蛇莓对人食管癌细胞作用的研究

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内容提要 本文以体外长期培养的Eca-109细胞系为实验材料，以分裂指数、集落形成、生成曲线、^{3}H-胸腺嘧啶核苷标记为观察指标，研究了中药蛇莓在体外对人食管癌细胞的生长、分裂、增殖、再繁殖能力和DNA合成的影响。结果表明，蛇莓水提取物相当于5mg/ml、10mg/ml、15mg/ml时，对细胞生长有较强的抑制效应；蛇莓15mg/ml作用48小时可使细胞完全丧失再繁殖能力。蛇莓10mg/ml、15mg/ml作用48小时对细胞有丝分裂的抑制率分别为46%和49%，但对细胞DNA合成仅呈轻度抑制效应。

蛇莓〔*Duchesnea indica*(Andr)Focke〕系蔷薇科植物，其味苦性寒，为清热解毒、治疗咽喉肿痛的中药，亦用于治疗癌肿和瘰疬[1]。林县食管癌防治研究所曾将蛇莓与龙葵等药联合应用于食管癌、贲门癌，观察到有缓解症状、延长存活时间的作用[2]。我们在对60种中草药的粗筛中，也观察到蛇莓的抗癌作用较强[3]。本研究的目的旨在探讨蛇莓对体外人食管癌细胞系的作用及其机理。

材料与方法

一、细胞及其培养：Eca-109细胞系[4]从中国医学科学院肿瘤研究所获得，常规培养于含20%小牛血清的199培养液内，每6天传代一次。

二、药物：蛇莓由本所在当地采集，阴干后称全草20g，切成0.5cm长的节段，粉碎后加水煮沸提取4次，去渣，将4次提取液混合浓缩至200ml，每毫升含生药100mg，滤纸过滤后，分装小瓶，高压灭菌，4 °C保存备用。5-氟尿嘧啶(5-Fu)为上海十三制药厂生产的注射针剂，在本实验中用作药物对照。上述两药用时都先以培养液稀释至高浓度，再依次二倍稀释至低浓度。先以蛇莓的不同浓度作用于细胞，观察各个浓度的作用，然后选择3个合适的浓度用于实验。根据我们过去的实验，5-Fu使用0.1mg/ml的浓度。

三、实验方法及观察指标

1. 形态观察及分裂指数：在细胞接种前，培养瓶内预先放入小玻片，分别于药物作用24、48小时后取片，甲醇固定，Giemsa染色，显微镜下观察细胞形态和结构的变化，并计数1000个细胞所含分裂相，求出分裂指数与对照组比较。

2. 生长曲线：将细胞定量接种，40万/瓶，于细胞接种后48小时，计数细胞数，然后换入含不同浓度药物的培养液，继续培养并逐日计数细胞数连续4天。药物作用后48小时换入常规培养液。观察各种浓度作用下细胞生长或死亡的动态改变，计算药物对细胞生长的抑制率，描绘生长曲线。

$$\text{抑制率}=\frac{\text{对照组}-\text{实验组}}{\text{对照组}}\times 100\%$$

3. 集落形成试验(colony forming test)，用来观察蛇莓作用后细胞的再繁殖能力。各组细胞经药物作用后两天，弃去培养液，消化计数，按2000细胞/cm^{2}的密度接种于培养瓶内（瓶底面积9 cm^2），一周后以甲醇固定，Giemsa染色，镜下计数集落形成数，凡50个细胞以上算一个集落。

4. ^{3}H-胸腺嘧啶核苷(^{3}H-TdR)参入实验：用来观察药物对细胞DNA合成的影响，按细胞培养同位素自显影术进行。分别于药物作用后24、48、72小时，换入含^{3}H-TdR(2μci/ml)的培养液，37°C培养6小时，取片，以生理盐水涮洗3次，以去除未参入的^{3}H-TdR，然后以甲醇固定1天，晾干后在暗室涂布核IV乳胶，曝光4天，D-76显影液显影15分钟，定影10分钟，Giemsa染色，树胶封固，在显微镜下计数1000个细胞，观察^{3}H-TdR参入细胞核的情况，得出百分比，与对照组进行比较。

结 果

一、蛇莓的不同浓度对细胞的作用

先以蛇莓0.1mg、0.5mg、2mg、5mg、10mg/ml作用于细胞，发现0.1mg、0.5mg、2mg/ml对细胞生长仅有轻度抑制作用，浓度达5mg/ml时，抑制作用才比较明显，因此，本实验选择蛇莓5mg、10mg、15mg/ml作为实验使用浓度，与不加药的正常对照作比较。

二、形态观察与分裂指数

1. 形态观察：对照组细胞轮廓清楚，间隙分明，

细胞密集，核仁2～3个，核内结构清晰可见，分裂相较多。实验组细胞较对照组差，分裂相少，结构不如对照组清晰，少数细胞有轻微固缩。

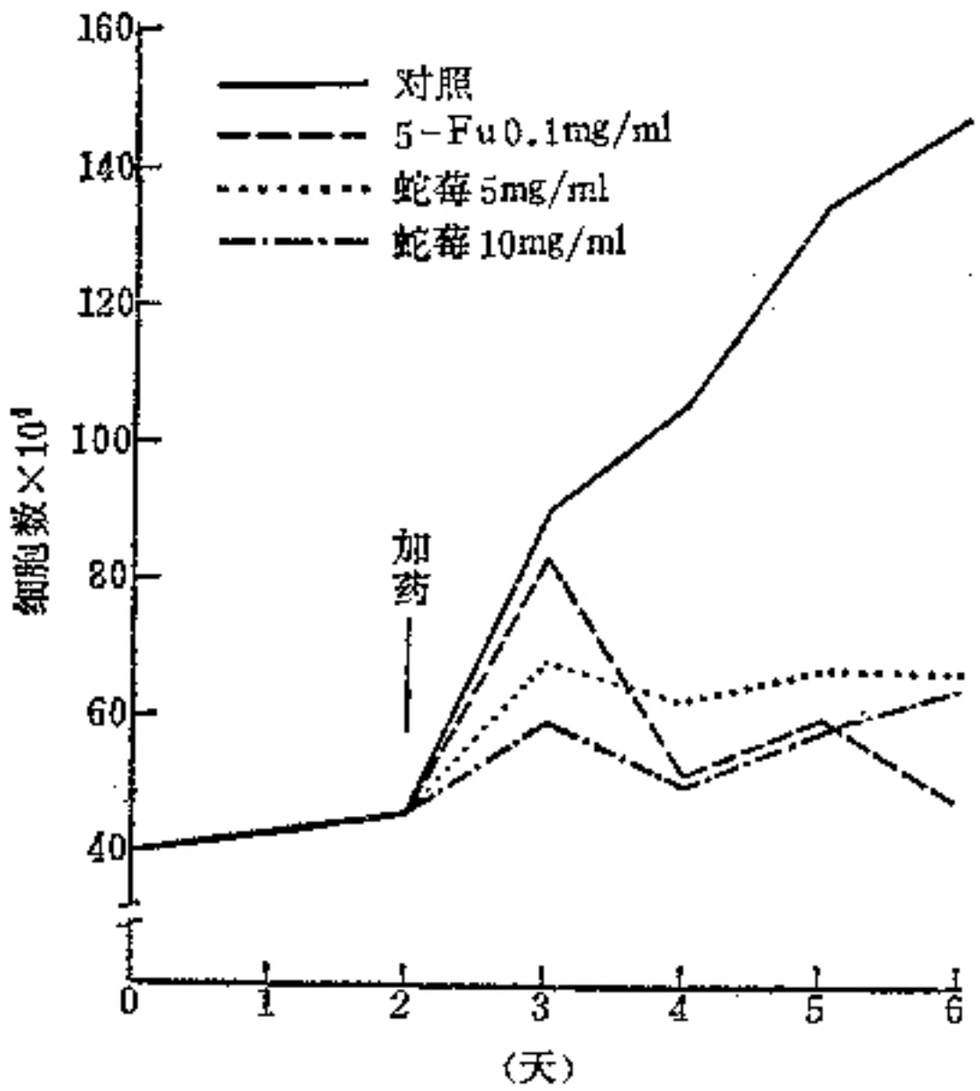
2. 分裂指数：如表1所示，蛇莓5mg/ml对细胞分裂的抑制不明显，10mg、15mg/ml对细胞分裂的抑制率分别为46%和49%。

表1 各组细胞分裂指数及细胞集落形成情况

组　别	分裂指数	抑制率(%)	集落形成数	抑制率(%)
对　照	41		75	
5-Fu0.1mg/ml	33	19	0	100
蛇莓5mg/ml	39	5	11	85
蛇莓10mg/ml	22	46	6	92
蛇莓15mg/ml	21	49	0	100

注：表内数据为三次实验结果平均值

三、生长曲线：从附图可见，蛇莓5mg、10mg/ml对细胞生长均有较强的抑制。蛇莓5mg、10mg/ml作用48小时的抑制率分别为39%和52%。



四、集落形成试验：三次实验结果表明，蛇莓15mg/ml和5-Fu0.1mg/ml均不能形成集落，而蛇莓在5mg、10mg时仍可有少量集落成形(表1)。

表2 蛇莓作用后Eca-109细胞的^{3}H-TdR标记率

组　别	细胞^{3}H-TdR标记率(‰)		
	24h	48h	72h
对照	469	495	471
蛇莓10mg/ml	474	461	423
蛇莓15mg/ml	427	401	336

五、^{3}H-TdR参入实验：如表2所示，蛇莓10mg、15mg/ml对Eca-109细胞的DNA合成抑制不明显，蛇莓15mg/ml作用72小时后，抑制率仅为29%，但蛇莓作用过的细胞参入的^{3}H-TdR颗粒不如对照组浓密。

讨　论

从生长曲线的结果看，蛇莓5mg、10mg/ml对Eca-109细胞的生长均有较强的抑制，而且以10mg/ml为强，这与葛铭等[3]对60种中草药的粗筛结果相似。上述情况在形态观察和集落形成实验中得到了进一步证明，蛇莓作用后的Eca-109细胞密度降低，结构模糊；蛇莓5mg、10mg/ml作用48小时对细胞集落形成的抑制率分别为85%和92%，至15mg/ml时，可使细胞完全丧失再繁殖能力。这些都说明，蛇莓5mg、10mg、15mg/ml对Eca-109细胞的生长、增殖均有一定的抑制作用，而且浓度愈大，作用愈强。

蛇莓10mg、15mg/ml作用48小时后，对细胞分裂指数的抑制率分别为46%和49%，这表明蛇莓对细胞分裂有影响，但不是全部阻止细胞分裂。^{3}H-TdR标记实验表明，蛇莓10mg、15mg/ml对Eca-109细胞的DNA合成有轻度抑制作用，15mg/ml作用72小时的抑制率为29%。这说明蛇莓对细胞的作用可能主要通过抑制细胞分裂和DNA合成这两个途径。

从我们过去的实验看出[4]，药物对细胞的作用受其浓度、作用时间、细胞数量等因素的影响，本实验中蛇莓抑制细胞生长增殖所需浓度较大，可能与药物提取过程中成份丢失和接种细胞数量较多有一定关系。虽然蛇莓5mg、10mg、15mg/ml对Eca-109细胞的生长、分裂有较强的抑制，但所需浓度较大，因此，提取其有效抗癌成分，对于降低用药量、减少毒性，提高疗效，均是必要的。

（本文承中国医学科学院基础医学研究所细胞生物学系章静波主任审阅，特此致谢）

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Clinical Observation and Experimental Study of Gossypol in Treatment of Dysfunctional Menorrhagia, Endometriosis and Fibromyoma of Uterus

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A total of 192 cases of dysfunctional menorrhagia, endometriosis and fibromyoma of uterus were treated with gossypol (20mg/day) for 3 weeks to 3 months or more with satisfactory results, the markedly effective rates were 91.5%, 90.3% and 79.5% respectively. 142 cases (74%) became amenorrheic, and the incidental rate varies with the accumulative amount of gossypol. Menstruation resumed after a period of 3~13 months of amenorrhea in 78 cases. Permanent amenorrhea resulted in 64 menopausal patients, and 10 of them have remained to be amenorrheic for over 5 years and enjoyed good health. 400~1200 mg gossypol were given to 14 cases of dysfunctional menorrhagia (anovulatory type) with the result of the menstruation normalized with ovulation, suggesting a proper and smaller accumulative dosage might produce menstruation regulatory and/or ovulation stimulating effects. Further observation is necessary. Results of the present study showed that immediate and longterm effects displayed in the following order: dysfunctional menorrhagia, endometriosis and uterine fibromyoma, the longterm result of the latter was unsatisfactory. However, the use of gossypol for premenopausal fibroids of smaller size might bring beneficial therapeutic results. No persistent adverse side-effects have been noted during treatment.

Animal experiments were carried out to explore the mechanism with following findings: (1) Rats receiving gossypol with accumulative dosis of 7 mg in a significant drop of Leucine-enkephalin and a marked increase of β-endorphin in the hypothalamus; plasma FSH and LH increased significantly with no change for the E_2 and PRL. (2) Large dosis of 40~48 mg gossypol caused marked atrophic changes of both the ovaries and the uteri of rats. The suppressive effect on the hypothalamus-pituitary-gonadal axis appeared to vary directly with the dosage of drug administered. Acidophils of the anterior pituitary lessened or even disappeared completely while that of the basophils increased both in number and size resembling that of the so-called "postmenopausal cells". Plasma E_2 diminished markedly and PRL was hardly detectable, suggesting that gossypol might be a valuable drug in treating the medium hyperprolactinemia. (3) Spontaneous remission of the above changes took place sooner or later following cessation of therapy. (4) No histological change was observed in the vital organs after medication.

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Observation on Effect of Liangxue-Huayu(凉血化瘀)Prescription on High Coagulation Phase of DIC in Rabbits

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The model of high coagulation phase of DIC in rabbit was established in our laboratory. It was used to study the relationship between "blood heat with stasis" syndrome and high coagulation phase of DIC, and the mechanism of liangxue-huayu (LXHY, cooling blood and relieving stasis) therapy. The results indicated that the"heat flaming in blood portion" syndrome and the "stagnation of blood channel" syndrome in TCM were closely related with the pathologic changes in DIC caused by the infection. The preventive effect of LXHY prescription for high coagulation phase of DIC caused by E. Coli endotoxin in rabbits was proved. The results of examining blood coagulation, hemorrheology and conjunctiva microcirculation showed that the pathologic changes of the therapeutic group could be alleviated, which suggested that the protective effect of LXHY therapy was related to the action of improving coagulation, microcirculation and blood viscosity of affected rabbits. Thus the pathologic process might be abated and blocked, the blood flow in some viscera increased and the shock would be prevented.

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Effect of *Duchesnea indica* on Extracorporeal Esophageal Cancer Cells

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The purpose of present study was to investigate the effect of *Duchesnea indica* on human esophageal cancer cell line (Eca-109). At the concentration of 10 mg, 15 mg/ml, the growth of the cells