物对消化道肿瘤有明显的抑制作用。最近的实验研究表明，清热解毒药一方面对肿瘤有抑制作用，具有抗肿瘤活性物质；另一方面，清热解毒药的抗癌作用，并不单纯是抑制癌细胞分裂或直接杀伤癌细胞，有些药物是通过提高机体的免疫防御机能为中介而产生抗肿瘤作用。文献还报道，虎杖具有抗病毒作用。关于用中药阻断大鼠胃癌前期病变发生的机理，目前研究尚少，本实验正是基于本方具有抗肿瘤作用的以前实验基础上来探讨在阻断癌前病变发生中，大鼠体内一些免疫生化变化，摸索其可能的机理。本实验表明大剂量加味四君子汤和维甲酸类药物的疗效相近，而小剂量无明显的阻断作用。至于本方在阻断癌前病变时，癌基因、抑癌基因(P53)等变化，及其组方分析和正校试验，尚待进一步实验研究。

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溶癌灵悬乳剂肿瘤内注射的抗癌作用实验研究

陈瑞川[1]　杨善民[1]　吴艳环[2]　江　锋[2]　陈　福[1]

内容提要　**目的**：探讨溶癌灵合并鸦胆子种皮毒性部分研制新型肿瘤内注射药物的抗癌效果。**方法**：经乙醇抽提的鸦胆子种皮水溶性部分，以聚乙烯吡咯烷酮为载体，混合消癌灵制成溶癌灵悬乳剂，并以体外细胞毒杀伤及荷瘤动物肿瘤内注射抑瘤实验观察。**结果**：细胞毒实验显示该剂对体外培养细胞的杀伤效果与鸦胆子种皮提取液相当，但优于消癌灵及 5-Fu；体内抑瘤实验表明在抑瘤作用及伤口愈合等方面，该制剂均优于消癌灵、酒精及 5-Fu 组。**结论**：溶癌灵是一种适于肿瘤局部注射治疗的细胞毒性物质，有一定的开发前景。

关键词　溶癌灵悬乳剂　肿瘤　瘤体注射

鸦胆子系苦木科鸦胆属[*Brucea javanicna*(L)Merr]植物的干燥成熟果实，其种皮含有细胞毒性较强的水溶性苦味物质[1]。本研究尝试以乙醇抽提法对鸦胆子种皮水溶性成分进行了粗提，与消癌灵[2]合并制成以聚乙烯吡咯烷酮(PVP)为载体的肿瘤内局部注射治疗剂——溶癌灵悬乳剂，并研究了该制剂对体外培养细胞及荷人肝癌裸鼠移植瘤模型局部注射的抗肿瘤效果。

材料与方法

1　材料

1.1　药物　消癌灵由厦门市中山医院吴艳环提供(批号：941108)；鸦胆子种皮由中山医院药房加工惠赠，PVP 及中性红购自上海华美生物工程公司。

1.2　细胞株　人肝癌细胞株 BEL-7402 和 H 9101 为本单位细胞培养室保存，培养条件为 15% 小牛血清 RPMI 1640 培养液(含 100Iu/ml 的青霉素及链霉素)，37℃、5%CO_2 下培养传代。移植瘤株为人肝癌裸鼠移植瘤株 HHC_{15}[3]。

1.3　动物　BABL/C 裸小鼠，4～6 周龄，体重 17±2g，雌雄兼用，由本单位医学实验动物室饲养及保种(SPF 级，合格证号为 009)。

1.4　仪器　450 型微孔板读数仪，美国 Bio-RAD 公司产品。

1.5　鸦胆子种皮提取及溶癌灵制备　(1)鸦胆子种皮提取：鸦胆子种皮经机械磨粉后，按 500g 加水 1000ml 煎煮 5～6h，以多层纱布过滤后，滤液按 1∶1 加入无水乙醇，室温搅拌 2h，再以多层纱布过滤，滤液煮沸浓缩至浓度达到每毫升含 2g 生药为止(250ml)，滤纸过滤，分装高压灭菌后 4℃贮存备用。(2)溶癌灵制备：取鸦胆子种皮提取液、消癌灵及 10% PVP 按 1∶1∶1

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配制，震荡混匀后即为溶癌灵悬乳剂，分装置 -20℃、4℃及室温存放，观察细胞毒性及外观变化。

2 方法

2.1 体外细胞毒实验 BEL-7402 及 H 9101 细胞按 10^4 个细胞/孔(0.2ml)接种于 96 孔微量培养板，培养 2 天后每孔吸去 0.1ml 培养上清，再分别加入 0.1ml 以细胞培养液系列稀释的药物，设鸦胆子种皮提取液、消癌灵、溶癌灵及 5-氟脲嘧啶(5-Fu)组，每组设 6 个剂量，每剂量 3 个平行孔；阴性对照组仅补加 0.1ml 培养液；继续培养 4 天后撤药，RPMI 1640 轻洗 2 次；细胞毒杀伤率测定则按 Borenfreund 方法[4]，略加修改如下：每孔加入含 80μg/ml 中性红的培养液 0.2ml，37℃培养 6h，去上清，迅速用固定液冲洗及固定后，各孔加 0.2ml 含 1%乙酸的 50%乙醇溶液，10min 后轻拍混匀，于微孔板读数仪测定 570nm 处 OD 值，计算细胞毒性杀伤率。杀伤率(%)=(对照组 OD 值 - 实验组 OD 值)÷对照组 OD 值 × 100%。

2.2 体内抑瘤实验 人肝癌移植瘤株 HHC_{15} 的接种：无菌条件下手术，取生长旺盛的 HHC_{15} 瘤组织数个，剔除坏死部分，剪碎混合后用套管针在 BALB/c 裸小鼠后背部或腋部皮下接种瘤组织(约 0.2mm × 0.2mm × 0.2mm 左右)，待大部分移植瘤长至 0.8cm × 0.7cm × 0.7cm 左右即剔除瘤体过大和过小的荷瘤鼠，随机分为 5 组，每组 4 只，设溶癌灵、消癌灵、无水酒精、5-Fu(100mg/ml)及生理盐水对照组，饲养 1 天后即分别于瘤体部分作环状封闭局部注射各药，每次剂量均为 0.1ml/只，共注射 3 次，每次间隔 4 天，于最后 1 次注射后观察 20 天，再脱臼处死照相。另取动物将实验重复 1 次，但观察 2 个月后再行处死照相。

结 果

1 药物稳定性观察 将鸦胆子种皮提取液、消癌灵及溶癌灵分别置 -20℃、4℃及室温 6 个月后，以体外细胞毒杀伤实验测定其药效稳定性。结果表明：置 -20℃、4℃及室温的 3 种药物的细胞毒杀伤效果均未见丧失，但置 -20℃的消癌灵及溶癌灵解冻后可见呈分层现象，表明该两种药物不宜冰冻存放。

2 药物对体外培养细胞的毒性杀伤作用 鸦胆子种皮提取液、消癌灵、溶癌灵及 5-Fu 各原液以 1:100、1:200、1:600 及 1:1000 稀释后(终稀释度)，各稀释液对人肝癌细胞 BEL-7402 和 H 9101 均有毒性杀伤作用(见图 1、2)，其杀伤效率均存在浓度依赖关系，并且 H 9101 对药物的敏感性较 BEL-7402 更明显；其中溶癌灵及鸦胆子种皮提取液的细胞毒性杀伤作用相近，均较消癌灵及 5-Fu 显著($P<0.01$)，而消癌灵的杀伤作用较 5-Fu 强($P<0.05$)。

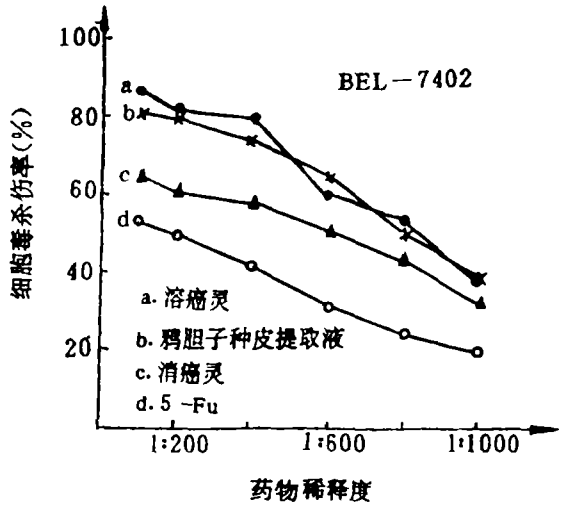

图 1 药物对 BEL-7402 细胞的毒性杀伤作用

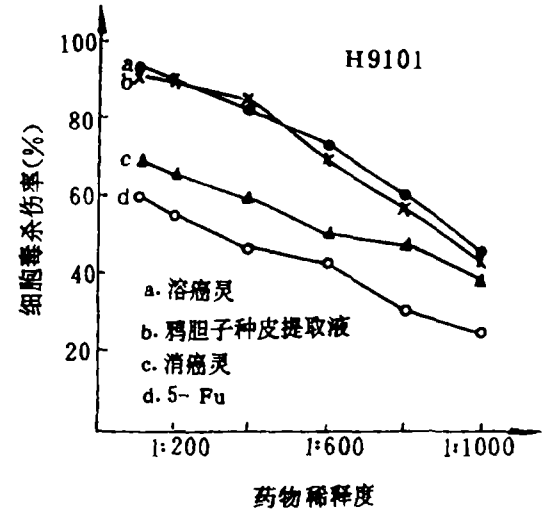

图 2 药物对 H 9101 细胞的毒性杀伤作用

3 溶癌灵对移植瘤的抑瘤作用 溶癌灵组在注射溶癌灵 2 次后，即可见移植瘤明显坏死、干涸并缩小，第 3 次注射后移植瘤全部脱落，伤口结痂，至 2 个月后疤痕消失，仅见不明显的凹痕，观察 20 天及 2 个月均未见移植瘤复发。消癌灵组局部注射消癌灵 3 次后，移植瘤体明显缩小，并干涸结疤，与溶癌灵组相似，2 个月后伤口无明显疤痕，但重复组观察 2 个月，结果有 2 只裸小鼠出现移植部位移植瘤复发，表明消癌灵 3 次局部注射尚未能完全杀死移植瘤细胞。酒精组 3 次局部注射酒精后，瘤体溃烂并大面积累及皮肤，其中严重者至 20 天仍未愈合，观察 20 天及 2 个月均未见移植瘤复发。5-Fu 组局部注射 5-Fu 后仅见瘤体生长速度减缓，但未见缩小，更无瘤块干涸脱落现象，且荷瘤鼠明显消瘦，重复实验时全组未到 2 个月即已全部

死亡。生理盐水对照组移植瘤生长旺盛，由于 HHG_{15} 移植瘤株为一恶性度较高的细胞株，瘤体迅速生长过程中可导致小鼠明显消瘦及衰竭，故该组小鼠观察尚未致 20 天即已出现死亡。

讨　论

肿瘤内局部注射药物治疗实体肿瘤是近年来开展的一项新技术，瘤体内注射局部化疗的动物试验及临床试用研究均已见报道[5,6]，其优点在于：可局部注射给予较高浓度的药物，从而对肿瘤形成有效的杀伤作用，并可大大降低常规给药方式给机体带来的毒副作用，对于一些不宜手术的肿瘤尤其有用。然而，由于水溶性化疗药物的迅速扩散作用，其疗效并不理想，以脂质体包载化疗药物局部注射给药，虽可明显延长药物在瘤体的滞留时间[7,8]，但因化疗药物本身的低毒副作用特殊要求，仍难以获得理想的疗效。

1993 年，吴艳环等报道[2]以脂质体包载抗肿瘤中药制成消癌灵乳剂，具有滞缓药物扩散作用及使肿瘤局部小血管收缩、血液凝固等作用，肿瘤内注射临床试用具有一定的抗肿瘤疗效，但需对患者进行多达 10 次以上的反复治疗才能奏效，分析可能亦是因为该乳剂对肿瘤细胞的毒性杀伤作用尚不够强的缘故。因此，本研究尝试从鸦胆子种皮中提取其细胞毒性成分，合并于消癌灵中，以加强其毒性杀伤作用，另以 PVP 为吸附载体固定种皮毒性物质，延长药物在瘤体内的滞留时间，同时减少因扩散而对机体造成毒副损害。实验表明，溶癌灵不仅具有较强的细胞毒性杀伤作用，同时也保留了消癌灵原有的减少瘤体溃烂、出血及促进瘤体干涸脱落的效果，仅注射 3 次即可使移植瘤消失，2 个月内未见复发，同时也保护皮肤的完好。本研究结果提示：影响肿瘤内注射药物局部治疗肿瘤的因素中，除应考虑药物在瘤体内的半衰期长短及立体分布均匀性[8]之外，还应考虑在保证药物低扩散的前提下，提高药物本身的细胞毒性杀伤作用。

本研究仅为初步尝试，有关鸦胆子种皮毒性成分的分离纯化、药物剂型的完善及药代动力学等方面尚待进一步深入研究。

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肿瘤抗原穴位注射抑制肿瘤生长的实验研究

刘亚革　蒲菲菲　曹建平　姜志明　孙亦红

内容提要　**目的**：探讨肿瘤抗原穴位注射对肿瘤的形成是否产生抑制作用。**方法**：采用小鼠 S180 细胞株粗提物（瘤苗）作为抗原，在后海穴间断免疫后，于小鼠腋下接种 10^6 个 S180 细胞。**结果**：发现穴位抗原注射组的未荷瘤且生存期达到 100 天的小鼠只数明显多于对照组。后海穴注射瘤苗和生理盐水（HS180 和 HNS）及股骨外侧中点肌肉注射瘤苗和生理盐水（MS180 和 MNS）各组未荷瘤鼠所占百分比分别为 80.0%、21.4%、71.4%、15.3%。HS180 组脾细胞培养上清的 IL-2 水平明显高于其他各组（$P<0.05$），ConA 和 LPS 诱发脾细胞增殖百分比，前者高于对照的各组，后者则低于 HNS 及 MNS 组（$P<0.05$）。**结论**：本法可有效抑制肿瘤的生长，此作用可能是细胞免疫参与所至。

关键词　肿瘤抗原　穴位注射　免疫治疗

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Objective: To observe the effect of Liandai Tablet(LDT, composed of Rhizoma Copitidis, Fructu Evodiae, Indigo Pulverata Levis, etc.) on preventing experimental gastric and duodenal cancer and interfering p21ras, c-erbB2, Rb, p53 oncogene expression. **Methods:** Administering N-methyl-N'-nitro-N-nitrosoguandidine(MNNG) to the drinking water for rats to induce gastric and duodenal cancers and then treating the rats with LDT for 3 months(gastrogavage, 1.5g/kg). The occurrence of carcinoma was observed by pathological examination and the expression of p21ras, c-erbB2, Rb, p53 in lesion tissues were examined by S-P or LSAB immunohistochemical technique. **Results:** The incidence of gastric and duodenal carcinoma in LDT group was 5.6% (1/18), which was significantly lower than that of the control group (38.9%, 7/18, $P<0.05$). The average score of pathological quantitive analysis in LDT group was also lower than that of the control group ($P<0.05$). Immunohistochemical examination found that in 8 rats of the control group, 5 rats showed positive p21 ras expression and 3 showed positive c-erbB2 expression, while in all the 7 rats of the LDT group, the above two expressions were negative. Rb and p53 were negative in all rats of both groups. **Conclusions:** LDT has a preventive effect on gastric and duodenal cancer in rats, which might be related to the depression of p21ras and c-erbB2 oncogene expression, but it could not protect the deficit of Rb. Besides, p53 is not the target gene of MNNG-induced cancer in rats.

Key words Liandai tablet, gastric cancer, oncogene

(Original article on page 252)

Experimental Research on the Mechanism of the Inhibitory Effect of Jiawei Sijunzi Decoction (加味四君子汤) in Rats with Gastric Precancerosis

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Objective: To study the mechanism of the inhibitory effect of Jiawei Sijunzi decoction (JSD) on gastric precancerosis of rats. **Methods:** N-methyl-NNG was used to induce the model of gastric precancerosis in Wistar rats, and JSD was given to the rats at the same time. **Results:** The precancerosis-inducing rate in the model group was 35.0%(7/20); in the JSD group and the control group, the precancerosis-inducing rates were 11.1%(2/18) and 10.5%(2/19) respectively, and the different was significant when compared with model group. **Conclusion:** The cell of gastric precancerosis has developed the structural change of specific epiglycosyl and the JSD could inhibit the precancerous process of gastric.

Key words gastric cancer, precancerosis, Jiawei Sijunzi decoction

(Original article on page 254)

Experimental Study on Anti-Tumor Effect of Intratumoral Injection with Rongailing(溶癌灵) Together with the Extract of Brucea Javanica Shell

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Objective: To prepare a new intratumoral injection -Rongailing(RAL) and study its anti-tumor effect. **Methods:** The cytotoxic component of Brucea javanica shell was extracted by ethanol extraction method. Then the component was mixed with polyvinylpyrrolidone (PVP) and Xiaoailing(XAL, a Chinese anti-tumor drug), to prepare a suspending emulsion RAL. The anti-tumor effects of the RAL were evaluated by cytotoxicity experiment in vitro, and tumor-inhibiting

experiments in vivo, which were performed on nude mice model bearing HHC15 hepatoma line. **Results:** Cytotoxicity experiments showed that the cytotoxicity of RAL was equivalent to that of the extract of Brucea javanica shell, but higher than that of XAL and 5-FU. The results of tumor inhibiting experiments showed that intratumoral injection with RAL was more effective than XAL, ethanol and 5-FU in respect of inhibiting tumor growth, minimizing skin lesion and healing of wound. **Conclusion:** RAL might be a valuable new drug suitable to intratumoral injection for tumor therapy.

Key words Rongailing suspending emulsion, tumor therapy, intratumoral injection, Brucea javanica shell

(Original article on page 256)

Experimental Study on the Inhibiting Effect of Tumor Growth by Injecting Tumor Antigen into the Acupuncture Point

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Objective: To study the inhibitory effect on tumor growth by injecting tumor antigen into the acupuncture point. **Methods:** The extract of S_{180} cell line was used as antigen, the mice were inoculated with 10^6 S_{180} cell at their axillary fossae. **Results:** The number of mice which did not bear the tumor and survived to 100 days in the group treated with injection of antigen into the point was significantly larger than that in the control groups. The percent of the mice which did not bear the tumor in HS_{180} (S_{180} cell was injected to Houhai point), MS180 (S_{180} cell was injected to control point), HNS (normal saline was injected into Houhai acupoint), and MNS (normal saline was injected into control point) groups were 80.0%, 71.4%, 21.4%, and 15.3% respectively. The interleukin-2 level of the spleen cell cultural supernatant in HS_{180} group was significantly higher than that in the control groups. The percent of T-cell proliferation in the test of Con-A and LPS stimulated spleen cell proliferation in HS_{180} group was higher than that in HNS and MNS groups ($P<0.05$). **Conclusion:** The tumor growth could be effectively inhibited by injection with tumor antigen into the acupuncture point and this effect might be attributed to the involvement of cell immunity.

Key words tumor antigen, acupuncture injection, immunotherapy,

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Experimental Study on Intratumoral Administration with Sustained-Release Preparation of Norcantharidin-Poloxamer 407

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Objective: To enhance therapeutic effects and reduce adverse effects of norcantharidin (NCTD) on anti-tumor. **Methods:** Poloxamer 407 (P407) gel was used as a sustained-release vehicle for topical administration of NCTD. The toxicity of different preparations of NCTD in mice were observed, respectively. The anti-tumor effects of NCTD or NCTD in P407 (NCTD/P) on SD rats implanted with W_{256} carcinoma were also studied. **Results:** (1) The toxicity of the sustained-release preparation of NCTD in P407 gel was lower than that of free NCTD. (2) There were significantly slower tumor growth, more extensive tumor necrosis and longer survival time in SD rats treated with NCTD/P than those treated with free NCTD. **Conclusion:** The NCTD in P407 gel appeared to be less toxic and have more tumoricidal effects than that equivulent dose of free NCTD, mainly because NCTD in P407 might stay in the injected location for a longer time and produce a lower peak level in plasma than free NCTD.

Key words neoplasm, norcantharidin, poloxalene, interventional therapy

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