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牛膝多糖的抗肿瘤活性及其免疫增强作用

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摘要 牛膝多糖(ABP) 50 mg·kg^{-1} ip 或250 mg·kg^{-1} ig 显著抑制小鼠移植性肉瘤S180生长，提高荷瘤小鼠低下的血清 IgG 含量和抗体形成细胞数量及脾淋巴细胞增殖反应. ABP ip 还提高荷瘤鼠 NK 细胞活性及 LPS 诱生的血清 TNF-α 产生. ABP 50—800 μg·ml^{-1} 体外对 S180细胞无直接细胞毒作用，但能增强 MΦ 对 S180的杀伤作用. 提示 ABP 抗肿瘤作用与其增强宿主免疫功能有关.

关键词 牛膝；多糖；抗体形成细胞；免疫球蛋白 G；淋巴细胞转化；自然杀伤细胞；肿瘤坏死因子

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Synergistic effect of oridonin and cisplatin on cytotoxicity and DNA cross-link against mouse sarcoma S180 cells in culture

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ABSTRACT Oridonin (Ori) is an active principle isolated from *Rabdosia rubescens*. The cytotoxic effect of the combination of Ori and cisplatin was tested by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazoliuim bromide] colorimetric assay. IC_{50} of cisplatin to cultured S180 cells in 24 h was 9.38 μg·ml^{-1}. When the cells were treated with cisplatin plus Ori 0.5 and 1 μg·ml^{-1}, the IC_{50} were 1/3.4 and 1/6.7, respectively, of that with cisplatin alone. Modified alkaline elution was used to detect the DNA interstrand cross-link and DNA-protein cross-link in S180 cells induced by the 2 drugs. A greater amount of DNA cross-link was detected when the cells were treated with cisplatin plus Ori than with cisplatin alone ($P<0.05$). After lysis by proteinase K, a reduction in DNA cross-link was seen, which suggested that the drugs could produce both kinds of DNA cross-link.

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KEY WORDS cisplatin; oridonin; drug combinations; cytotoxins; DNA; cross-linking reagents

Oridonin (Ori) is a diterpenoid compound isolated from *Rabdosia rubescens* (hemsl). It has been used alone or in combination with other drugs to treat human cancers, especially

esophageal carcinoma[1]. Cisplatin causes a nephrotoxicity and the rapidly developing resistance of cancer cells to cisplatin restricted its clinical usage[2]. We found that Ori potentiated the antitumor activity of cisplatin *in vivo* on ECA, S180, and leukemia P388[3]. In order to elucidate the interaction between the 2 drugs, their cytotoxicity and DNA cross-linking were tested.

MATERIALS AND METHODS

Drug and cell line Cisplatin was purchased from the First Pharmaceutical Factory of Jinzhou. Oridonin was gifted by the Chemical Drug Factory of Zhengzhou. Stock solution of the 2 drugs were prepared by vigorous stirring in phosphate buffered solution immediately prior to use. The mouse sarcoma S180 cell line (ascites type) has been maintained in our laboratory for several years by weekly abdominal passages. The cells were taken from the peritoneal cavity of mice on d7 after inoculation, then washed twice and cultured at a cell density of $10\times10^4-30\times10^4$ cell·ml^{-1} in RPMI-1640 medium (Gibco) supplemented with 10% calf serum and HEPES 15 mmol.

Methyl thiazolyl tetrazolium colorimetric assay MTT (Fluka) colorimetric assay for cell survival was performed[4,5]. The S180 cells were incubated with the drugs for 1 or 24 h in a 200 μl medium, and then centrifugalized ($700\times g$) for 5 min. The supernatant 100 μl was drawn and MTT-medium (5 mg·ml^{-1}) 10 μl was added. Then the cells were incubated for another 4 — 6 h in the medium containing MTT, and lysed with 10% HCl-isopropanol 100 μl. Viable cells were monitored for conversion of MTT to formazan by a multiscan 96-well microtiter plate reader at 570 nm. Growth inhibition rates were calculated by the following formula:

$$\text{Cytostasis}\ (\%)=(1-\frac{\text{Absorbance of treated}}{\text{Absorbance of control}})\times100\%$$

Alkaline elution method for DNA interstrand cross-link and DNA protein cross-link DNA of the S180 cells (30×10^4 cell·ml^{-1}) was labeled by growing the cells for 24 h in 11.1 kBq [^{3}H]thymidine (Research Institute of Isotopes, Chinese Academy of Atomic Sciences, specific activity: 1.0 TBq·ml^{-1}). Then the cells were incubated in a nonradioactive medium containing the drugs for 4 h. The drug-treated cells were washed and incubated for an additional 12 h in a fresh medium. Then modified alkaline elution[6] was performed to monitor the two kinds of DNA cross-link. The cells which had been subjected to 3000 rad ^{60}Co γ-rays were lysed, with or without proteinase K (E Merck). After lysis, the radioactivity remaining on the filters and in the eluted fractions was determined by counting it in a FJ-2101G liquid scintillator (№ 262 Factory in Xi-an). The increase in DNA retention on the filters reflected the total DNA cross-link. The part of DNA retention on filters after lysis with proteinase K was DNA interstrand cross-link (ISC). The total minus ISC left DNA-protein cross-link (DPC). The aim of background irradiation was to lyse out most of the DNA strains without DNA cross-linking.

RESULTS

Cytotoxicity studies The cytotoxic effect of cisplatin and Ori used singly or together at various concentrations was estimated by MTT assay. When the duration of treatment was 1 h, there was no significant difference between the effects of the single and combination treatment groups ($P>0.05$ in most of the tested concentrations). The dose-response effect of a 24 h incubation with Ori and/or cisplatin on S180 cells population was depicted in Fig 1. Both drugs produced a dose-related cytotoxicity when used singly. When Ori at lower concentrations was used together with various concentrations of cisplatin, a sharp increase in cytotoxicity was seen in comparison with that of cisplatin alone, especially at lower levels of cisplatin. IC_{50} of cisplatin or cisplatin in combination with Ori 0.5 and 1.0 μg·ml^{-1} were 9.38, 2.72, and 1.41 μg·ml^{-1}, respectively (Fig 1).

DNA cross-link No increase in DNA retention on filter was noted when the cells were treated with Ori for 4 h, indicating that Ori did not cause any DNA cross-link. Cisplatin caused a dose-dependent increase in DNA

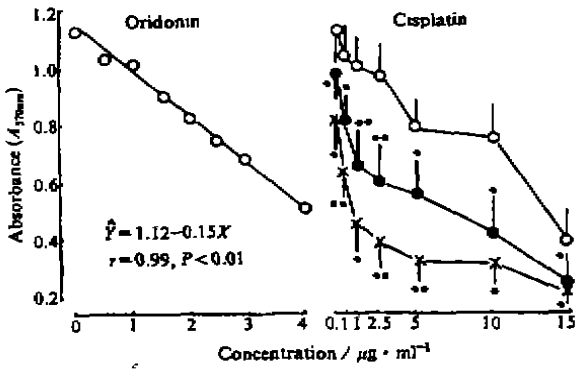

Fig 1. The cytotoxic effect of oridonin and cisplatin used singly or in combination on S180 cells tested by MTT colorimetric assay. ○ cisplatin or oridonin, ● cisplatin + oridonin 0.5 μg·ml⁻¹, × cisplatin + oridonin 1 μg·ml⁻¹. $n=4$ samples/dose. $\bar{x}\pm s$, $^*P>0.05$, $^{**}P<0.05$ vs single drug groups.

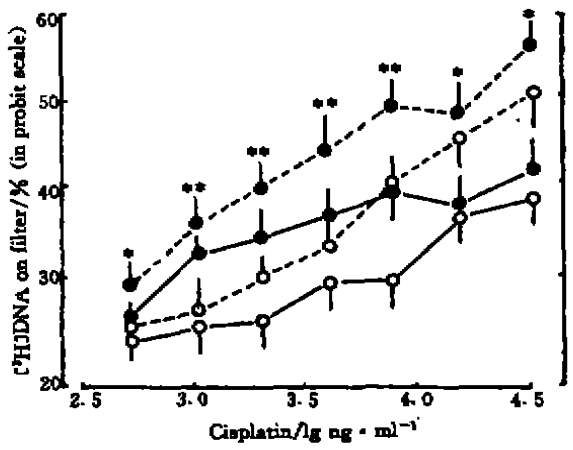

Fig 2. Effect of oridonin on S180 cell DNA cross-link induced by cisplatin. The cells were treated with cisplatin without (○) or with oridonin 2.0 μg·ml⁻¹(●), then lysed with (—) or without (···) proteinase K. $n=4$ samples/dose, $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$ vs cisplatin alone.

cross-link. A lesser amount of DNA cross-link was obtained when the cells were digested with proteinase K, suggesting the existence of DNA-protein cross-link. When cisplatin was simultaneously used with Ori 2.0 μg·ml⁻¹, a prominent increase in DNA cross-linking was found at low concentrations of cisplatin. DNA interstrand cross-link constituted the bulk of the increment in DNA cross-link induced by the combination (Fig 2).

DISCUSSION

The effect of the combination in this test was defined by the following equation[7,8]:

$$q=E_{A+B}/(E_A+E_B)$$

($q>1.15$: synergism; $q<0.85$: antagonism; $0.85<q<1.15$: addition)

Where E_A and E_B are the inhibitory effect of each drug used singly, and E_{A+B}, the effect of the combination. The combination produced synergistic cytotoxic effect at most concentrations chosen for this study, with q up to about 2.90 at the best combination regimen (cisplatin: 1 μg·ml⁻¹, Ori: 1 μg·ml⁻¹). Cisplatin at concentration much below the common clinical peak serum level[9,10] could produce prominent synergistic cytotoxicity when used together with Ori. Moreover, the 24 h or longer continuous exposure schedule may be a particularly attractive model for clinical use, since low dose with prolonged course was important for the synergism.

The technique used in this study did not permit the assessment of DNA intrastrand cross-link, which constituted more than 98% of cisplatin adduct[11]. However, there was a positive correlation between the extent of ISC and DPC formation and cisplatin's cytotoxic activity[12,13]. At least, an increase in ISC and DPC could be viewed as an indication of altered cellular response to platinum-DNA adducts in the presence of Ori. Ori was demonstrated to inhibit DNA synthesis by reducing the activity of DNA polymerase I[14], which was also essential to the repairment of DNA-platinum adducts. The increase in DNA cross-link may be a result of the delayed restoration of the damaged DNA. Still, the increased quantity of DNA cross-link detected in this test was perhaps not sufficient to explain the markedly increased cytotoxicity in

vitro. Other activity of the combination, such as their activity on DNA chain breakage and DNA polymerases, should be studied.

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冬凌草甲素和顺铂对体外小鼠肉瘤S180细胞的细胞毒和DNA交联的协同作用

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A **摘要** 冬凌草甲素(Ori)是从碎米桠中提取的有效成分. 用MTT比色法测定Ori与顺铂合用的细胞毒性. Ori 0.5, 1.0 μg·ml^{-1}与顺铂合用于S180细胞24小时的IC_{50}值分别为顺铂单用的1/3.4, 1/6.7. 用改良的碱性洗脱法测定两药引起的DNA链间交联和DNA蛋白质交联. 合用较单用引起的交联量明显增加($P<0.05$). 蛋白酶K洗脱后，交联减少，提示有两种DNA交联存在.

关键词 顺铂；冬凌草甲素；合并用药；细胞毒素；脱氧核糖核酸；交联试剂