复方龙葵注射液对肝癌(H_{22})细胞的影响

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内容提要　本实验应用复方龙葵注射液（龙葵、蛇莓、白英、当归、丹参、郁金的提取成份）连续作用于小白鼠肝癌(H_{22})腹水型癌细胞，观察到本药对肝癌腹水型癌细胞的增殖有明显的阻抑作用，抑制率可达87.35%；P值＜0.001，效果非常显著。同时观察到癌细胞膜表面上的磷酸二酯酶和(N_a^+-K^+)-ATP酶的活性明显下降，膜表面上的微绒毛明显消退。实验结果说明，本药具有高效的抗癌作用，其作用机理可能是通过抑制膜表面的磷酸二酯酶和(N_a^+-K^+)-ATP酶活性以提高细胞内cAMP水平，调控细胞的增殖和分化。

近些年来，发现不少中草药含有3′,5′环腺苷酸的组成成分[1]，有的中草药具有提高细胞内3′,5′环腺苷酸水平[2]和抑制细胞内磷酸二酯酶活性的作用[3,4]。本实验进一步研究了复方龙葵注射液（龙葵、蛇莓、白英、当归、丹参、郁金的提取成分）对肝癌(H_{22})腹水型癌细胞增殖的抑制作用，并用电镜细胞化学的方法观察了本药对肝癌(H_{22})细胞膜表面磷酸二酯酶及(N_a^+-K^+)-ATP酶活性的影响，以了解其作用机理，现将结果报道如下。

材料与方法

采用昆明种健康的小白鼠（体重18～22g），按常规腹腔接种，造成肝癌(H_{22})腹水的模型。次日将动物分成两组，实验组（10只）每日腹腔注射0.2ml复方龙葵注射液，对照组（9只）每日腹腔注射等量的生理盐水；连续注射8天后，分别将两组动物断头处死，取腹水用台盼蓝染色检查死活细胞，并用盐水把每只动物腹腔内的癌细胞冲洗出来计数，求出抑制率和P值。同时取出新鲜腹水滴入冷藏的1%戊二醛（0.1M二甲砷酸钠缓冲液配制成）的离心管内，离心3分钟（1000转/分），弃上清，用0.05M二甲砷酸钠缓冲液将沉淀洗涤两次后，弃上清，将沉淀分别放入磷酸二酯酶[5]或(N_a^+-K^+)-ATP酶[6]孵育液内，在37°C温箱内保温3小时或1小时，用0.2M三羟甲基氨基甲烷缓冲液将沉淀洗涤2次，弃上清，再经1%四氧化锇在冰浴中固定2小时，梯度酒精脱水，再经环氧丙烷置换，Epon812包埋，LKB-V型超薄切片机切片，醋酸双氧铀单染，电镜观察。

结　果

一、复方龙葵注射液对小鼠肝癌(H_{22})腹水型癌细胞增殖的影响

实验结果表明复方龙葵注射液对肝癌腹水型癌细胞的增殖有明显的抑制作用，在给药的第9天时，实验组（10例）癌细胞为101±33.2/ml（×10^6），对照组（9例）癌细胞为799±118.5/ml（×10^6），抑瘤率可达87.35%，P值＜0.001，效果非常显著。

二、复方龙葵注射液对肝癌(H_{22})腹水型癌细胞膜表面磷酸二酯酶的影响

从电镜细胞化学显示的cAMP-PDE活性反应，可以看出肝癌(H_{22})腹水型癌细胞膜表面具有较多的微绒毛和较强的磷酸二酯酶活性反应（图1），但经复方龙葵连续作用后癌细胞膜表面绒毛明显减少，磷酸二酯酶活性明显下降（图2）。

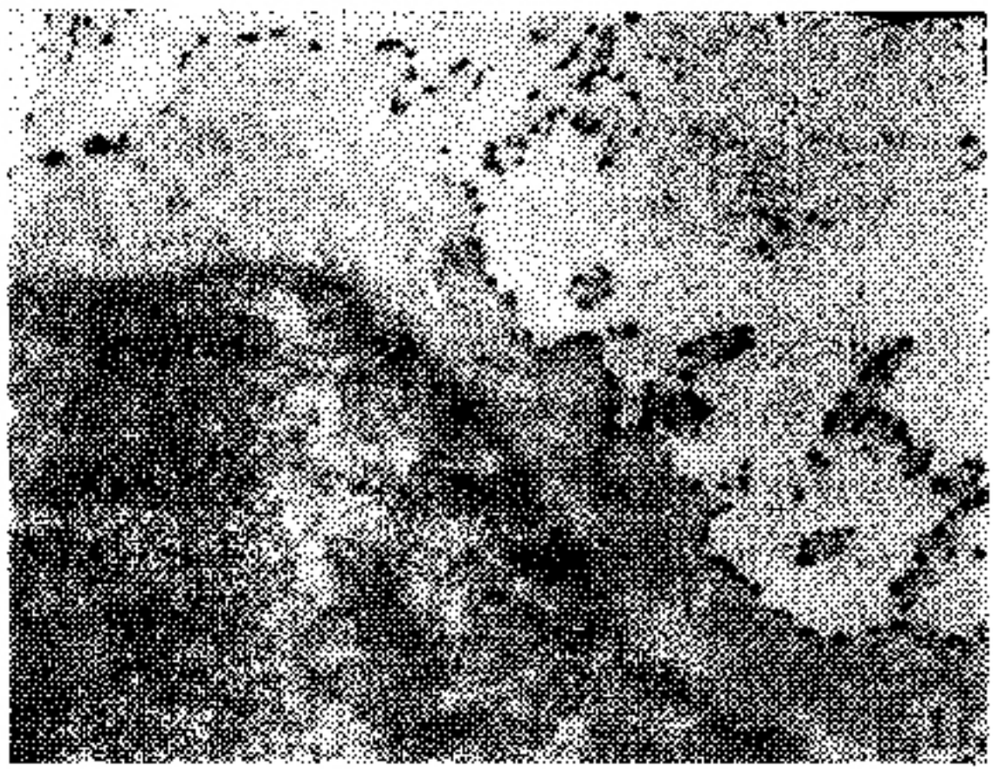

图1　对照组癌细胞膜表面有多而长的微绒毛，磷酸二酯酶呈强阳性反应　×6700

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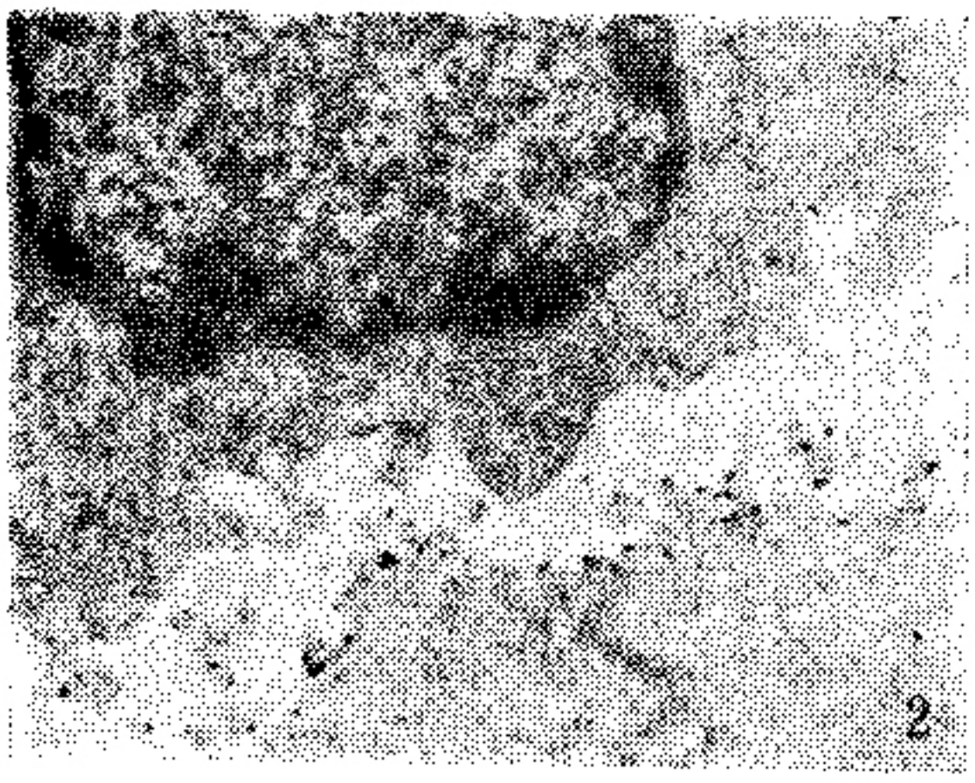

图 2　实验组癌细胞膜表面较光滑，磷酸二酯酶呈弱阳性反应　×5000

图 3　对照组癌细胞膜表面(N_a^+-K^+)-ATP酶呈强阳性反应　×10000

图 4　实验组癌细胞膜表面(N_a^+-K^+)-ATP酶活性明显减弱呈弱阳性反应　×4800

三、复方龙葵注射液对肝癌(H_{22})腹水型癌细胞膜表面(N_a^+-K^+)-ATP酶活性的影响

用电镜细胞化学的方法显示出肝癌(H_{22})腹水型癌细胞膜表面具有强的(N_a^+-K^+)-ATP酶活性反应，(N_a^+-K^+)-ATP酶活性反应呈大颗粒状的沉淀分布在癌细胞膜表面和微绒毛上(图3)；实验组肝癌(H_{22})腹水型癌细胞膜表面微绒毛减少，(N_a^+-K^+)-ATP酶活性也明显降低(图4)。

讨　论

中草药的抑瘤效果，有关文献报道甚多[4,7]。本文所用的中草药，其抑瘤率达80%以上，在国内外抗癌药物中尚少见。复方龙葵的抗癌机理非属直接杀伤癌细胞的作用，用台盼蓝染色测试对照组和实验组动物的肝癌(H_{22})腹水型癌细胞，只有少数死细胞，这说明复方龙葵的抗癌作用，不是对癌细胞的直接杀伤作用。我们的实验结果进一步观察到该复方中药对癌细胞膜表面的磷酸二酯酶活性具有明显的抑制作用(图1、2)。癌细胞在该复方中药的连续作用下，磷酸二酯酶活性下降，说明细胞内cAMP的分解作用受到障碍，从而使细胞内cAMP水平升高，阻止细胞的增殖。我们的实验结果进一步证实癌细胞膜表面上有强的ATP酶活性[8~11]，说明碱性金属K^+参与细胞的增殖调节过程[10]，而细胞内K^+浓度变化与膜上(N_a^+-K^+)-ATP酶功能有关。本实验显示出肝癌细胞上ATP酶活性较高，其细胞内K^+的浓度也应较高，使癌细胞处于持续的增殖状态。经中药处理后(N_a^+-K^+)-ATP酶活性显著降低，与抑制肝癌细胞的增殖有关。肝癌(H_{22})腹水型癌细胞膜表面上(N_a^+-K^+)-ATP酶活性增强的同时其膜表面上的腺苷环化酶活性会明显降低，这可能与该两种酶竞争底物ATP有关，癌细胞内cAMP水平低就促使癌细胞不断地处于增殖状态，而经复方龙葵连续作用的癌细胞(N_a^+-K^+)-ATP酶活性较低，这可能说明该复方中药有提高膜上腺苷环化酶活性的作用。Sheppard等[12]已发现儿茶酚胺、糖皮质激素等在抑制(N_a^+-K^+)ATP酶活性的同时，可提高腺苷环化酶的活性，从而使细胞内cAMP处于高水平，阻止细胞的增殖。该复方中药抑制肝癌(H_{22})腹水型癌细胞增殖的同时，并且有抑制膜表面磷酸二酯酶和(N_a^+-K^+)-ATP酶活性的作用，而使细胞内cAMP水平升高，从而

抑制癌细胞的增殖；而膜表面微绒毛的减少，则可能呈现出癌细胞在复方龙葵作用下逆转后形态学上改变的重要表现之一[11,13,14]。

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中西医结合治疗巩膜大面积坏死脱落 1 例

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巩膜大面积坏死脱落，脉络膜暴露，症属罕见。本例经中西药合治而愈，现报道如下。

患者张××，女，54岁，农民，住院号：3212。右侧头痛眼胀，心烦呕吐，1978 年 9 月 5 日来诊，经检查，以右眼慢性单纯性青光眼急性发作收治。9 月18日局麻下行巩膜板层下咬切术，术中顺利。6 天拆去球结膜缝线，一般情况尚好，但自觉症状未减，瞳孔仍椭圆中等散大上移，前房仍浅。术后10天发现少许虹膜色脱点，次日巩膜瓣上方球结膜似白色膜样物复盖，范围黄豆大，边界清。半月后白色膜样物变为淡黄色苔状物，虹膜色素脱失增多，范围继有扩大。术后一月苔状物范围 11×21mm，似不规则半球形将巩膜包绕半圈，中部有两处球结膜脱落，从而可见病溃的巩膜组织似含气泡之海绵状，可推移，后自行脱落，从溃脱区可见紫蓝色脉络膜显露，虹膜萎缩。术后五周糜烂巩膜全部脱落，范围 12×22mm，中部高而宽，两端低且窄，周界清，未侵及巩膜瓣，边缘有少许淡黄色渗出物，中部呈结节状隆起，全身与局部使用激素，各种抗生素，眼部缩扩瞳药交替应用效果不理想，遂加用中药治疗。证见目赤眼胀，身胸不适，心烦作呕，口苦、便结、舌质红、苔黄腻、脉弦数，乃属肝经湿热。药用：菊花、黄芩、大黄、木贼草各 10g，青葙子、草决明、谷精草各 12g，黄连、甘草、薄荷、枸杞子各 6 g。五剂药后患者觉证减轻，依上方加僵蚕 10g，丹参 12g，苡仁 20g。三剂后继有好转，但眼部病变未改善，恐脉络膜继向外突，后果难收，故于术后85天拟行巩膜修补术，因患者畏惧手术，而坚持门诊药物治疗，继服强的松、维生素，并配服中药；见上证俱存，舌边瘀点，苔薄黄，脉弦微数，以逍遥散加越鞠丸，去苍术、白术、川芎，加菊花、黄芩各 8 g，生地、赤芍各 10g，桃仁 18g，用以疏肝解郁、凉血活血逐瘀。连服15剂，脉络膜暴露区灰白膜形成，患者心悸、失眠、咽干、舌红无苔。激素按常规减至维持量，配用中药益正养阴、宁心安神，方用天王补心丹去丹参，加菊花 8 g，玉竹、石斛各10g，服 15 剂，仅觉头晕，视物昏朦、流泪，巩膜缺损周边薄膜变厚，则宜滋补肝肾。方用杞菊地黄丸加草决明、女贞子各15g，天冬10g，连服20剂后，诸证悉减，巩膜病溃缺损区为灰白色膜样物复盖，停止用药。两年后随访，原见巩膜缺损区为黄白色再生巩膜组织所修复，中部稍隆，呈瓷白色，质坚硬。坚持随访七年余，情况稳定。

Protective Effect of Astragalus Polysaccharide on Ribonuclease and Ribonuclease Inhibitor System

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Astragalus Polysaccharide (APS) is a mixture isolated from *Astragalus mongolicus*, which has two polysaccharides as its major components. APS enhanced the activity of the ribonuclease inhibitor (RI) and regulated RNA metabolism and thereby affected protein biosynthesis. For ribonuclease (RNase), there existed two states, a free form and a latent form which was a compound complexed with the RI. Tissue homogenates were incubated in the medium containing p-chloromercuribenzoic acid (PCMB), 1×10^{-4} M, its activity of RI was lost and thus the latent RNase which was formerly complexed with the RI was released. The latent RNase activity reflected the RI level. The RI level was different among various tissues, kidney > liver > lung > spleen. APS-2, ip 200 mg/kg per day in ICR mice for three days did not cause a significant change in total activity of RNase (latent+free) except spleen. It showed that the RI activity was enhanced by APS-2, hence the activity of free RNase decreased. The order of the inhibition rate of RNase activity by APS-2, from high to low, was spleen, lung, liver and kidney. PCMB was subcutaneously given to mice 40 mg/kg, the RNase activity increased significantly in kidney, serum and lymph node 18h later. After the medication of APS-2 ip 200mg/kg × 3, the same dosage of PCMB did not cause the increase of free RNase activity in both plasma and kidney, there existed no significant difference with the control group. Therefore, the effect of APS-2 was to counteract PCMB. The results indicated that APS may play an important role in maintaining balance between RNase and RNase-RI system.

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Effect of Long Kui (龙葵) Injection Co. on Hepatoma H_{22} Ascites Tumour Cells in Mice

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This experiment was carried out in adult mice. Hepatoma H_{22} ascites tumour cells was injected intraperitoneally to produce a hepatoma H_{22} ascites tumour model. On the 2nd day after inoculation, these animals were divided into two groups. Group 1 was injected intraperitoneally with 0.2 ml Long Kui injection co. every day for 8 days, which contained *Solanum nigrum, Solanum lyratum, Duchesnea Indica, Angelica Sinensis, Curcuma aromatica* and *Salvia miltiorrhiza*. Group 2 received same dose of saline as control. Results showed that this injection inhibited markedly the proliferation of hepatoma H_{22} ascites tumour cells. The inhibitory rate was 87%, $P<0.001$. At the same time, the cAMP-phosphodiesterase and (Na^+-K^+)-ATPase activity on the cell membrane surface was decreased and the microvilli showed marked regression. These results indicated that the Long Kui injection co. was a potent anti-cancer drug, and it may increase intracellular cAMP level by inhibiting 3',5'cAMP-PDE and (Na^+-K^+)-ATPase activity to modulate the proliferation and the differentiation of the cancer cells.

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Effect of Tonifying Recipes on Platelet Aggregation

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The effect following injection of Sijunzi Decoction (四君子汤, a Qi tonic), Siwu Decoction (四物汤, a Blood tonic) and Bazen Decoction (八珍汤, a mixture of Qi tonic and Blood tonic) was estimated by measuring degrees of inhibition of rabbit platelet aggregation induced by ADP. The result of this study are as follows: The platelet aggregation was inhibited with Sijunzi Decoction, Siwu Decoction and Bazhen Decoction ($P<0.01$), with the last being more effective. This is due to the combined prescription of Qi tonics and Blood tonics, which generates inter-related pharmacologic action. The result indicates that the tonifying recipes may be effective in the clinical treatment with anti-platelet aggregation.

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