

中國1號天仙液 對移植性S₁₈₀ 肉瘤和肝癌抑制作用的研究

研究單位:FRC生物醫學研究中心

Free Radical Biology & Medical Research Center

聯絡地址:台北市信義區和平東路三段213號4F

聯絡電話:(02)7390550

El estudio de los Efectos Inhibitorios de FRC001 (Líquido Tian Xian China No. 1, para Sarcoma) sobre el Transplante S₁₈₀ y el Hepatocarcinoma

¹ Prof. Kexiang Ding, 1Chung-Wan Su, Ph.D.,

² Prof. Robert W Bradford

¹ Free Radical Biology & Medical Research Center

² Bradford Research Institute

Capital University, Washington D.C.

ABSTRACTO

El Sarcoma S₁₈₀ (por transplante) y el Hepatocarcinoma del ratón son los modelos más importantes y populares para el estudio de las medicinas antineoplásicas. Este trabajo estudió los efectos inhibitorios del FRC001 en estos dos modelos. Los resultados demostraron que el FRC001 tiene efectos inhibitorios hasta cierto grado sobre el Sarcoma S₁₈₀ y el Hepatocarcinoma. Entre estos encontramos que las tasas de inhibición sobre el S₁₈₀ en dosis altas, medianas y bajas del FRC001 fueron en el orden de 59.73%, 52.57% y 41.33% respectivamente, lo que demuestra efectos obvios dependientes de la dosis. El FRC001 también tuvo efectos sobre focos de Hepatocarcinoma, con una tasa de inhibición de 47.81%. Después del tratamiento con el FRC001, el peso promedio disminuyó y los focos de carcinoma se redujeron aparentemente en comparación con el grupo control ($p<0.05$ o $p<0.01$). El FRC001 es una medicina antineoplásica que utiliza medicinas chinas tradicionales como ingrediente principal.

Palabras clave: FRC001; Tumor por transplante, Sarcoma S₁₈₀; Efecto inhibidor sobre el carcinoma hepático (Hepatocarcinoma).

INTRODUCCIÓN

Tumores malignos son parte de una serie de enfermedades comunes que hacen daño a los seres humanos. En la actualidad su tratamiento es un problema difícil para el mundo de la medicina. Además de cirugía, radioterapia y quimioterapia, los papeles de las medicinas tradicionales chinas y la combinación de las medicinas China y de Occidente son reconocidas

ampliamente en círculos médicos. El FRC001 se prepara utilizando principalmente medicinas chinas tradicionales que pueden eliminar el factor patogénico y apoyar la energía saludable. Esta energía saludable de apoyo incluye el fortalecimiento del Ki y el enriquecimiento de la sangre, calienta el Yang y alimenta el Yin, La eliminación del factor patogénico incluye la estimulación de la circulación de la sangre para eliminar el estancamiento, elimina el calor y los materiales tóxicos y suavizan y resuelven la masa dura. Estos métodos tienen mejor efecto inhibidor sobre los tumores.

Este trabajo estudia los efectos del FRC001 sobre el carcinoma S₁₈₀ (por transplante) y el Hepatocarcinoma de ratones.

MATERIALES Y MÉTODOS

1. Materiales

- 1.1. Droga experimental: FRC001 (extracto líquido de medicinas Chinas).
- 1.2. Animales experimentales: Ratones Kunming de pura sangre C57, peso de 18-22 gr., sanos de 2-3 meses, machos y hembras proporcionados por el Centro de Animales Experimentales del Instituto de Investigaciones Profiláctico terapéuticos Tumorales Jiangsu.
- 1.3. Variedad de Tumor Experimental: (1) Sarcoma S₁₈₀ (2) Carcinoma Hepático. proporcionados por el Departamento de Investigaciones Medicas del Instituto de Investigaciones Profilactico-Terapéuticas Tumorales Jiangsu.

2. Métodos de Preparación del Patrón Animal

2.1. Sarcoma S₁₈₀

Se extrajo líquido ascítico de Sarcoma S₁₈₀ de ratones a los cuales se les había inoculado con el S₁₈₀ 7-9 días antes, diluidos con solución salina normal al 1x108/ml de solución de células de Sarcoma. Al día siguiente, los ratones fueron inoculados por vía subcutánea con 0.2 ml de la solución de células de Sarcoma en la pata derecha delantera usando técnica aséptica; luego fueron agrupadas al azar e incluidas en el experimento. A los ratones se les dio medicamentos tales como FRC001, y otras por vía endogástrica una vez al día durante los siguientes 12 días, y luego disecados el día 13 para obtener el Sarcoma. Los sarcomas fueron pesados exactamente (gm) y se calculó la tasa de inhibición de las medicinas sobre Sarcoma por medio de la siguiente formula.

$$A\% = \frac{X - Y}{X} * 100\%$$

A	Tasa de Inhibición del FRC001 sobre el Sarcoma S ₁₈₀
X	Peso promedio del S ₁₈₀ en el grupo control (gm)
Y	Peso promedio del S ₁₈₀ en el grupo experimental (gm)

2.2. Carcinoma Hepático

La cepa de carcinoma hepático se diluyó con solución salina normal al 1X108/ml de suspensión de células cancerosas. A los ratones se les inoculó con 0,2 ml de dicha solución en la pata anterior derecha usando técnica aséptica. Al día siguiente fueron agrupados al azar y se les dio medicinas como FRC001 una vez al día por 8 días consecutivos y disecados el día 9. Los ratones y el Hepatocarcinoma fueron pesados exactamente. Se utilizó la siguiente fórmula para calcular las tasas de inhibición de medicamentos sobre el carcinoma hepático

$$C\% = \left(1 - \frac{W}{Z}\right) * 100\%$$

C%	Tasa DE Inhibición del medicamento sobre el carcinoma hepático
W	Peso promedio del Hepatocarcinoma del grupo experimental (gm)
Z	Peso promedio del Hepatocarcinoma del grupo control (gm)

RESULTADOS Y DISCUSIÓN

1. El efecto inhibidor del FRC001 sobre el Sarcoma S₁₈₀ del ratón:

Para los efectos de determinar la dosis óptima y estudiar la relación de dosis y efecto del FRC001 sobre S₁₈₀, usamos tres dosis: alta (6.0 ml/kg. de peso corporal); mediana (3.0 ml/Kg. de peso corporal) y baja (1.5 ml/kg. de peso corporal).

Los resultados se muestran en la Tabla 1 y la Figura 1,2.

Tabla 1. Los efectos del FRC001 sobre el Sarcoma S₁₈₀ Transplantado a Ratones

Grupo	Número (n)	Dosis (ml/kg.pc)	Días del Experimento (d)	Promedio de S ₁₈₀	Peso ($\bar{X} \pm SD$)	Tasa de Inhibición (%)
Grupo Control	10	0	12	3.75±1.53		-
Dosis Alta	10	6.0	12	1.51±0.43***		59.73
Dosis Mediana	10	10	3.0	1.78±0.58***		52.53

Dosis Baja	10	1.5	12	$2.20 \pm 0.93^{**}$		41.33
Quimioterápia	10	25mg/kg.bw	12	$1.96 \pm 1.00^{***}$		47.73

Comparado al grupo control, *P >0.05, **P <0.05, ***P<0.01, ****P<0.001

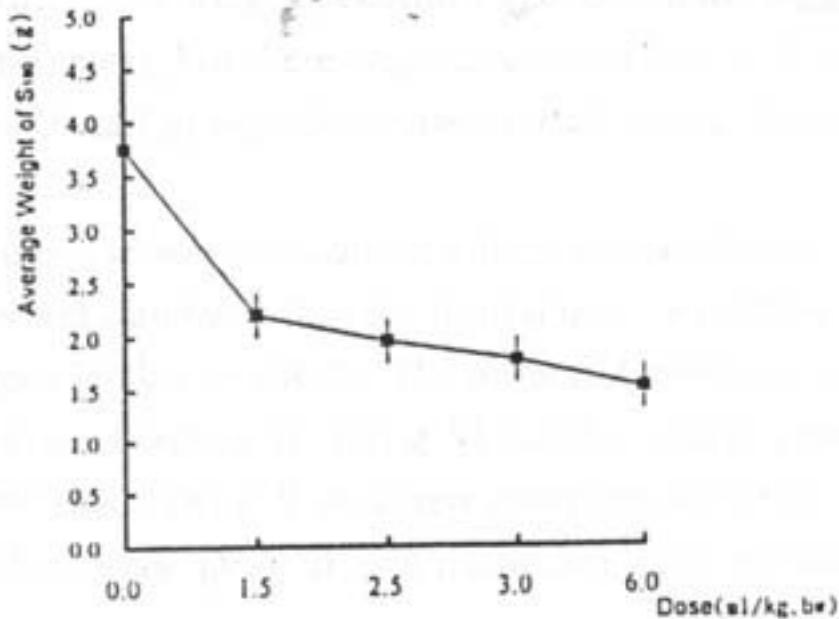


Figura 1 Curva de Dosis-Efecto del FRC001 sobre S₁₈₀

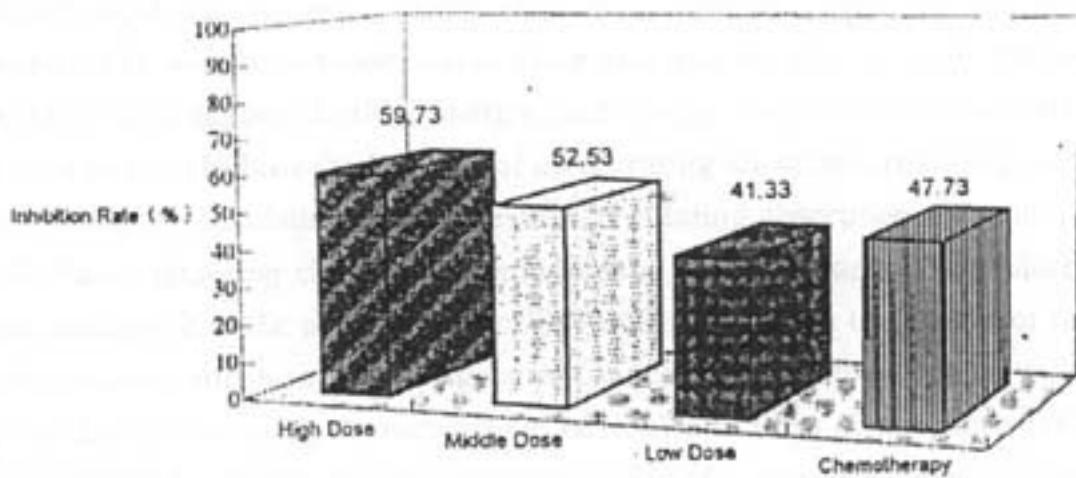


Figura 2. Tasas de Inhibición de Varias Dosis de FRC001 sobre S₁₈₀

De acuerdo con la Tabla 1 tres dosis de FRC001 tuvieron diferentes efectos de inhibición sobre el Sarcoma S₁₈₀ transplantado al ratón. Los pesos promedio de S₁₈₀ fueron significativamente mas pequeños que los del grupo control (P<0.05 o P<0.01). Estos hallazgos muestran que el FRC001 tuvo un efecto inhibidor sobre el Sarcoma S₁₈₀.

De acuerdo con la Figura 1, la inhibición del FRC001 sobre el Sarcoma S₁₈₀ transplantado al ratón depende de la dosis, por ejemplo al aumentar la dosis aumentaron los efectos inhibitorios sobre S₁₈₀, disminuyó el peso del Sarcoma y el tamaño de los focos del tumor.

Basados en las investigaciones de dosis-efecto, fue seleccionada la alta dosis de FRC001 (6.0 ml/kg peso corporal) para estudiar sus efectos sobre el peso corporal, el peso del carcinoma en el animal experimental del carcinoma hepático y la tasa de inhibición, (mostrados en Tabla 2 y Figuras 3 y 4)

De acuerdo con la Tabla 2, estas medicinas tuvieron efectos inhibitorios sobre los focos de carcinoma hepático en los animales experimentales que mostraron una disminución del peso del carcinoma. El peso promedio de carcinomas de Drogas I a vi FUERON 1.41gm, 1.09gm, 1,26gm, 1.49gm, 1.02gm, 0.71gm, respectivamente, disminuyeron 0.65gm, 0.97gm, 0.80gm, 0.59gm, 1.04gm, 1.035gm respectivamente cuando se compara con el grupo control. Comparados con el grupo control, los efectos de droga i y IV no fueron estadísticamente significativos (P>0.05), mientras que drogas II,III,V Y VI tuvieron efectos significativos (P<0.01 o P<0.001)

La figura 3 muestra que el peso del carcinoma del grupo control fue el mayor, los pesos del carcinoma de drogas I-VI disminuyeron en algo, pero hubieron focos de carcinoma en todos los grupos de medicamentos. El orden del peso del carcinoma de todos los grupos fue: Control > droga IV > droga I > droga III > droga II > droga V > droga VI.

Estos resultados muestran que como un agente antineoplásico, FRC001 tuvo efectos sobre Sarcoma S₁₈₀ y Hepatocarcinoma. Los mecanismos pueden ser:

(1) FRC001 es una preparación compleja de medicamentos Chinos y Occidentales, que puede estimular la circulación sanguínea para eliminar el estancamiento de la sangre, eliminar el calor y materias tóxicas, ablandar y resolver masas duras, estimular el Ki, enriquecer la sangre, calentar el Yang y nutrir el Yin, para ayudar al cuerpo a eliminar el factor patogénico y apoyar energía saludable, eliminar materias tóxicas y resistir tumores, (2) Los componentes en el Edfrann podrían tener la función de vitalizar el Ki, el bazo, resolver la humedad, aliviar el hígado y regular la circulación del Ki, digestivo, regula absorción, elimina el desbalance del metabolismo de sustancias, facilitando circulación, hemopoyesis, inmunidad y pueden regular las funciones neuronales, endocrinas, de electrolitos y nucleósidos cíclicos, etc. (3) FRC001 puede inhibir el crecimiento del tumor por medio de la regulación de las funciones inmunológicas, favoreciendo la función fagocítica del sistema retículo endotelial . (4) Quizás el

FRC001 tiene la función de inhibir el metabolismo del ADN y ARN de la célula cancerosa para de esta manera matar la célula cancerosa o inhibir su crecimiento. (5) Quizás el FRC001 tenga la función de eliminar los radicales libres, ya que los radicales libres juegan un papel importante en la mutagenesis y carcinogenesis. FRC001 elimina macromoléculas y macromoléculas. Por supuesto que el crecimiento , desarrollo o inhibición es un curso biológico complicado, los efectos y mecanismos exactos del FRC001 requieren mas estudios todavía.

The Study on the Inhibition Effects of FRC001(China No.1 Tian Xian Liquid) on Transplantation Sarcoma S₁₈₀ and Hepatocarcinoma

¹ Prof. Kexiang Ding, ¹Chung-Wan Su, Ph.D.,

² Prof. Robert W. Bradford

¹Free Radical Biology & Medical Research Center

²Bradford Research Institute

Capital University, Washington D.C.

ABSTRACT

The transplantation sarcoma S₁₈₀ and hepatic carcinoma of mouse are the most popular and important models in the screening of antineoplastic drugs. This paper studied the inhibition effects of FRC001 on these two models. The results showed that on FRC001 had inhibition effects, to some extent, sarcoma S₁₈₀ and hepatic carcinoma. Among them, the inhibition rate of high, middle and low dose of FRC001 on S₁₈₀ were 59.73%, 52.57%, 41.33%, respectively which showed obvious dose-dependent effects; FRC001 also had some effects on hepatic carcinoma foci, and the inhibition rate was 47.81%; after the treatment of FRC001, the average weight decreased and the carcinoma foci shrank apparently compared with the control group (P<0.05 or P<0.01). FRC001 is an antineoplastic using traditional Chinese medicine as the main ingredient.

Key Words: FRC001; Transplantation tumor; Sarcoma S₁₈₀; Inhibition effect on hepatic carcinoma

BACKGROUND

Malignant tumor is a series of commonly encountered diseases which harm the human. The treatment of it is a difficult problem of today's world medical science. Besides operation, radiotherapy, chemotherapy, the roles of TCM and the combination of Chinese and Western medicine are widely noted by medical circles. FRC001 is prepared mainly using traditional Chinese drugs which can eliminate the pathogenic factor and support healthy energy. The supporting healthy energy includes invigorating Qi and enriching the blood, warming Yang and nourishing Yin; the eliminating the pathogenic factor includes promoting blood circulation to remove stasis, clearing away heat and toxic material and softening and resolving hard mass. These methods have better inhibition effect on tumors.

This paper studied the effects of FRC001 on transplantation carcinoma S₁₈₀ and hepatic carcinoma of mice.

Address correspondence to: Dr.solomon J.F. Chiou, FRC Radical Biology & Medical Research Center, 4F, No.213, Sec.

3, Hoping E. RD, Taipei ,Taipei ,Taiwan ,R,O .C

TEL:886-2-7390550

MATERIALS AND METHODS

1. Materials

1.1 Test Drug: FRC001 (liquid extract of Chinese drugs) , provided by China-Japan Feida Union Co.,LTD .

1.2 Experimental Animal: mouse, pure kunming bred C₅₇,18-22g, healthy, 2-3 months, male and female, provided by Experimental Animal Center of Jiangsu Tumor Prophylactico-therapeutic Research Institution.

1.3 Test Tumor Strain: ①Sarcoma S₁₈₀ strain; ②Hepatic carcinoma strain, provided by Medicine Research Department of Jiangsu Tumor Prophylactico-therapeutic Research Institution.

2. Methods of Preparing Animal Pattern

2.1 Sarcoma S₁₈₀

Ascites S₁₈₀ sarcoma were drawn from mice in which S₁₈₀ had been inoculated 7-9 days ago, diluted by normal saline to be 1×10⁸/ml. sarcoma cell solution. The next day, mice were inoculated the sarcoma cell solution 0.2 ml subcutaneously in their right forefeet by aseptic manipulation; then were randomly grouped and put into experiment. The mice were endogastricly given drugs such as FRC001, etc, one time daily for successive 12 days, and dissected to get sarcoma on the 13th day. The sarcoma were weighted precisely (g) , and calculate the inhibition rates of drugs on sarcoma by following formula

$$A\% = \frac{X - Y}{X} \times 100\%$$

A -- Inhibition rate of FRC001 on sarcoma S₁₈₀;
X -- The average S₁₈₀ weight of control group (g) ;
Y -- The average S₁₈₀ weight of experimental group.

2.2 Hepatic Carcinoma:

Hepatic carcinoma strain was diluted to 1×10⁸/ml carcinoma cell suspenstion solution by normal saline. The mice were inoculated above carcinoma solution 0. 2ml in their right forefeet by aseptic manipulation. The next day, they were randomly grouped and given drug such as FRC001, etc/one time daily for successive 8 days, and dissected on the 9th day. The body weight and hepatocarcinoma weight were got accurately. To calculate the inhibition rates of drugs on hepatic carcinoma by following formula:

$$C\% = \frac{W - Z}{Z} \times 100\%$$

C% -- Inhibition rate of drug on hepatic carcinoma;
W -- Average hepatocarcinoma weight of experimental group (g)
Z -- Average hepatocarcinoma weight of control group (g)

RESULTS AND DISCUSSION

1. The inhibition effect of FRC001 on transplatalation Sarcoma S₁₈₀ of mice:

To explore the dose-effect of FRC001 on S₁₈₀ and find the optimal dose, we tested three doses,i.e. high dose (6.0ml/kg.bw) , middle dose (3.0ml/kg.bw) and low dose (1.5ml/kg.bw) of FRC001 on

Table 1 . The Effects of FRC001 on Transplantation Sarcoma S₁₈₀ of Mice

Group	Number (n)	Dosage (ml/kg.bw)	Experiment Days(d)	Average of S ₁₈₀	Weight ($\bar{X} \pm SD$)	Inhibition Rate (%)
Control Group	10	0	12	3.75±1.53		—
High Dose	10	6.0	12	1.51±0.43****		59.73
Middle Dose	10	3.0	12	1.78±0.58***		52.53
Low Dose	10	1.5	12	2.20±0.93**		41.33
Chemotherapy	10	25mg/kg.bw	12	1.96±1.00***		47.73

Compared to control group, *P>0.05; **P<0.05; ***P<0.01; ****P<0.001

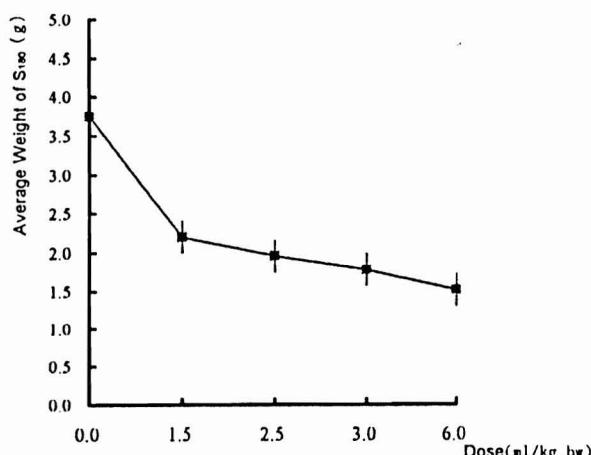


Figure 1 . Dose-effect Curve of FRC001 on S₁₈₀

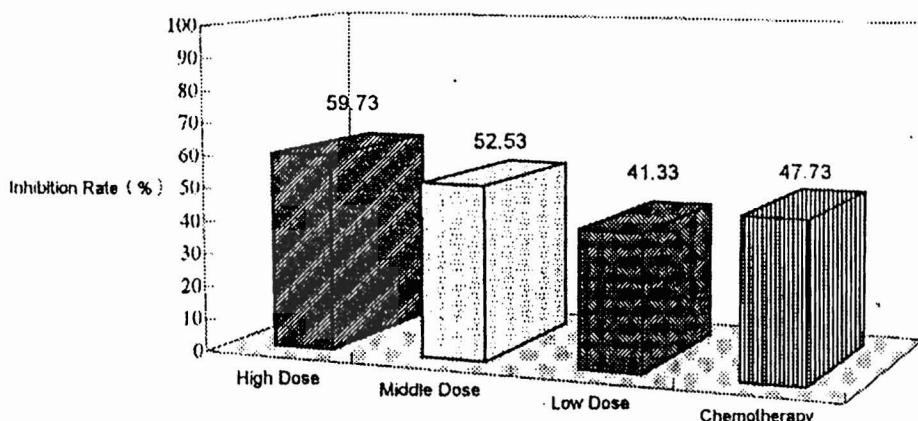


Figure 2 . Inhibition rates of Different Doses of FRC001 on S₁₈₀

transplantation sarcoma S₁₈₀ of mice (results showed in Table 1 and Figure 1,2)

According to Table 1, three doses of FRC001 had different inhibition effects on transplantation Sarcoma S₁₈₀ of mouse. The average weights of S₁₈₀ of the three doses were significantly smaller than that of control group (P<0.05 or P<0.01) . These findings showed that FRC001 had some inhibition effect on sarcoma S₁₈₀.

According to Figure 1, the inhibition of FRC001 on transplantation sarcoma S₁₈₀ of mouse were dose-dependent, i.e. as the increasing of the dose of FRC001, the inhibition effects on S₁₈₀ enhanced, the weight of sarcoma decreased and the foci shranked.

On the basis of the dose-effect research, high dose of FRC001 (6.0ml/kg.bw) was chosen to test and its effects on the body weight, carcinoma weight of experimental animal of hepatic carcinoma and the inhibition rate were investigated (showed in Table 2 and Figure 3,4) .

According to Table 2, these drugs had some inhibititon effects on hepatic carcinoma foci of experimental animals which showed in the decrease of carcinoma weights. The average carcinoma weights of drug I - VI were 1.41g,1.09g,1.26g,1.49g,1.02g,0.71g,respectively, decreased 0.65g,0.97g,0.80g,0.59g,1.04g,1.35g,respectively compared with control group. Compared to the control group, the effects of drug I , IV were not significant statistically (P>0.05) ,whereas drug II,III,V,VI had significant effects (P<0.01 or P<0.001) .

Figure 3 showed that the carcinoma weight of control group was the biggest, the carcinoma weights of drug I -VI decreased to some extent, but there were carcinoma foci in all drug groups. The orders of carcinoma weights of all experimental groups:Control>drug IV>drug I >drug III>drug II >drug V >drug VI.

Figure 4 showed these drugs had some inhibition effects on carcinoma foci. According to the anti-neoplastic screening procedures and standards that the inhibition rate must be up to 30%,except for drug IV (27.67%) ,all drugs had good inhibition effects. The orders of inhibition rates of drug I -VI:drug VI >drug V >drug II >drug III>drug I >drug IV. Drug VI is 5-Fu which is one of the chemotherapeutic drugs having good antieoplastic effect. Drug V is a new complex preparation of Chinese and Western drugs which is being screened by author. Drug II is a marketing antineoplastic, and its effect is weaker than drug VI and V.

These results showed that, as a antineoplastic, FRC001 had some effects on sarcoma S₁₈₀ and hepatocarcinoma. The mechanisms may be:①FRC001 is a complex preparation of Chinese and Western drugs, can promote blood circulation to remove stasis, clear away heat and toxic material, soften and resolve hard mass, invigorate Qi, enrich the blood, warm Yang and nourish Yin, so it can help body to eliminate the pathogenic factor and support healthy energy, clear away toxic material and resist tumors;②The components in Edfrann maybe have the function of invigorating QI, spleen, resolving dampness, soothing the liver and regulating the circulation of Qi, digestant, regulating absorption, eliminating disturbance of substance metabolism, enhancing circulation, hemopoiesis, immunity, and can regulate the function of nerve, endocrine, electrolyte, cyclic nucleoside, etc;③FRC001 can inhibit the growth of tumor by regulating immunologic function, enhancing the phagocytic function of reticuloendothelial system; ④ FRC001 maybe has the function of inhibiting the metabolism of RNA and DNA of carcinoma cell so as to kill the carcinoma cell or inhibit the growth of carcinoma cell;⑤FRC001 maybe has the function of eliminating free radicals, as the free radicals play an important role in mutagenesis and carcinogenesis. FRC001 contains macromolecule and micromolecule radical scavenge. Of course, the growth, development or inhibition is a complicated biologic course, the effects and accurate mechanism of FRC001 on tumor are awaited to further study.

REFERENCES

- [1] Han Rui, The way and Tactics of Antineoplastic Screening,Cancer 1990,9 (3) :229-234
- [2] Wang Yixian, et al, The Study on Antitumor Function of Receptaculum FiCi Caricae, Cancer 1990,9 (3) :223-225
- [3] Liu Yunying, The Progress of Clinical Pharmacology and Antineoplastic, Cancer, 1989,8 (2) :117-119
- [4] Li Xuetang, Clinical Trial of Anticarcinogen, Cancer 1989,8 (2) :116-117

- [5] Huang Qiang, et al. The Suggestion for the Screening routine of Anticarcinogen Using H-tumor Mouse, Cancer, 1991,10 (3) :178-189
- [6] Sun Zhiyong, Active Oxygen and Carcinogenesis, Abroad medicine (Tumor) 1990,19 (2) :65
- [7] Han Zhihong, Liu Xiaokang, Wu Yongfang, The Effects of green Tea Extracts TP-91 on S180 of Mouse and the Activity of SOD in RBC, Free Radicals Bioscience Progress, 1995,3 (3) :182
- [8] Zhu Jian-hong , et al. preclinal trials with monoclonal antibody immunoconjugate for the tragecing chemotherapy of human brain glioma. 9th Int. congress of neurological surgery october, 1989.new Delhi-India. Resident's Award Papers.No. 8000001
- [9] Rayner AA. et al. Lymphokine-Activated killer (LAK) cells Analysis of factors relefv ant to the immunotherapy of human cancer. cancer, 1989;55:1327
- [10] Charles K, Grieshaber et al. Relation of preclinical Toxicology to findings in Early Clinical Trials. Cancer Treat Rep. 1986;70:65-72
- [11] Jerry M. Collins et al. Potential Rgoles for Preclinical Pharmacology in phase I Clinical Trials. Cancer Treat Rep. 1986;70:73-80
- [12] Prentiss AM. Chemical in War: A Treatise on Chemical warfare. New York. Mc Graw-Hill,1973.
- [13] Greene RF et al. Plasma Pharmacokinetics of Adriamycin and Adriamycinol:Implications for the Design of in Vitro Experiments and Treatment protocols. Cancer Res. 1983;43:3417-3421
- [14] Goldsmith MA et al. Quantitative Prediction of drug toxicity inhuman from toxicology in small and large animals cancer Res. 1975;35:1354-1364
- [15] Skipper HE et al. Some thoughts regarding the modes of action of drugs on cell and on application of available Pharmacokinetic data (anticancer drugs) .Booklet. 199:10.P 5-9.Southern Research Institute, Birming-ham.
- [16] Zhang Zhe, Bian Lijun, Fu Hongjie, et al, The Activity of GST and γ -GT in Sera and Tissure of Experimetnal Hepatic Carcinoma Rats, Cancer, 1989,8 (1) :17-19
- [17] Xie Bingfen, Pan Qichao, Cheng Haiying, et al. The Relation of Methionine with the Growth of Transplatation Carcinoma of Mouse and the Action of Anticarcinogen, Cancer, 1989,8 (6) :438-441
- [18] Zhu Yingeng, et al. The pathologic Effects of Pretreatment of Hepatocarcinoma Vaccine on Transplatation Hepatocarcinoma of Mouse, Tumor Protective and Treatment Research, 1984,11 (1) :1
- [19] Wang Daqing, et al. The Effects of Active Immunity of Carcinoma Vaccine on the Growth and Metastasis of Ascite Hepatocarcinoma of Mouse, Journal of Zunyi Medical College, 1987, (1) :10
- [20] Yan Ruiqi, et al. The Inhibition of Green Tea on Hepatocarcinoma of Rat Induced by Aflatoxin B1, Cancer 1987, 6 (2) :83
- [21] Setsuo Tabeuchi et al. Benzaldehyde as a Carcinostatic Principle Figs. Agric. Biol Chem, 1978;42 (7) :1449.
- [22] Marks PA et al. Report from Memorial Sloan-Kettering Cancer center, 1986;184.
- [23] Lapis Kand Eckhardt S (eds) .Lectrues and Symposia 14TH Int Cancer Congr Budapest, 1986;Vol.1
- [24] Xu Bin et al. In " First Beijing Int. Symposium on New Trends of Cancer Chemotherapy" . 1986:78.
- [25] Beckman WC, et al. Differential retention of rhodmaine 123 by avian Sarcoma virusinduced glioma and normal brain tissue of the rat in vivo. Cancer, 1987;59:266-270.
- [26] Chang WI, Chen CF. Cytotoxic activity of tanshinone aganst human carcinoma cell lines. Am J Chin Med, 1991,19:207
- [27] Van Dierendonck JH, Cornelisse CJ, Willem PG, et al Characterization of a slow-growing, trans-plantable rat mammary tumor (MCR-83) : a model for endocrine-related cell kinetic studies.

- Cancer Res, 1987,47-4093.
- [28] Connolly KM, Bogdanffy MS. Evaluation of proliferation cell nuclear antigen (PCNA) as an endogenous marker of cell proliferation in rat liver: a dual stain comparison with 5-bromo-2'-deoxyuridine. J Histochem Cytochem, 1993,41:1
- [29] Teodori L, Trinca ML, Goghde W, et al. Cytokinetic investigation of lung tumors using the antibromo-deoxyuridine (BudR) monoclonal antibody method:comparison with DNA flow cytometric data. Int J Cancer, 1990,45:995
- [30] Bravo R, Frank R, Blundell PA, et al. Cyclin/PCNA is the auxiliary protein of DNA polymerase δ . Nature, 1987,326:515
- [31] Del Bino G. Morphological and clinical significance of cell kinetics in non-Hodgkins lymphomas. Basic Appl Histochem, 1986,30:197
- [32] Campling BG, Pym J, Baker HM, et al. Chemosensitivity testing of small cell lung cancer using MTT assay. Br J Cancer, 1991,63:75
- [33] Dosik Gm, Barlogid B, Smith TL, et al. Pretreatment flow cytometry of DNA content in adult acute leukemia, Blood, 1980,55:474
- [34] Racker DK, Hoffman RE, Forther JG. Transmission electron microscopy:methods of application. Illinois: Charles C Thomas Publisher, 1983:14
- [35] Kaufman S. Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other anticancer drugs: a cautionary note cancer Res, 1989,49:5870
- [36] Barry NA, Behnke CA, Eastman A, et al. Activation of Programmed cell death (apoptosis) by cis-platin, other anticancer drugs, toxins and hyperthermia. Biochem Pharmac, 1990,40:2353
- [37] Freund J. The mode of action of immunologic adjuvants Adv Tuberc Res, 1956,7:130