ±153.57** (52.39%)	424.25 ±241.05** (57.35%)
Kanglaite Injection High dose + Gemzar	25 ml/kg +100 mg/kg	iv x 10 ip x 1	6	6	65.00 ±13.69	89.92 ±29.45** (40.91%)	150.42 ±47.38** (56.71%)	215.17 ±71.47** (66.60%)	307.75 ±96.61** (69.06%)

Compared with the Negative Control Group: * $p<0.05$, ** $p<0.01$. Number in () is tumor inhibition Rate.

3 Discussion and Conclusions

The tumor inhibition rates of Kanglaite Injection for SW1990 human pancreatic cancer xenograft in nude mice, when administered iv at the dose of 25, 12.5 and 6.25 ml/kg for 10 consecutive days after the tumor was visible, were respectively 52.42%, 43.62% and 22.72%. Statistical analysis of the data demonstrates that the differences between the tumor inhibition effects of all Kanglaite Injection dose groups and that of the negative control group are very significant.

The tumor inhibition rates for Kanglaite Injection at the dose of 25, 12.5 and 6.25 ml/kg respectively in combination with Gemzar are all higher than those for Gemzar or Kanglaite Injection when they are used alone. The q values are all above 0.85, indicating that there is an apparent additive effects when the two drugs are used together.

This pharmacodynamics study indicates that Kanglaite Injection might be further clinically studied in the treatment of pancreatic cancer, either being used alone or in combination with chemotherapy agents.

References:

1. XU Shu Yun: Methodology of Pharmacology Studies, 1991, People's Health Printing House.

2. CHEN Qi: Methodology of Traditional Chinese Medicine Pharmacology Studies, 1993, People's Health Printing House.
3. The Bureau of the Drug Administration, the Ministry of Health of China: Guidance for Traditional Chinese Medicine New Drug Studies (Pharmacy, Pharmacology and Toxicology), July 1993.
4. HAN Rui: Chemoprevention and Chemotherapy for Cancers, First Edition, The Printing House of Beijing Medical University and the Peking Union Medical University, December 1991.
5. JIN Zheng Jun: Additive Effects in Drug Combinations: Chinese Journal of Pharmacology, 1980; 1(2): 70