中國1號天仙液 對小鼠非特異性免疫功能影響的實驗研究

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The Experimental Study on the Effect of FRC001 （ China No.1 Tian Xian Liquid ） on Nonspecific Immunity of Mice

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ABSTRACT

This paper investigated the effect of FRC001 on nonspecific immunity of mice and graft versus host reaction (GVHR).The results shows that FRC001 had some effect on phagocytic function of mononuclear macrophage,spleen index and thymus index, splenic lymphocyte transformation, serum hemolysin formation, host spleen reaction coefficient and stimulate confficient and stimulus index i.e FRC001 can increase and enchance humoral immunity and cellular immunity of experimental basis for the use of FRC001 in regulating immunity and preventing and curing tumor.
Key Word FRC001 Nonspecific immunity graft versus host reaction(GVHR)

BACKGROUND

Tumor formation is related to immunologic inadequacy.un recentt year ,there are many report on immunologic inadequacy index which was regarded as an important indrx in the screening of antineoplastic The study investigated the effect of FRC001 on nonspecific immunity of mice and GVHR.

MATERIALS AND METHODS

1 Materials

1.1 Subject:FRC001,provided by China-Japan Feida Union Co., LTD.

1.2 Experimental Animal: Mouse,Kunming species,18~22g, half male and half female ,provided by Experimental Animal Center of Jiangsu Tumor Prophtlactico-therapeutic Research Institute.

2 Method:

2.1 Phagocytic function of mononuclear macrophage(mouse carbon clearance test)

2 1.1 Experimental principle:

Experimental mouse was injected with Indian ink which was used asgranular foreign body.The Indian ink was phagocytized and cleared by mononuclear macrophage after it went into circulation.Ninety percent of it was phagocytized by liver Kupffer cell while the rest was

phagocytized by spleen macrophage.The clearance rate of granular foreign body in ciculation reflected the phagocytic function of mononuclear macrophage. Within given scope,the clearance rate of granular foreign body had exponential function relation with granular dose,i.e. phagocytosis rate had direct propotion relation with granular concentration in circulation.They have linear relation in system of coordinates with the time as the abscissa and granular concertration as the vertical ordinate The slope K of the line is the phagocytosis rate(or clearance rate).

2.1.2Manipulation

Mice were randomly divided into negative control group,postive control grope and different dose of FRC001 groups.They were fed with distilles water,elemene milk and different dose of FRC001 for succussive 10 days.Each mouse was injected Indian ink(diluted to 1~5 times)0 05ml/1/0g bw from mouse tail vein in 30 minutes after the last feeding.In the first minute(t1)and 5th minute(t5) after injection,20 μ l blood was drawn from postobital vein with suction tube in which was moistened with heparin solution and diluted in 2ml 0.1% sodium carbonate solution .Then absorbance (A) of blood solution was assayed at 680nm .To calculate the value of K by following fomular

(lgA1-lgA5)/ (t5-t1)=(lgA1/A5)/ 4

2.2GVHR test

Two pure line mice ♂ C57BL ♀ JCR hybridized to give birth to newborn mouse.Beginning at the first day of the birth of the newborn mouse, experimental male parent were fed with FRC001 for 10 days while control male parent were not given any treatment.On the 11th day after the birth of newborn mouse,male parent mice were killed by dislocation to take out spleens.Spleen cell suspension (1 × 10s/ml) was prepared by aseptic technique.The newborn mice were divided into experimental group,control group and normal group .The experimental newborn mice were inoculated 0 1ml spleen cell suspention of experimental male parent,the control newborn mice were jnoculated0 1ml spleen cell suspension of control male parent,and the normal group were not given any treatment .All these newborn mice were killed at seventh day after inoculation,and their spleens were weighed to calculate spleen coefficient (mg spleen/10g.bw).Then calculation spleen stmulus index (SI) by following formula ·

SI=(Average Spleen Coefficient of Experimental or Control)/ Average Spleen Coefficient of Normal Group

. 2.3 Spleen index and thymus index test

Kunming species mice (18~22g) were randomly divided into negative control group (fed with N>S?) positive control group and FRC001 group.They were fed with resepective subject for successive 10 days,and killed by orbital bleeding on the second day after the last feeding.Their spleen and thymuses were stripped out and weighed accurately with torsion balance .The results

showed with organ index-weight of spleen or thymus(mg)/10g.bw

2.4 Mouse lymphocyte transformation test-co;orimetry of mouse splenic MTT

Pure species kunming mouse was killed by orbital bleeding and its spleen was stripped out to make 5 × 10(8)/ml cell suspension with Hank's solution and RPM 1640 medium cell suspension 100 μl was added to pore of culture dish,and every pore was added PHA 100 μl and experimental drugs,in the meantime ,control pore was set up.They were cultured in 37~38 ℃.5% CO2 incubator for 72hrs.Every pore was added 50 μl MTT 4~5hrs before the ending of culture,then continue to culture 24h,36h,48h,respectively.They were put into 4 ℃ refrigerator for one night. Supernate 150 μl was sucked, added 150 μl acid isopropanol and blown to homogeneous solution. Absorbance (A) of every tube was assayed at 630nm of ELISA photometer.To see detail in reference[10].

2 5 Serum hemolysin test

1 Mice (Kunming species,18~22g) were randomly divided into nonimmunologic group,immunologic group immunology and drug(low,middle and high dose) group.Every mouse of the last two groups was injected intropert itoneally 0.2ml sheep red blood cell(SRBC)which diluted to 3:5(V/V) with N.S Mice of drug group were given drug before or after immunization. Four days after immuization. 1 ml blood was taken to get serum. To see the detail in reference[8].

Result and Discussion

1. The effect of FRC001 on phagocytic function of mononuclearmacrophage in mice (Figure 1)

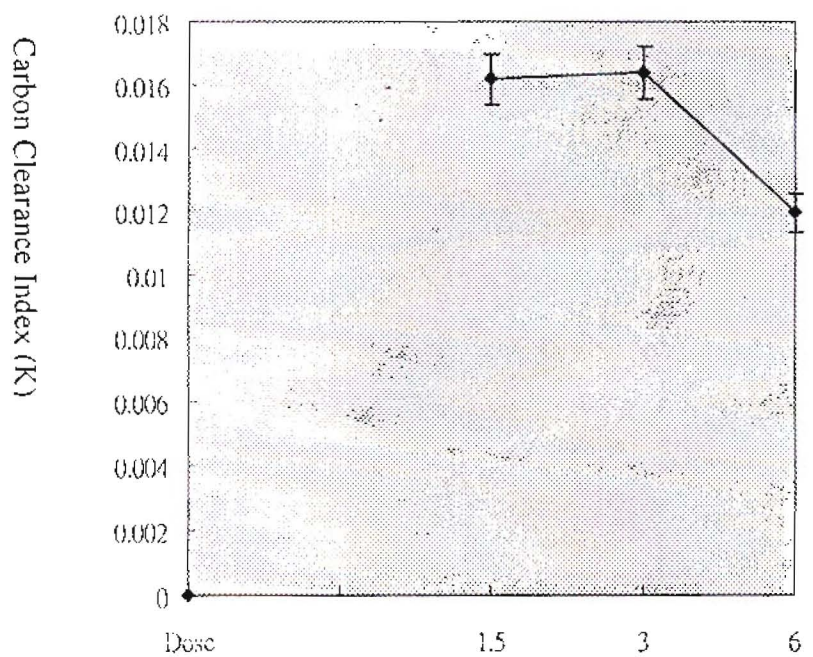


Figure 1 The effect curve of FRC001 on Phagocytic Function of Mononuclearmacrophage in

The increase of carbon clearance index (K) reflects the enhancement of phagocytic function of mononuclearmacrophage and nonspecific immunity.According to Figure 1, low,middle and high dose of FRC001 increased K value significantly($p<0.01$).The result showed FRC001 can enhance the phagocytic function of mononuclearmacrophage and nonspecific immunity.

2 The effect of FRC001 on spleen index and thymus index (Table 1)

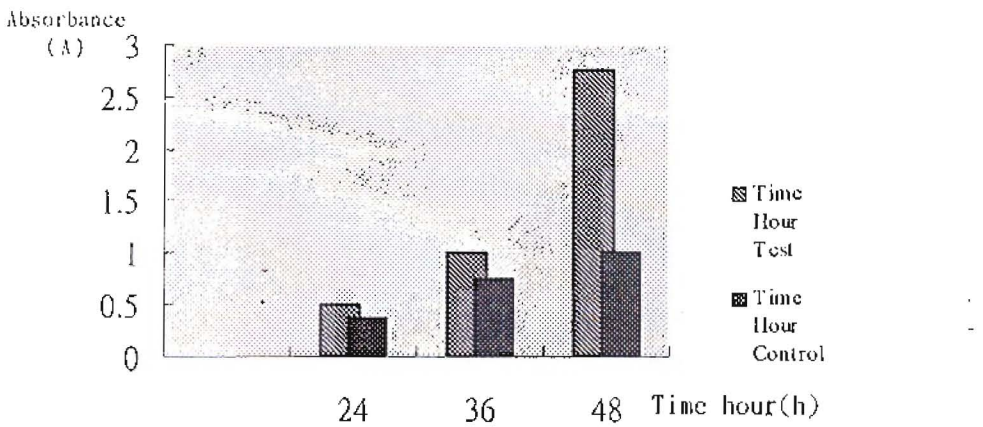
Table 1 The effect of FRC001 on spleen index (SI)and thymus index(TI)

Group	Number (n)	Dose	X±SD(mg/10g.bw)	
			SI	TI
Control	10	0	69.59±25.59	26.23±9.01
FRC001	10	6.0 × 10(mg/kg × d)	64.92±35.91 *	27.03±13.03 *
Elemene Milk	10	100 × 10(mg/kg × d)	78.26±22.82 *	16.15±2.60 * * *

Compared with control, * P>0.05; * * P<0.05; * * * P<0.01

Spleen and thymus are important immunologic organs.The degeneration and atrophy of them will influence their normal function .According to Table 1 ,FRC001 had no significant effect on SI and TI ($P>0.05$) The result showed Edfrann had no evident influence on immunologic organs. The authors thought it may be related to the shot time.

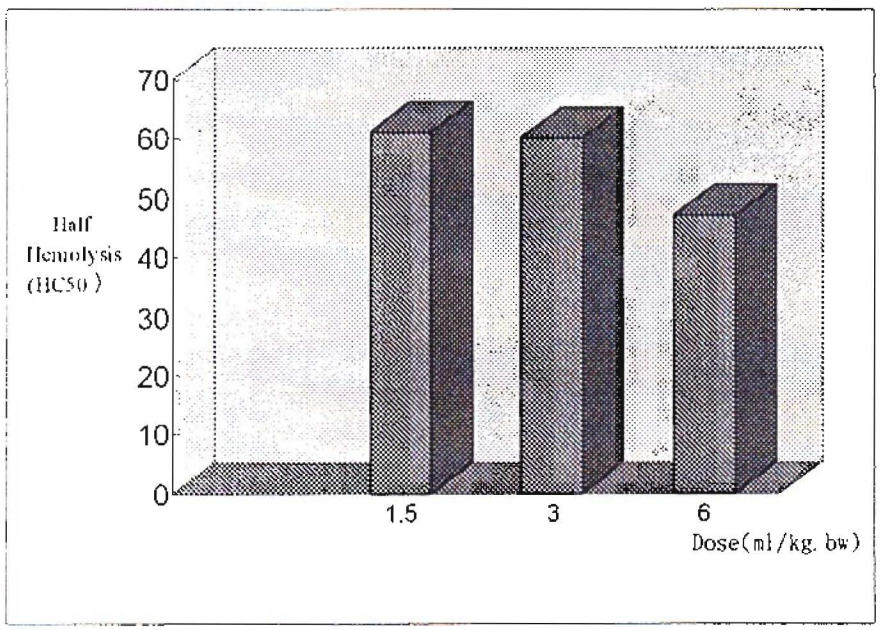
3.The effect of FRC001 on splenic lyphocyte transformation in mouse(Figure 2)



(Figure 2) The effect of FRC001 on splenic lyphocyte transformation in mouse

Splenic lymphocyte transformation reflects cellular immunologic function.From Figure 2, FRC001 had evident influence on splenic lymphocyte transformation. Splenic lymphocyte transformation went up as the increasing of time.The increasing amplitude between 26h and 48h was the biggest Compared with control,splenic lymphocyte transformation at 24h,36h,and 48h increased significantly($P<0.01$).The result showed FRC001 can enhance cellular immunologic function.

4.The effect of FRC001 on serum hemolysin (Figure 3)



(Figure 3) The effect Curve of FRC001 on serum hemolysin Formation.

Hemolysin(IgM) reflects humoral immunity.Increasing of absorbance at the time half hemolysin takes place(HC50) shows the increasing of hemolysin and enhancement of humoral immunity.According to Figure 3,HC50 went up gradually as the increasing of FRC001.The difference between experiment group and control group was significant (P<0.01).The result showed Edfrann had enhance humoral immunity.

5.The effect of FRC001 on GVHR in mice (Table 2)

(Table 2) The effect of FRC001 on GVHR in mice

Group	Number	Administration way	Dose (mg/kg × d)	Host Reaction Index(mg spleen/g.w)	Stimulu Index (SI)
Normal	8	ig	0	7.76±1.14***	-
Control	8	ig	6.0 × 10	9.82±1.36**	1.22
FRC001	8	ig	6.0 × 10	12.96±1.88	1 96

Compared with FRC001 group ,*P>0.05; **P<0.05; ***P<0.01

GVHR reflects cellular immunologic function by host reaction index and stimulus index. According to Table e, host reacyion index and stimulus index of FRC001 group was obviously higher than normal group(without any treatment) and control group (fed with distilled water only)(P<0.05 or P<0.01).The result shows Edfrann can increase and enhance cellular immunologic function.

All of these results shows FRC001 had some influence on nonspecific immunity of experimental mice.The regulation function of FRC001 to immunity may be related to its componets.It is reported that Chinese drugs with the function of invigprating Qi,b/ood and warming Yang,etc contain many bioactive components which can enhance the function of reticuloendothelial system,antibody formation,specific and nonspecific immunity.In addition,free radical scavenges in Chinese drugs have cooperative function in protecting immulogic organs,regulating immunity,enhancing immunologic factors,etc. FRC001 contains components which can invigorate Qi and blood,warm blood and scavenge free radicals .then the componens which play roles in regulating immunity and their action intensity,best bioeffect are waiting for future study.

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