Review

STUDIES ON THE PHARMACODYNAMICS AND PHARMACOKINETICS OF PACLITAXEL(Zisu®)

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ABSTRACT

Pharmacological studies demonstrated that paclitaxel (Zisu®) was very active in the inhibition of the growth of human cancer cell panel including KB cells, HCT-8, A2780, and MCF-7 cells. The IC_{50} was as low as 0.0019, 0.0019, 0.0036 and 0.01 μ g/ml respectively. Experimental therapeutic studies indicated that paclitaxel(Zisu®) significantly inhibited the growth of melanoma B-16, Walker carcinomsarcoma and heterotransplanted human ovarian cancer in nude mice. Biochemical pharmacological studies showed that paclitaxel (Zisu®) could accelerate microtubule assembly and inhibit its deassembly; population in G_1 was decreased while the cell population in G_2+M phase was increased significantly. In addition, a polyploid cell population appeared. Pharmacokinetic studies demonstrated that the $t_{1/2\alpha}$ was 0.12 h and $t_{1/2\beta}$ was 5.02 h when it was injected intravenously at a dose of 5 mg/kg in rats. The AUC, Vc and CLs were 11.82(μ g.h)/ml, 0.50L/kg and 0.42L(h.kg) respectively.

Key words: Paclitaxel(Zisu®), Pharmacodynamics, Pharmacokinetics

Since 1984 the research of paclitaxel(Zisu®), an effective component of *Taxus yunannensis* had been started. This research has been listed in the Seventh Five-Year Scientific Plan of National Pharmaceutical Industry Administration bureau. During the fifteen years we have systematically screened the anti-tumor activity of the extracts and components from four species of *Taxus* bark and activity of paclitaxel(Zisu®) *in vitro* and *in vivo*. At the same time the anti-tumor mechanism of action were studied, especially the effect of paclitaxel(Zisu®) on microtubule assembly and cell cycle traverse of mouse leukemia cell line L-1210. In addition, the pharmacokinetics was studied by HPLC. This research was rewarded by the National Pharmaceutical Industry bureau. In February 1995 paclitaxel was approved for clinical trial by the Ministry of public Health. In September of the same year the production license was issued. The discovery of taxol has been regarded as one of the three big achievements in anti-tumor drugs research of 1990s. It has unique therapeutic effect on breast cancer and ovarian cancer.

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In vitro Anti-tumor Activity

It was proved by MTT assay that oral epithelium cancer cell line KB, colon cancer cell line HCT-8, ovarian cancer cell line A2780 were the most sensitive cell lines to paclitaxel(Zisu®), the IC_{50} was 0.0019, 0.0019 and 0.0036μ g/ml respectively. Breast cancer cell line MCF-7 and stomach cancer cell line MGC-803 were sensitive. Lung adenocarcinoma cell line A549 and hepatocarcinoma cell line Bel7402 were less sensitive. The two kinds of cancer cell lines KB/VCR and HCT-8/$_{VCR}$ which were resistant to Vincristin were not sensitive (Table 1). The clone formation in soft agar studies showed that paclitaxel(Zisu®) significantly inhibited the clone formation ability of mouse leukemia cell line L-1210 and melanoma cell line B-16. This effect was dose-dependent.

Growth Inhibitory Effect of Paclitaxel (Zisu®) on Transplantable Tumors

It showed that paclitaxel(Zisu®) 10mg/kg *i.p.* for 10 days had significant inhibitory effect on transplantable mouse melanoma cell line B16. The life span of mice with S180 was significantly prolonged. Walker Carcinosarcoma 256 was also sensitive to paclitaxel(Zisu®). Paclitaxel(Zisu®) at the dose of 8mg/kg could obviously inhibit its growth (Table 2)

Effect of Paclitaxel(Zisu®) on Heterotransplanted Human Ovarian Carcinoma in Nude Mice

Paclitaxel(Zisu®) at the dose of 5mg/kg had obvious growth inhibitory effect on heterotransplanted human ovarian carcinoma in nude mice (Table 3). At higher dose the effect was more obvious.

Table 1. Cytotoxic effect of paclitaxel(Zisu®) on human cancer cell lines

Cell lines	IC_{50}(μ g/ml)
Human oral epithelium cancer cell line KB	0.0019
Human colon cancer cell line HCT-8	0.0019
Human ovarian cancer cell line A2780	0.0036
Human stomach cancer cell line MGC-803	0.005
Human breast cancer cell line MCF-7	0.01
Human lung adenoma cell line A549	0.05
Human hepatocarcinoma cell line Bel7402	0.07
Human oral epithelium cancer cell line resistant to Vincristin KB/VCR	0.2
Human colon cancer cell line resistant to Vincristin HCT-8/VCR	0.11

Table 2. Growth inhibitory effect of paclitaxel(Zisu®) on animal transplantable tumor

Groups	Dose (mg/kg)	Animal number (begin/end)	Weight of tumor (g)/average lifespan(d)	Inhibitory rate/life span prolonged rate	*P value*
Melanoma	Control	10/10	1.63± 0.48g		
Paclitaxel(Zisu®)	10×10d	10/10	0.74± 0.13g	55	<0.01
Sarcoma 180	Control	10/10	14.3± 2.3d		
Paclitaxel(Zisu®)	10×10d	10/10	35.2± 13.4d	146	<0.01
Walker 256	Control	10/10	9.3± 1.3g		
Paclitaxel(Zisu®)	8×3d	10/10	1.3± 0.8g	86	<0.01

Table 3. Inhibitory effect of paclitaxel(Zisu®) on heterotransplanted human ovarian carcinoma in nude mice

Groups	Animal numbers		Body weight		Tumor weight(g)	Inhibitory	*P* value
	Begin	End	Begin	End	X± SD	rate(%)	
Control	5	5	26.76± 1.51	27.56± 0.90	0.86± 0.90		
Paclitaxel(Zisu®)							
5mg/kg×10	6	6	26.58± 0.74	27.16± 0.71	0.30± 0.10	65	<0.01
10mg/kg×10	8	8	26.18± 1.49	26.80± 1.57	0.11± 0.08	87	<0.01

Biochemical Mechanism of anti-tumor Activity

The anti-tumor activity of taxol was reported by Wall and Wani in early 1970s. But researchers paid little attention about it. Until the middle of 1980s when Susan Horwitz, professor in Albert Einstein Medical college, reported its unique action which attracted attracted researchers' attention. She and her colleagues found that taxol selectively promoted microtubule assembly and inhibited its deassembly thereby affect the function of spindle and inhibit mitosis of tumor cells, which is complete different from traditional anti-tumor drugs. It didn't affect the synthesis of DNA and RNA of tumor cells, and damage DNA molecules.

Effect on microtubule assembly

Microtubules of pig brain was prepared and purified according to literature. The effect of paclitaxel(Zisu®) *in vitro* on microtubule assembly and deassembly was studied. Paclitaxel(Zisu®) at a concentratioin of 5 μ mol/L significantly inhibited microtubule deassembly at low temperature(Figure 1).

Effect on cell cycle dynamics of mouse leukemia L-1210 cells

The Log-phase growth mouse leukemia L-1210 cells were divided into control group and different concentrations of paclitaxel(Zisu®) treated groups. The cell pellet was collected, fixed with 95% ethanol.

After being stained with DAPI and SR101, the DNA content of each cell was determined by PAS-II flow-cytometer. For each sample 20,000 cells were determined. The result showed that paclitaxel(Zisu®) at a concentration of 25nmol/L significantly affected cell cycle traverse of L-1210 and blocked the cell population in G_2+M phase (Figure 2). The tumor cells couldn't divide and enlarged after treatment by paclitaxel(Zisu®). At the same time polynucleus cell population appeared and the mitosis index significantly increased.

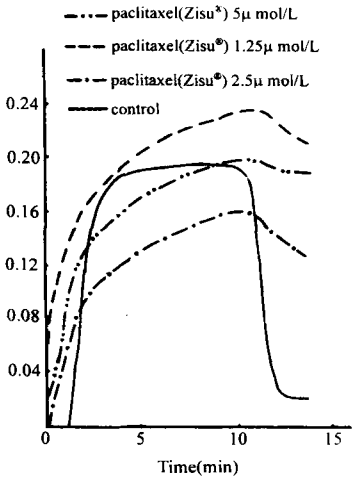

Fig. 1. Effect of paclitaxel(Zisu®) on microtubule assembly. Paclitaxel(Zisu®) significantly promoted microtubule assembly and shorten the latent time of assembly at 37℃, pH6.4 with of ATP. The optical density at 350nm was increased obviously. While cooled off till 0-4 ℃ microtubule deassembly was seen and the optical density at 350nm was decreased.

General Pharmacology

Effect on mice behavior and performance activity

Fort ICR mice were randomly divided into four groups. When paclitaxel(Zisu®) was administered *i.p.* for single dose of 20 mg/kg no obvious side effect was observed in one week. While administered *i.p.* at the dose of 30 mg/kg, 3 mice died after 5 days. Main symptom were difficulty in movement, sleepy and the righting-reflect lost.

Another 128 ICR mice were randomly divided into four groups, male and female in half each. After subcutaneous injection the mouse was put in the free activity counter. No obvious effect on mice performance activity was observed when paclitaxel(Zisu®) was administered at the dose of 10,15 and 20mg/kg.

Effect on respiration, blood pressure, heart rate and electrocardiogram of anaesthetized dog

Three healthy dogs (one female and two male) were anaesthetized by pentobarbital and respiration, blood pressure, heart rate and electrocardiogram were recorded. Equal volume of solvent and different doses of paclitaxel(Zisu®) 3, 6, 12, 24 and 48 mg/kg were given intravenously, for each group the dog was observed for at least 60 min. It was observed that after *i.v.* of solvent the blood pressure decreased in a short time, respiration was accelerated. Ten minutes later the parameters gradually recovered. Paclitaxel(Zisu®) at 3mg/kg had no obvious effect on the above parameters, 6mg/kg accelerated the respiration, 12 and 24mg/kg caused similar effect but had no obvious effect on blood pressure, heart rate and electrocardiogram.

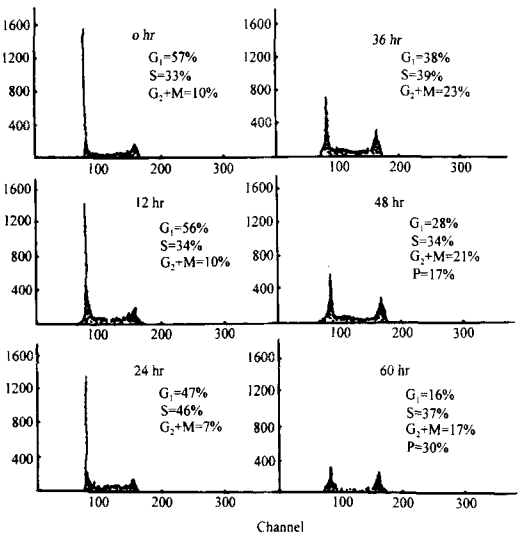

Fig 2. Effect of paclitaxel(Zisu®)(25nol/L) on cytokinetics of mouse leukemia L-1210. Paclitaxel(Zisu®) at a concentration of 25nmol/L significantly affected cell cycle traverse of L-1210 and blocked the cell population in G_2+M phase cells and a polyploid cell population (P) appeared in DNA histograms.

Pharmacokinetic Studies in Rats

Sixty three Wistar rats were randomly divided into 3 groups. Paclitaxel(Zisu®) 5, 10, 20mg/kg was injected from tail vein. The heads of rats were cut down to collect blood after 5, 15, 30 min, 1, 3, 6, 12 h of administration. The original drug concentration in the serum was determined by HPLC at wavelength of 227 nm, mobile phase was methanol: pure water (70:30). The minimum detectable limit was 5 ng. 10-deacetylcephalomannin was used as internal standard. The analytical software 3P87 was adopted

to calculate pharmacokinetic parameters. Results were shown in Table 4 and Table 5.

Table 4. Drug concentration in the blood of rats after i.v. of paclitaxel(Zisu®) 5, 10 and 20mg/kg

Time after administration	Blood drug concentration (X± SD, μ g/ml, n=5)		
	5mg/kg	10mg/kg	20mg/kg
5 min	6.62± 1.37	14.03± 0.90	26.72± 1.77
15 min	3.56± 0.43	6.20± 0.36	15.26± 1.39
30 min	1.62± 0.31	3.96± 0.55	7.73± 1.21
1 h	1.43± 0.37	2.56± 0.26	3.99± 0.59
3 h	0.93± 0.25	1.03± 0.25	2.72± 0.43
6 h	0.56± 0.25	0.80± 0.33	0.98± 0.27
12 h	0.31± 0.05	0.49± 0.34	0.80± 0.41

Table 5. Pharmacolinetic parameters in rats after i.v. paclitaxel(Zisu®) 5, 10 and 20mg/kg

Index	Unit	Result		
		5mg/kg	10mg/kg	20mg/kg
A	μ g/ml	8.59	17.69	31.97
α	1/h	5.86	5.50	4.13
B	μ g/ml	1.43	2.49	4.05
β	1/h	0.14	0.18	0.18
Vc	L/kg	0.50	0.50	0.56
$T_{1/2\alpha}$	H	0.12	0.13	0.17
$T_{1/2\beta}$	H	5.02	3.80	3.85
K_{21}	1/h	0.95	0.84	0.62
K_{10}	1/h	0.85	1.20	1.19
K_{12}	1/h	4.20	3.65	2.49
AUC	(μ g.h)/ml	11.82	16.87	30.27
CLs	L/(h.kg)	0.42	0.59	0.66

Serum drug concentration decreased quickly after *i.v.* paclitaxel(Zisu®) at 10mg/kg. Twelve hours later the blood drug concentration was less than 0.5 μ g.h) /ml and CLs was 0.59L(h.kg)

In addition, [^{3}H] labeled paclitaxel(Zisu®) was used for monitoring tissue distribution and excretion of taxol in Wistar rats. It was proved that [^{3}H] labeled paclitaxel(Zisu®) widely distributed in all tissues after intravenous injectioin. The radioactivity in liver, spleen, kidney, lung and large intestine was highest, radioactivity in small intestine, fat and bone marrow was lower and the radioactivity in brain and muscle was the lowest.

Twelve hours after administration, rats excreted more [^{3}H] labeled paclitaxel(Zisu®) in urine than in faeces. Total excretion quantity in urine and faeces accounted for 74% of administrated drug 72 h later. Three hours after administration the excretion quantity in bile accounted for 59.4% of administrated drug.

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