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• LIVER CANCER •

Anti-hepatoma activity of resveratrol in vitro

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Abstract

AIM: **To study the anti-tumor effect of resveratrol alone and the synergistic effects of resveratrol with 5-FU on the growth of H22 cells line *in vitro*.**

METHODS: **The number of cells was measured by MTT method,the morphological changes of H_{22} cells were investigated under microscopy and electron microscopyq.**

RESULTS: **Resveratrol inhibited the growth of hepatoma cells line H_{22} in a dose- and time-dependent manner. IC_{50} of the resveratrol on H_{22} cells was 6.57mg·L^{-1}. The synergistic anti-tumor effects of resveratrol with 5-FU increased to a greater extent than for H22 cells treated with 5-FU alone (70.2% *vs* 28.4%)(P<0.05).Under microscope and electron microscope, characteristics of apoptosis such as typical apoptotic bodies were commonly found in tumor cells in the drug-treated groups.**

CONCLUSION: **Resveratrol can suppresses the growth of H_{22} cells *in vitro*, its anti-tumor activity may occur through the induction of apoptosis.**

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INTRODUCTION

Hepatoma is common in China[1-20],but only a few chemotherapeutic drugs hold a high place in the treatment of human primary hepatocellular carcinoma (PHC).Resveratrol, a phytoalexin found in grapes, fruits, and root extracts of the weed Polygonum cuspidatum, has been an important constituent of Japanese and Chinese folk medicine. Indirect evidence suggests that the presence of resveratrol in white and rose wine may explain for the reduced risk of coronary heart disease associated with moderate wine consumption.This effect has been attributed to the inhibition of platelet aggregation and coagulation, in addition to the antioxidant and anti-inflammatory activity of resveratrol[21-28].Moreover, a recent report shows that resveratrol is a potent cancer chemopreventive agent in assays representing three major stages of carcinogenesis[29-35].The ability to inhibit cellular events associated with tumor initiation, promotion, and progression has been attributed to the anticyclooxygenase activity (COX-1) of resveratrol[36]. We report here the results of our findings showing that resveratrol inhibited the growth of hepatoma cells line H_{22}.

MATERIALS AND METHODS

Reagents

Resveratrol was kindly provided by Prof Li(Environment and Chemical Engineering School,Xi'an Jiaotong University)and dissolved in dimethylsulfoxide (DMSO);MTT was obtained from Sigma. PRMI1640 containing 100 mL·L^{-1} fetal bovine serum (FBS) was bought from Gibco. All other chemicals were standard commercial products of analytical grade.

Cell culture

H_{22} cells were obtained from Center of Molecular Biology of First Hospital, Xi'an Jiaotong University and routinely cultured in RPMI 1640 containing 100 mL·L^{-1} FBS at 37℃ in an atmosphere with 50mL·L^{-1} CO_2.

Assay of cell proliferation

H_{22} cells were plated in 96-well plates (2×10^4/well) for 24 h before the addition of resveratrol. Medium was then aspirated and replaced with fresh RPMI 1640 + 100 mL·L^{-1}FBS containing resveratrol for 48 h. Different compositions to be tested were added according to designed groups: group A (cell control group) with nothing added, group B (DMSO control group) with DMSO 5 mL·L^{-1}, group C1-5 with Resveratrol (1.25,2.50,5.0, 10.0 and 20.0 mg ·L^{-1}),group D 1-25-FU (2400 and 1200 mg·L^{-1}), group E with Resveratrol 5.0 mg ·L^{-1}+5-FU 1200 mg ·L^{-1} .Each group had 4 wells and was cultured for 48 h. The number of cells was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) method as described in Sigma Technical Bulletin (Sigma, MO). Absorbance at 570nm (A) was assayed at different time points.The A value was adjusted with the living cell number. Each sample was assayed three times. Inhibition rate(%)=(1-experimental A/control A)×100%.

Morphologic observation

After the cellular culture for 48h, cells in groups A,C and E were observed and photographed with an Olympus BH-I microscope and a Hitachi-600 electron microscope.

RESULTS

Growth inhibition of H_{22} cells

H_{22} cells at 2×10^4/well were incubated with different concentrations of resveratrol for 8 - 48 h and the effect of resveratrol on the cells growth was examined by MTT assay. The growth of H_{22} cells was markedly inhibited by resveratrol with the IC50 value of 6.57 mg·L^{-1}. Moreover,the cytotoxicity of resveratrol was in concentration-dependent and time-dependent manners (Table 1) . The inhibition ability of 5-FU was 49.2%(2400mg·L^{-1}),28.4% (1200 mg·L^{-1}) respectively;The inhibiting ability of resveratrol (5.0 mg·L^{-1})combined with 5-FU(1200mg·L^{-1}) was higher than that of 5-FU alone(70.2% *vs* 28.4%,P<0.05).

Morphology observation

Apoptotic cells were found in cells incubated with resveratrol. Light microscopic observation showed that apoptotic cells were characterized with cytoplasmic condensation, vacant bubbles, and condensed nuclei(Figure 1). Under electron microscope, H_{22} cells exhibited the characteristics of apoptosis including cytoplasmic condensatin, pyknotic nuclei, condensed chromatin and apoptotic bodies(Figures 2,

3). Compared with control groups, groupC and E had much more cells with the apoptotic characteristics.

Table 1 Effect of various concentrations of resveratrol on the growth of hepatoma cells H_{22}

c(resveratrol) / mg ·L^{-1}	8h	12h	24h	48h
0.00	-	-	-	-
1.25	11.4	12.6	13.4	16.8
2.50	23.4	29.3	30.2[a]	32.6[a]
5.00	30.5[a]	31.2[a]	36.4[a]	43.5[a]
10.0	32.2[a]	38.3[a]	45.1[a]	62.2
20.0	38.2[a]	45.9[a]	65.2[b]	74.9[b]

[a]P<0.05;[b]P<0.01 *vs* control

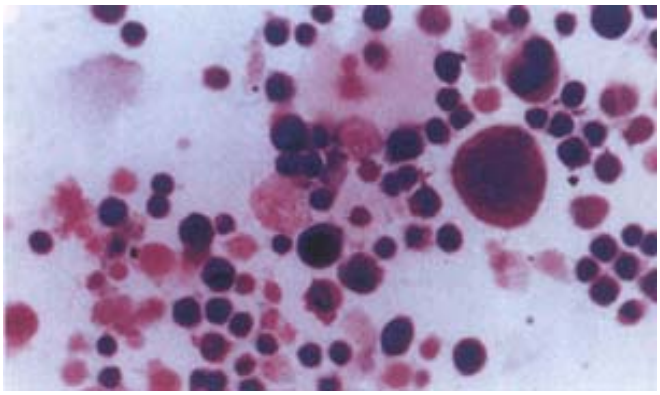

Figure 1 Treatment with resveratrol for 48 hours: apoptotic cell with condensed nuclei and cytoplasmic condensation (×200).

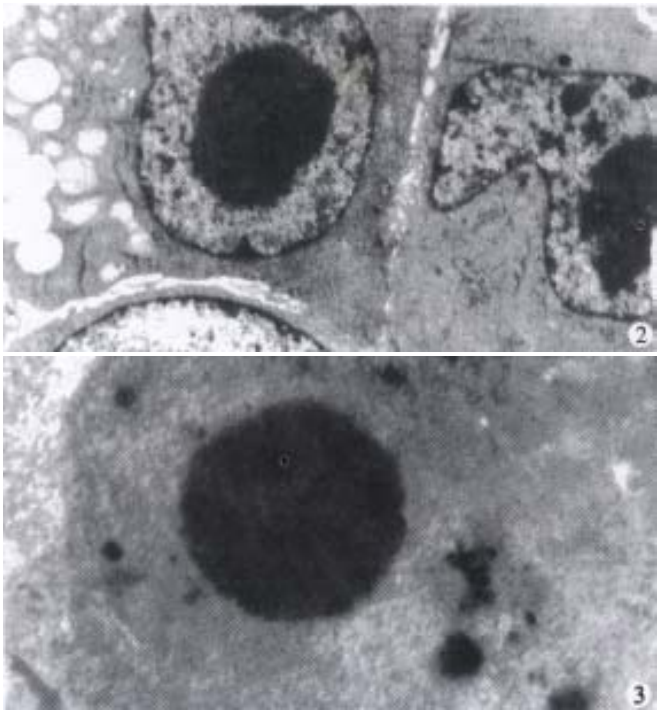

Figure 2 Cytoplasmic condensation with vacant bubbles, pyknotic nuclei, some with condensed chromatin inside (×5000).
Figure 3 Apoptotic body (×10 000).

DISCUSSION

To date, only a few chemotherapeutic drugs hold a high place in the treatment of human primary hepatocellular carcinoma (PHC) and there is clearly a need for evaluation of new anti-hepatoma drugs. Resveratrol (3,5,4'-trihydroxystibene), a natural compound present in grapes and other food, has been shown to provide cancer chemopreventive effects in different systems based on its striking inhibition of diverse cellular events associated with tumor initiation, promotion, and progression[29-35,37]. At the molecular level, these effects were related to the inhibition of free radical formation and cyclooxygenase activity[36], as well as induction of differentiation. In addition, resveratrol was shown to be a remarkable inhibitor of ribonucleotide reductase and DNA synthesis with cellular arrest in the S phase or the S-G2 phase transition[38-40]. In the present study, MTT assay was used to observe the effect of resveratrol on the growth of H_{22} mouse hepatoma cells *in vitro*, indicating that the drug could inhibit the growth of hepatoma cells. Its concentration- and time-effect relationships were also significant. Compared with control groups, group C had much more cells with apoptotic characteristics. The plausible mechanisms that could account for the anti-tumor activity of resveratrol might be related to induce apoptosis of tumor cells[41-48].

Resveratrol combined with 5-FU inhibited the cell growth much more strongly than each agent used alone. At a certain concentration, resveratrol inhibits H_{22} cell growth with the same effect as using 5-FU alone. Combination of resveratrol and 5-FU could have a cooperative effect. Both drugs inhibit cell growth at different phases of the cell cycle, i.e, resveratrol mainly causes G2/M arrest[39-40] and 5-FU mainly inhibits DNA synthesis(S phase) which naturally decreases the cellular growth more significantly. Our study indicates that combined use of resveratrol and 5-FU at low concentration that is used to treat hepatoma may be more efficient than using a single drug at higher concentration.The side-effects produced by 5-FU at the high doses can be avoided by its combination at lowdoses. These results suggest that resveratrol, may be potentially useful as a biochemical modulator to enhance the therapeutic effects of 5-FU in cancer chemotherapy.

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