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健脾理气、清热解毒、软坚化痰中药对二乙基亚硝胺致大鼠肝癌的作用

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内容提要　在二乙基亚硝胺诱发大鼠肝癌的过程中测定肝内酶 ACT、γ-GT、OCT、CPS_1以及AKP，并作病理检查，观察灌喂健脾理气、清热解毒、软坚化痰方剂的中药组、西药组(Vit. C + Vit. E + Vit. A)和对照组的酶以及病理变化。结果：对照组在致癌过程中AKP、ACT、γ-GT 增高，而 CPS_1、OCT 下降，中药能改变这种情况。西药组与中药组 γ-GT、ACT、CPS_1存在显著差异($P<0.05$)。病理证实：肝组织脂变、纤维间变、假小叶形成等变化以对照组最严重、西药组次之，中药组最轻。

健脾理气、清热解毒、软坚化痰中药组成的方剂治疗晚期肝癌的一年生存率为32.5%[1]，为了探讨中药作用的机理，本实验采用二乙基亚硝胺致大鼠肝癌作模型，观察在致癌过程中大鼠肝的形态以及与分裂、分化有关酶类的变化。

材料与方法

一、动物分组：Wistar 雄性健康大鼠 30 只，体重140g左右，随机分为对照、西药、中药组各 10 只。对照组诱癌后喂水，西药组诱癌后每日喂 Vit. A 500 u/kg，Vit·C 10mg/kg、Vit·E 0.1mg/kg，中药组诱癌后每日喂中药 2ml/只(预初实验结果提示上述中药和西药的剂量对抑制诱癌有较好的作用) 三组鼠均连续喂药物(或水)直到死亡 (最后终止实验时间为 1 年 8 个月)。

二、肝癌的诱发：实验大鼠 1 周 6 日喂食由医科院基础所按 Rogel 法合成的二乙基亚硝胺 (沸点77～79°C/13～14mmkg、n_D^{15}1.4406)、由于考虑到能够使被观察的中、西药物在二乙基亚硝胺致大鼠肝癌过程中充分发挥作用，肝癌诱发的速度不宜过快，依据过去预初实验的结果，我们选用了二乙基亚硝胺的剂量为 20mg/kg·日，总剂量为 600mg/kg，达到总剂量后在食物中添加50mg/kg之戊苯巴比妥钠每日 1 次共 3 个月。第 1 次取材后(第 5 个月)再追加二乙基亚硝胺上述用量 1 次。

三、中药制备：取上海中医学院附属龙华医院中药库房提供的中药组成下列方剂：太子参12g　珠儿参12g　炒白术12g　茯苓30g　生牡蛎30g先煎　夏枯草12g　岩柏30g　马兰根30g　炙山甲各12g　玫瑰花 9 g　绿萼梅 12g　八月札 12g　莫蓉叶 30g　地鳖虫 9 g。取上述中药，用温水浸泡半小时后用急火煮沸，即改为文火煎 30 分钟取汁，加水适量再用急火煮沸改用文火煎 30 分钟、再取出汁，合 2 次药液用文火浓缩。校正浓度为0.1g/ml(按最大剂量中药计算，余按比例推算)。

四、取材：用乙醚麻醉大鼠剖腹暴露肝脏，肉眼观察，切除部分肝癌，止血、缝合，大鼠继续喂养。取出之肝脏立即分成 3 份：(1)低温 (0～1°C) 生理盐水洗去所含血液，低温下制成均浆，进行酶测。(2) 10%福尔马林固定待做病理检查。(3)低温洗去血液后保存于-80°C 备其他用途。第 1 次取材于诱癌后第 5 个月，第 2 次取材于诱癌后第 7 个月。

五、酶活性测定：(1)氨甲酰磷酸合成酶I(CPS_1)。(2)鸟氨酸氨甲酰转移酶 (OCT)。(3)天门冬氨酸甲酰转移酶(ACT)。以上三种酶活性测定分别按 Richard[2]法和Bresnick[3]等法改良测定。方法及试制均由医科院基础所帮助建立和提供。(4)γ-谷氨酰胺转肽酶 (γ-GT)用上海化学试剂研究所提供的药盒测定。(5)碱性磷酸酶(AKP) 用改良金氏法[4]。(6)病理切片：10%福尔马林固定，石蜡包埋，常规切片 HE 染色，光镜读片。

结　果

一、各组大鼠肝内酶的变化

1. 两次取材肝内碱性磷酸酶(AKP)，γ-谷氨酰胺转肽酶(γ-GT)的变化，见表 1。

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表 1　两次取材肝内AKP、γ-GT的变化
(M±SD，下同)

组别	AKP(u)		γ-GT(u)	
	诱癌第5月	诱癌第7月	诱癌第5月	诱癌第7月
中药	8.12±0.70	7.67±1.47	23.48±2.44	23.60±1.67
西药	6.83±0.61	10.75±0.98*	22.65±1.36	35.78±1.75*
对照	6.45±0.49	23.00±2.39*	23.30±1.45	33.90±1.61*

* 两次取材自身比较，P<0.05

2. 两次取材肝内氨甲酰磷酸合成酶I (CPS$_1$)，鸟氨酸氨甲酰转移酶(OCT)、天门冬氨酸甲酰转移酶(ACT)的变化，见表2、表3。

表 2　两次取材肝内CPS$_1$、ACT的变化

组别	CPS$_1$ (nmol/g·h)		ACT(nmol/g·h)	
	诱癌第5月	诱癌第7月	诱癌第5月	诱癌第7月
中药	147.80±3.79	134.80±5.75	30.00±2.23	31.10±3.18
西药	168.70±7.17	70.40±3.50*	30.80±2.39	45.10±2.33*
对照	166.30±4.93	47.50±4.86*	49.95±1.98	78.00±2.58*

* 两次取材自身比较，P<0.05

表 3　两次取材肝内OCT的变化

组别	OCT(nmol/g·h)	
	诱癌第5月	诱癌第7月
中药	12320±758	10590±967*
西药	12610±561	9160±532*
对照	8085±225	4980±382*

* 两次取材自身比较，P<0.05

二、各组大鼠肝脏病理变化

病理观察，第1次取材3组区别不大。肉眼见部份肝脏轻度水肿、充血、表面偶见细小结节。镜下见肝细胞结构大部份完好、有少量脂变、水样变性等。第2次取材则变化较为明显，各组之间差别较大。各组大鼠肝脏病理变化百分率（例）见表4。

表 4　两次取材各组大鼠肝脏病理变化百分率比较

组别	结构乱、假小叶形成	脂变	水样变	纤维间隔	再生	炎侵
中药	40	60	0	40	80	0
西药	85	50	66.7	50	83.3	16.6
对照	71	85.7	85.7	100	100	28.5

讨　论

中药起效时间较晚，但药效持续时间长，鉴于这个特点，我们选用较大鼠龄和间断给药，中间辅以启动子(戊苯巴比妥钠)的方法，以求充分发挥中药的作用。方中部分中药在过去的实验研究中发现具有反突变作用[5]、细胞毒作用和降低C$_{57}$BL近交系小鼠Lewis肺癌的肺转移作用等；实验选用文献报道中经常提到的具有阻断诱癌作用的Vit. A、Vit. C与Vit. E作为西药对照组，以观察中药与西药在二乙基亚硝胺致大鼠肝癌过程中的作用。

实验结果表明：对照组随着诱癌时间的延长，AKP、γ-GT、ACT有显著的增高(P<0.05)，CPS$_1$、OCT有明显的减少(P<0.05)与文献报道[6]一致。西药组变化与对照一致的有γ-GT，CPS$_1$虽比对照组下降得少但相差不大，而ACT虽有上升但上升幅度也不大，OCT，AKP的变化与对照组有显著差异(P<0.05)。中药组在诱癌后第7个月观察的酶均与对照组存在着差异(P<0.05)。中药组的AKP、OCT与西药组大体相似，而γ-GT、ACP、CPS$_1$、则存在显著差异(P<0.05)。

病理结果所示：对照组大鼠肝脏病理变化较为明显，西药组次之，中药组最轻。表4百分比之计算仅单纯以该项病理变化存在与否计算。若以该病变存在的程度综合考虑，则可明显地表现出上述情况的真实性。结合两次取材病理和肝内酶的变化，可以认为健脾为主的中药在二乙基亚硝胺诱癌过程中能够发挥一定的阻断作用。

综上提示，中药不但能对二乙基亚硝胺诱发肝癌发生影响，而且其作用较维生素A，维生素E，维生素C组成的西药组强。在病理检查中，西药组大鼠第2次取材的肝脏标本中有42.8%见到癌巢，而中药组则无一例发现。这也可以从另一个侧面加以佐证。

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参 考 文 献

1. 邱佳信，等。健脾理气、清热解毒、软坚化瘀方剂治疗晚期肝癌的临床观察及实验研究。中西医结合杂志 1987; 7(5):275.
2. Richard P. Ornithine carbamyl transferase from rat liver. Acta Chem Scand 1957; 11:523.
3. Bresnick E. Aspartate carbamyl transferase from rat liver. Biochem J 1966; 101:63.
4. 朱忠勇，等，主编。临床医学检验。第1版。上海：上海科学技术出版社，1978：330—333.
5. 邱佳信。中医中药反突变作用的研究。上海中医药杂志 1985; 9:46.
6. 李士谔。癌变原理研究I，二乙基亚硝胺诱发大鼠肝癌过程及发育过程中肝细胞增殖酶和组织特异酶活性的变化及其相互关系。生物化学与生物物理学报 1977; 2:113.

Anti-Viral and Interferon-Inducing Effect of Kangli Powder(抗戾散)

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This paper presents the anti-viral and interferon-inducing effect of Kangli Powder (KLP) with the model of VSV-L929 cells. KLP was composed of Chinese herbal drugs according to the pestilence theory of TCM. Result: (1) It inactivated VSV directly and the effect of inactivation was strengthened with the increase of consistency. 1:100 consistency was the maximal non-toxic dosage limit. This consistency that acted on cells for 24 hours was able to protect them effectively from the attack of 100 $TCID_{50}$ VSVs. The test of inhibiting virus reproduction proved that inhibiting titre was two logarithmic value. (2) KLP promoted NDV inducing the interferon of mice. 100%KLP 0.4ml fed through gastric tube in the mice, once a day in the experimental group, this dosage was used for seven days successively. In the seventh day, 1280HAU/ml NDV 0.5 ml was injected into the abdominal cavity of mice. The mice were let out the blood till death six hours later. The titre of interferon in serum was examined. The mean titre was $2^{8.01}\pm0.22$ in the control group and $2^{9.17}\pm0.32$ (u/ml, $\bar{x}\pm SD$) in the experimental group; by comparison between this group and control, the difference was highly significant, $P<0.001$. (3) KLP induced the interferon directly. 100%KLP 0.6 ml was injected into the abdominal cavity of the experimental group and the same volume of normal saline was injected in the control. The mice of both groups were let out the blood till death after 8, 16, 24 or 36 hours separately. The mean titre of serum was 100 u/ml in 8 hours group and reached the peak (160 u/ml) in 16 hours group. The titre dropped in 24 hours group and would not be able to check out in the control. The induced interferon in 16 hours group that was treated with pancreatin for one hour under the condition of pH=2.0 and 56°C, which indicated that it might be α-interferon.

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The Effect of "SRRS" Recipe* on the Formation of Rat Liver Cancer

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30 rats were divided into three groups. The TCM group was fed with "SRRS" recipe. The WM group was fed with vitamin A, E and C. The control group was fed with water. In the process of formation of rat liver cancer induced by Diethylnitrosamine, the action of some enzymes of the liver (ACT, γ-GT, OCT, CPS_1 and AKP) were determined and pathologic examinations were done at intervals. The results showed that AKP, γ-GT, ACT were prominently increased and CPS_1, OCT were notably reduced in the process of the hepatocarcinogenesis in the control, but these phenomena can be changed in the TCM group. In the WM group, AKP and OCT were the same as the TCM group, but γ-GT, ACT and CPS_1 were different between these two groups ($P<0.05$). The results of pathologic examination indicated that the change in the control group was most serious, while those in the WM group were moderately serious and those in the TCM group were not serious at all, no matter what they were fatty degeneration, fiber sepium, regeneration or inflammation infiltration, etc. It is suggested that the TCM not only can influence the process of formation of liver cancer induced by diethylnitrosamine but also was more effective than some WM group (vitamin A, E and C).

*SRRS recipe: The recipe for strengthening the Spleen, regulating the Qi(气), removing the toxic heat and softening the hard lumps and resolving the Phlegm.

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Effects of *Cistanche deserticola* on Immune Function of Mice

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The effects of *Cistanche deserticola* on immune systems in normal mice were studied. At the per os doses of 50 or 100 mg/kg twice a day for 7 days, *Cistanche deserticola* significantly increased the weights of spleen and thymus from 85±12 and 37±6 mg/kg to 140±12 and 58±6 mg/kg respectively. It also increased the phagocytosis of macrophage % from 53±5% to 78±3%, HC_{50} and PFC from 140±47 and 0.05±0.01 to 367±62 and 0.18±0.01 respectively, cAMP of intraperitoneal macrophage from 100±8.6 pmol/ml to 152±10.9 pmol/ml. It decreased cGMP of intraperitoneal macrophage from 62±12 pmol/ml to 39±7 pmol/ml. It increased lymphocyte transformation of ^{3}H-TdR incorporation induced by PHA in vitro from 178±19 cpm to 589±139, DTH from 0.54±0.15 mm to 0.82±12 mm.

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