

Anticancer Effect of PS-T on the Experimental Hepatocellular Carcinoma

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Received 31 October 2003

Abstract Objective: To apply PS-T in different phases of carcinoma formation and development, and research the mechanism of anti-carcinoma of PS-T in the cytological level. **Methods:** N-nitrosodiethylamine (DENa) and CCl₄ were applied jointly to duplicate the rat hepatocirrhosis and hepatic cancer model. The rats were divided into 7 groups and were administrated via nasal-stomach tube with PS-T in different phases to interfere the cancer genesis and development. All the rats were killed in 20 weeks for pathological observation. **Results:** The loss of body weight of rats slowed down in the PS-T-treated group, and the carcinogenesis rate was significantly decreased correspondingly. PS-T could also inhibit the carcinogenesis by supressing the hepatocirrhosis, which showed the positive correlation between the curative effect and the curative period. **Conclusion:** Application of PS-T during cancer induction showed a significant effect on preventing and supressing cancer. PS-T might be an ideal drug for clinical anti-cancer therapy. And it will be a main drug in both combined and single treatments for tumor.

Key words: PS-T; hepatocellular carcinoma model; hepatocirrhosis; pathological change

Hepatocellular carcinoma (HCC) threatens the human health. 260 000 cases of HCC arises every year, and among them, 42.5% occur in China^[1]. The incidence of the disease is still rising in China and the world. The research of the prevention and treatment of HCC is very popular these years. As a new generation of anticarcinoma medicine, PS-T, national first class new medicine, has been applied clinically to treat many kinds of carcinoma with significant effect^[2]. The research of anticarcinoma mechanism of PS-T is still limited in clinical observation and immunological determination. The histological and cytological description of PS-T treatment hasn't been found out so far. In this project, N-nitrosodiethylamine (DENa) and CCl₄ are applied jointly to duplicate the HCC model of rats to interfere with the carcinogenesis by applying PS-T, and to observe the effect of preventing carcinogenesis and anticarcinoma in the cytological level pathologically.

Materials and methods

Materials

78 normal male SD rats (offered by experimental animal centre, Nantong Medical College), weighting 100-120 g were selected. The rats whose liver function determined

before experiment were abnormal were excluded in this study. Cancer induction reagent, DENa (No. 0756, Sigma Co. USA); PS-T with main component being extract of Huaier hypha contained many kinds of organic matter and minerals and was s a kind of brown or yellow-brown granules with fishy, sweet and slightly bitter (BA24, offered by Gaitianli Pharmacy Co., Qidong, China); Carbon tetrachloride (Shanghai chemical reagents stock and accomodate centre, lot number: 20011018), and Sabaro olive oil (product of Spain).

The duplication of rat HCC model and the grouping

SD rats were raised in common animal laboratory, 3 per cage. The rats were fed by common feed and drinking water freely. The enviroment temperature was 20-25 °C, with humidity 50%-60%. The rats were divided into 7 groups randomly: (1) control group, 10 rats, administrated via nasal stomach tube with equal volumn of natural saline, once a week, for 15 weeks continuously^[3]; (2) carcinoma induction group, 10 rats, administrated via nasal stomach tube with DENa, 70 mg/kg, once a week, for 15 weeks continuously; (3) carcinoma induction and hepatocirrhosis group, 12 rats with the same carcinoma inducing method as in group 2, injected on the abdomen with CCl₄ twice a week, 0.30 mL/100 g once, 75% CCl₄-olive oil for the first time, and 50% for the rest times, for 15 weeks continuously; (4) carcinoma induction and PS-T-

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treated group, 10 rats, with the same carcinoma inducing method as in group 2, administrated via nasal stomach tube with PS-T twice a week, 2 mL/rat once (original liquid diluted for 1:1 with ddH₂O), for 15 weeks continuously; (5) carcinoma induction and PS-T-treated for 9 weeks group, 12 rats with the same carcinoma inducing method as in group 2, application of PS-T after 9 weeks, with the same method and dose as in group 4; (6) carcinoma induction, hepatocirrhosis and PS-T-treated group, 12 rats, with the same methods of carcinoma induction and hepatocirrhosis induction as in group 3. The application of PS-T was the same as in group 4; (7) carcinoma induction, hepatocirrhosis and PS-T-treated for 9 weeks group, 12 rats, with the same methods of carcinoma induction and hepatocirrhosis induction as in group 3, application of PS-T after 9 weeks, with the same method and dose as in group 4.

Routine pathological examination

All the rats were killed in 20 weeks to measure body weights and the liver weights, and sample the livers for general observation. The samples were fixed in 10% neutral formalin, paraffin embedded, sliced up, HE stained and observed under microscopy.

Statistical analysis

ANOVA and ranksum test (stata 6.0).

This experiment was done twice repeatedly on the basis of pre-experiment to ensure the validity of the results.

Results

Seven rats died during the experiment (2 rats in group 2, 3 and 6 respectively, 1 in group 7). The main reasons for death were oesophagitis and/or thorax and celiac infection caused by perforation because of pouring reagent

Table 1 The comparison of body weight and liver weight in 20 weeks

Groups	Cases	Body weight ($\bar{x}\pm s$)*	Liver weight ($\bar{x}\pm s$)**
1	10	454.6±18.7	16.8±1.0
2	8	283.3±73.2	14.3±5.6
3	10	155.8±68.9	14.6±7.4
4	10	382.9±25.7	13.6±1.7
5	12	338.4±81.2	15.4±4.1
6	10	359.5±38.1	15.6±2.3
7	11	312.0±85.1	13.8±4.7

* $F_1=12.14$, $P<0.001$; ** $F_2=0.48$, $P>0.05$

There was significant difference in the body weights among the 7 groups through ANOVA ($P<0.001$). The inter-group comparison showed there was significant difference between group 1 and 2, 3, 7 ($P<0.01$, $P<0.01$ and $P<0.05$ respectively), group 3 and 4, 5, 6, 7 (all the $P<0.001$). No significant differences in liver weights were found among the 7 groups ($P>0.05$)

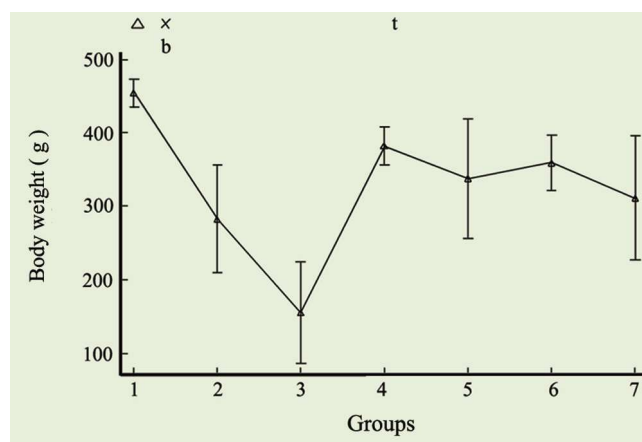


Fig. 1 The comparison of body weight in week 20

into the stomach. The total mortality rate of this group was 8.97% (7/78).

The comparison of body weight and liver weight in 20 weeks

The results were shown in Table 1 and Fig. 1.

The pathological observation of liver tissues

In group 1 (control group), the structure of liver tissue was normal, the hepatic cells were mono-nucleus, the hepatic plates were radiated from the central vein, and no degeneration and necrosis was found.

In group 2 (carcinoma induction group), the livers were coarse and rigid. Hyperplastic hepatic cell nodi, which had inequable volumn, were seen in the sections. Most of the cells were transparent having big nuclei, significant nucleoli or multiple nucleoli. Mitosis could be seen easily. There were big and rough granules in the cell plasma. The typical carcinoma nodi were found in most cases (Fig. 2).

In group 3 (carcinoma induction and hepatocirrhosis group), except the features in group 2, the nodi on the liver surface were distributed diffusely. The volumn of the nodus was from rice-like to bean-like. Under the microscopy, hepatic fibrosis was significant. The hyperplastic nodi were divided discordantly. In the centre of the nodi, there were atypical hyperplastic cells. Mitosis were plenty. Many carcinoma nodi were seen (Fig. 3), forming transparent cell type, small grider type, pseudo-glandular type, bile duct cell with small focus of necrosis type.

In group 4 (carcinoma induction and PS-T-treated group), degeneration at different levels with active cell hyperplasia were seen. The hyperplastic hepatic cells had big, lightly stained nucleoli. The chromatin were distributed around the nucleus membrane. Expansile growth nodi oppressing the surrounding were seen casually.

In group 5 (carcinoma induction and PS-T-treated for 9 weeks group), hyperplastic nodi with active mitosis were seen, and most of the cells were transparent. There were hyperplasia of olivary cells around the lobule. The olivary cells were small, lack of plasma, and the nuclei were empty and lightly stained. Some "transitional cells" and olivary

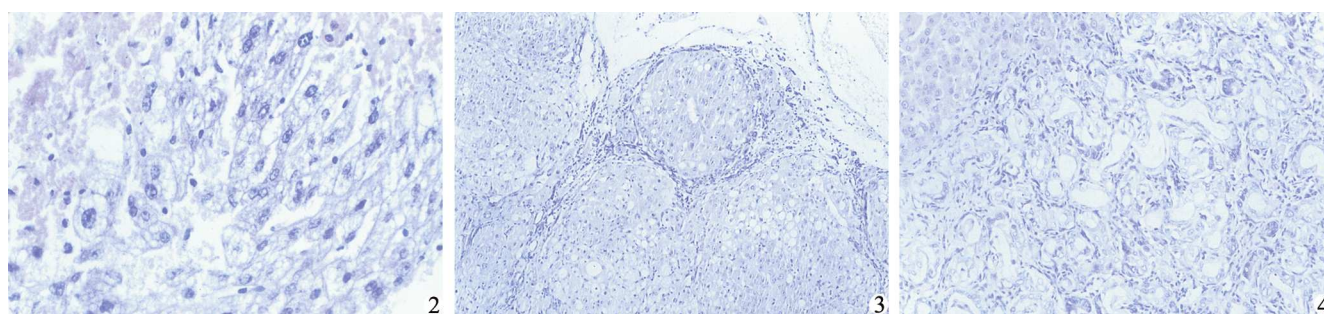


Fig. 2 The typical carcinoma nodi with necrosis were found in group 2 (HE stain×500)

Fig. 3 Many carcinoma nodi with fibrosis were found in group 3 (HE stain×125)

Fig. 4 The transparent cells (olivary cells) around the lobule were found in group 5 (HE stain×250)

Table 2 The comparison of atypical hyperplasia and carcinoma in different groups

Groups	Cases	Normal	Atypical hyperplasia	Carcinoma(%)
1	10	10	0	0 (0.0)
2	8	0	3	5 (62.5)
3	10	0	0	10 (100.0)
4	10	6	3	1 (10.0)
5	12	0	7	5 (41.67)
6	10	4	2	4 (40.0)
7	11	0	2	9 (81.82)
Total	71	10	18	33 (46.48)

χ^2 (Kruskal-Wallis Test)=36.221 with 6 d. f. $P=0.0001$

Note: The most serious pathological changes were listed above. For example, if there was carcinoma, the other pathological changes were neglected.

From table 2, there were significant differences in the carcinogenesis rate among different groups ($\chi^2=36.221$, $P=0.0001$)

cells that had hepatic cell differentiation (big, plenty of plasma and big nucleoli) could be seen. The hepatic cell hyperplastic areas gathered many olivary cells, forming the small bile duct and carcinoma (Fig. 4).

In group 6 (carcinoma induction, hepatocirrhosis and PS-T treated group), the livers were rough generally. The number of “transitional cells” was increased and the light hepatocirrhosis was seen.

In group 7 (carcinoma induction, hepatocirrhosis and PS-T-treated for 9 weeks group), the features were similar to those in group 3.

The comparison of atypical hyperplasia and carcinoma

The results were shown in Table 2 and Table 3.

Rank correlation analysis

The rank correlation analysis was performed to compare the PS-T’s curative effect among different groups. There was significant difference in carcinogenesis rates

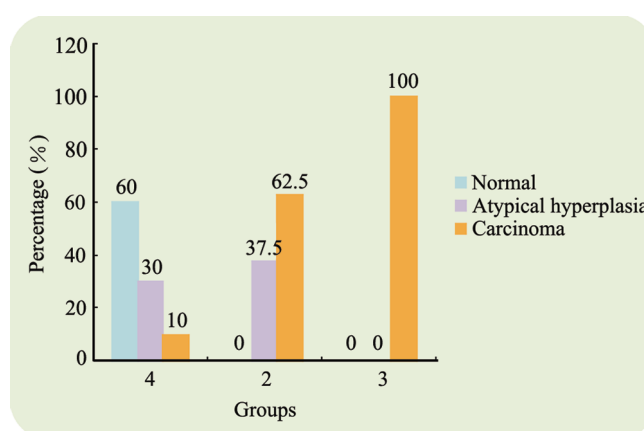


Fig. 5 Rank correlation analysis the carcinogenesis rates of group 4, 2, 3

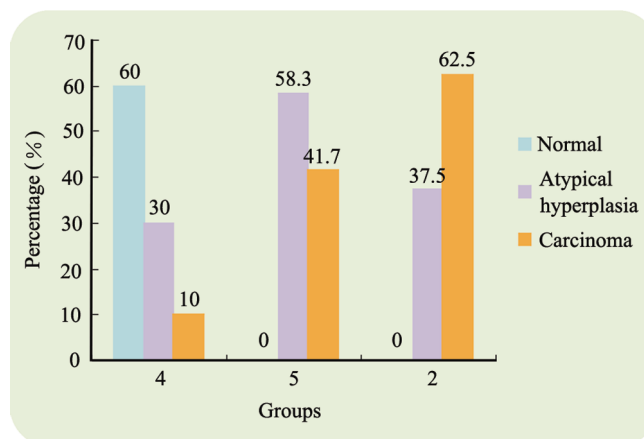


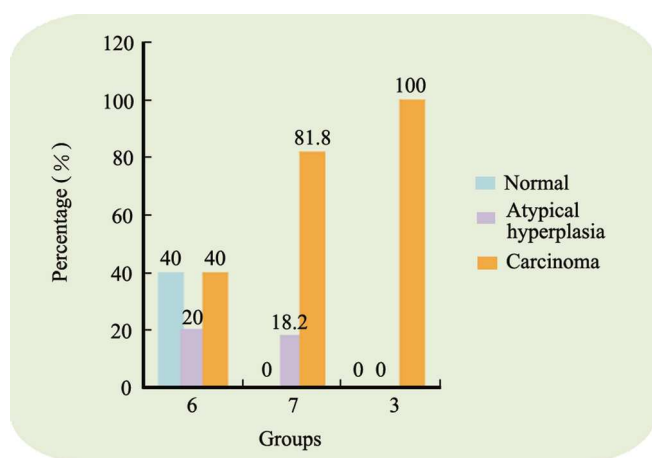
Fig. 6 Rank correlation analysis the carcinogenesis rates of group 4, 5, 2

among groups 4, 2, 3 ($P=0.000$), and the curative effect of PS-T had significantly negative correlation with the carcinogenesis rates (Cramer’s $V=0.7121$, Fig. 5). Histologically, there were the gradual changes from normal to atypical hyperplasia to carcinoma. There was significant difference in carcinogenesis rates among group 4, 5, 2 ($P=0.004$), and the curative effect of PS-T had significant correlation with the carcinogenesis rates (Cramer’s

Table 3 Comparison between groups (Nemenyi test)

Groups	1	2	3	4	5	6
2	34.4* ($P<0.05$)					
3	44.0* ($P<0.05$)	9.6 ($P>0.05$)				
4	10.0 ($P>0.05$)	24.4 ($P>0.05$)	34.0* ($P<0.05$)			
5	29.1* ($P<0.05$)	5.3 ($P>0.05$)	14.9 ($P>0.05$)	19.1 ($P>0.05$)		
6	21.3 ($P>0.05$)	13.1 ($P>0.05$)	22.7 ($P>0.05$)	11.3 ($P>0.05$)	7.8 ($P>0.05$)	
7	39.4* ($P<0.05$)	5.0 ($P>0.05$)	4.6 ($P>0.05$)	29.4* ($P<0.05$)	10.3 ($P>0.05$)	18.1 ($P>0.05$)

* $P<0.05$. From table 3, there were significant differences between group 3 and 4 ($P<0.05$), as well as group 4 and 7 ($P<0.05$). Group 4 and group 6 had no significant differences with the control group (group 1) ($P>0.05$). All the rest groups had significant differences with the control group ($P<0.05$)

**Fig. 7** Rank correlation analysis the carcinogenesis rates of group 6, 7, 3

$V=0.5256$, Fig. 6), suggesting that the curative time of PS-T had positive correlation with the curative effect. There was significant difference in carcinogenesis rates among group 6, 7, 3 ($P=0.006$), and the curative effect of PS-T had significant correlation with the carcinogenesis rates (Cramer's $V=0.4557$, Fig. 7). PS-T had the effect of inhibiting hepatocirrhosis, while the HCC increased with the development of hepatocirrhosis. The effect had positive correlation with the curative time. It suggested that PS-T could interfere carcinogenesis by inhibiting hepatocirrhosis.

Discussion

The effect of PS-T is to supply the healthy energy and to promote blood circulation to the removed blood stasis. It could be applied to the cases of primary liver cancer (that were inoperable) as an assisting medicine as it relieved such symptoms like pain in hepatic region, abdominal distension and fatigue. The extracted PS-T had a good curative effect in the treatment of primary HCC^[4]. Recent research confirmed that PS-T had an apoptosis inducing effect to A-549, a lung adenocarcinoma cell line,

and a direct killing effect on tumor cells. And it had significant anti-cancer effect to mouse S-180 solid and ascites type cell line. In this experiment, the histological changes were observed pathologically when using PS-T to prevent and treat the HCC. The loss of body weight slowed down when the patients were treated with PS-T. There was no significant difference in the body weight changes in PS-T-treated groups and control group (except group 7). In group 3, which had carcinoma induction and hepatocirrhosis at the same time, there was significant body weight loss with the difference being significant among the PS-T treated groups (group 4, 5, 6, 7).

Histopathologically, PS-T could significantly prevent and antagonize carcinoma. The carcinogenesis rate in the PS-T treated group (group 4) was only 10%, showing no significant difference with the control group. And there was significant difference in the carcinogenesis rates between group 2 (62.5%) and 3 (100%). Even treated with PS-T for 9 weeks when the cancer had been formed, PS-T still showed the effect of inhibiting the carcinoma histologically (the carcinogenesis rate in group 5 was 41.67%), and the malignant change was lighter than that in no treated PS-T group (group 2). It suggested that the carcinogenesis rate of PS-T treated groups was significantly lower than that of carcinoma induction groups, showing the gradual changes from normal to atypical hyperplasia to carcinoma histologically, in other words, from the PS-T treated group to the carcinoma induction group. On the other hand, it showed that the curative effect of PS-T had a significantly positive correlation with the curative time when PS-T was used to interfere the carcinogenesis process.

It is well-known that HCC had great relationship with hepatocirrhosis. Hepatocirrhosis could promote carcinogenesis. The incidence of HCC combined with hepatocirrhosis was 50%–85%^[5]. In the experiment, the results showed that PS-T not only had the effect of prevention and anti-cancer, but also could prevent and inhibit the forming of hepatocirrhosis. The carcinogenesis rate in the PS-T treated group (group 6) was significantly lower than that of carcinoma induction group (group 2) and carcinoma induction and hepatocirrhosis group (group 3). The rank correlation analysis revealed that PS-T had stronger effects on inhibiting hepatocirrhosis when comparing the

effect of anticarcinoma of PS-T in different phases of carcinoma induction and hepatocirrhosis forming process. The effect had significantly positive correlation with the curative time. It suggested that PS-T could also interfere with the forming of carcinoma by preventing hepatocirrhosis.

All together, using PS-T in the early stage of duplicating rat HCC model showed a significant effect of preventing carcinoma and alleviating pathological change. PS-T also had a significant effect on anticarcinoma in the forming and developing of carcinoma, and could interfere with the forming of carcinoma by inhibiting hepatocirrhosis, too. The curative effect had positive correlation with curative time. The observation of the effect on PS-T treated rat HCC indicated that PS-T had an exact anti-cancer effect. It suggested that PS-T work by the activating of immune system in the internal environment. Meanwhile, the main component of the PS-T was polysaccharide which was the main prosthetic group of nucleic acid and protein. It could adjust the synthesis of DNA and protein, and worked on the second messenger system (Ca^{2+} , protein kinase, etc.) as a "environmental signal", so that the cytotoxicity could be produced to kill or restrain tumor

cells. It was an ideal drug of clinical anti-cancer therapy. And it would be a main drug in both combined and single treatments for tumor.

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以hTERT基因反义核酸抑制端粒酶活性增加白血病细胞对CDDP诱导凋亡的敏感性

Inhibition of Telomerase with hTERT Antisense Increases Susceptibility of Leukemic Cells to CDDP-induced Apoptosis

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目的 以hTERT反义核酸抑制白血病细胞(HL-60和K562)端粒酶活性,研究CDDP诱导凋亡敏感性的变化。

方法 全硫代反义核酸由上海生物化学研究所合成和纯化;端粒酶活性用试剂盒测定(宝灵曼公司产品);用形态学方法和流式细胞仪检测细胞凋亡。

结果 实验结果显示,hTERT全硫代反义核酸,通过下调hTERT基因表达,显著地抑制端粒酶活性;端粒酶活性下降以后,白血病细胞对CDDP诱导凋亡的敏感性显著升高。

结论 以hTERT基因反义核酸抑制端粒酶活性增加白血病细胞对CDDP诱导凋亡的敏感性。

关键词 hTERT(端粒酶逆转录酶);全硫代反义核酸(AS PS-ODN);端粒酶;白血病细胞;顺铂(CDDP)

Chinese—German J Clin Oncol, 2004, 3(1): 42-46

新型白血病疫苗对小鼠巨噬细胞作用的研究

Effect of Leukemia Vaccine on the Macrophage of Mice

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目的 探讨自制的一种白血病疫苗对C57 / BL6小鼠M₁ p杀伤功能的影响。

方法 建立白血病荷瘤小鼠模型,用自制的3种不同的白血病疫苗进行预防或主动免疫治疗,用M₁ p比色法检测预防或治疗2周、4周后小鼠M₁ p杀伤活性,并与对照组比较。

结果 (1)随着白血病细胞在小鼠体内的生长,小鼠的M₁ p免疫功能受到严重抑制。(2)灭活肿瘤细胞+IFA(不完全福氏佐剂)+细胞因子(rGM—csF+rIL-2+rIL-6)的白血病疫苗在提高小鼠的M₁ p免疫功能方面,优于灭活白血病细胞+IFA疫苗,而仅含灭活白血病细胞的疫苗作用不明显。

结论 灭活白血病细胞+IFA+细胞因子(rGM—CsF+rIL-2+rIL-6)的肿瘤疫苗可以激活以M₁ p为代表的非特异性细胞免疫功能,如非特异性细胞毒、免疫监视、抗原递呈等功能,为进一步活化T淋巴细胞打下基础,此种疫苗在血液恶性肿瘤的特异性主动免疫治疗中很有潜力。

关键词 白血病疫苗;巨噬细胞;小鼠

Chinese-German J Clin Oncol, 2004, 3(1): 47—50

冬凌草甲素对NB4细胞的生长抑制作用及其作用机制

Inhibitory Effect of oridonin on the Proliferation of NB4 cells and Its Mechanism

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目的 探讨冬凌草甲素对白血病NB4细胞的生长抑制作用及其作用机制。

方法 以不同浓度的冬凌草甲素作用于体外培养的NB4细胞,应用MTT法检测细胞生长抑制率,流式细胞仪检测细胞凋亡率,Flow—cytometry 33258荧光染色法观察细胞凋亡,TRAP-PCF-ELISA法检测细胞凋亡前后的端粒酶活性。

结果 8 μmol / L以上的冬凌草甲素可显著降低NB4细胞端粒酶活性,抑制细胞的生长及诱导细胞发生凋亡,并呈现出明显的量—效与时—效关系。药物(16 μmol / L)作用48~60 h在Hoechst染色图片上可见核浓缩及核碎裂等典型的凋亡改变。

结论 冬凌草甲素能抑制NB4细胞的生长并诱导细胞发生凋亡,降低细胞端粒酶活性可能是其重要作用机制之一;这为冬凌草甲素进一步应用于临床治疗急性白血病提供了有力的试验依据。

关键词 冬凌草甲素;白血病;端粒酶;细胞凋亡

Chinese—German J Clin Oncol, 2004, 3(1): 51—54

对实验性肝癌中槐耳抗癌作用的研究

Anticancer Effect of PS-T On the Experimental Hepatocellular Carcinoma. <http://www.cnki.net>

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目的 本文从病理角度在细胞水平上观察槐耳清膏的防癌与抑癌作用。

方法 联合应用二乙基亚硝胺(DENA)、四氯化碳复制鼠肝硬化肝癌模型,分为7组,在不同阶段灌服槐耳清膏进行干预癌形成,20周后处死鼠,进行病理观察。

结果 灌服槐耳清膏减缓鼠体重的下降,减轻癌变的病理变化,癌变率显著降低。槐耳也能通过抑制肝硬化干预癌的形成,其疗程与疗效呈显著正相关。

结论 在鼠肝癌模型制备中灌服槐耳清膏具有确切的防癌抑癌作用,槐耳清膏是临床抗肿瘤的理想药物,将成为肿瘤综合治疗及单独治疗的主干药物。

关键词 槐耳清膏(PS—T);肝癌模型;肝硬化;病理变化

Chinese—German J Clin Oncol, 2004, 3(1): 55—59

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