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• LIVER CANCER •

Antitumor and immunomodulatory activity of resveratrol on experimentally implanted tumor of H22 in Balb/c mice

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Abstract

AIM: To study the antitumor and immunomodulatory activity of resveratrol on experimentally implanted tumor of H22 in Balb/c mice

METHODS: The cytotoxicity of peritoneal macrophages (M ϕ) against H22 cells was measured by the radioactivity of [^{3}H]TdR assay, mice with H22 tumor were injected with different concentrations of resveratrol, and the inhibitory rates were calculated and IgG contents were determined by single immunodiffusion method. the plaque forming cell (PFC) was measured by improved Cunningham method, the levels of serum tumor necrosis factor-α (TNF-α) were measured by cytotoxic assay against L929 cells.

RESULTS: Resveratrol 2.5 mg·L^{-1}, 5.0 mg·L^{-1}, 10.0 mg·L^{-1}, 20.0 mg·L^{-1} (E:T=10:1, 20:1) promoted the cytotoxicity of M ϕ against H22 cells. Resveratrol ip 500 mg·kg^{-1}, 1 000 mg·kg^{-1} and 1 500 mg·kg^{-1} could curb the growth of the implanted tumor of H22 in mice. The inhibitory rates were 31.5 %, 45.6 % and 48.7 %, respectively (P<0.05), which could raise the level of serum IgG and PFC response to sheep red blood cell. Resveratrol 1 000 mg·kg^{-1} and 1 500 mg·kg^{-1} and BCG 200 mg·kg^{-1} ip could increase the production of serum TNF-α in mice H22 tumor. However, the effect of resveratrol was insignificant (P >0.05).

CONCLUSION: Resveratrol could inhibit the growth of H22 tumor in Balb/c mice. The antitumor effect of resveratrol might be related to directly inhibiting the growth of H22 cells and indirectly inhibiting its potential effect on nonspecific host immunomodulatory activity.

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INTRODUCTION

Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the multi-stage carcinogenesis. Resveratrol (3,5,4' -trihydroxy-trans-stilbene), a phytoalexin found in grapes and other dietary and medicinal plants, has been shown to have anti-inflammatory, antioxidant and antitumor activities[1-7]. Many of these beneficial effects of resveratrol require participation of the cells of the immune system. However, the effect of resveratrol on the development of immuological responses remains unknown.

In the present study, the antitumor activity and immunomodulatory actions of resveratrol, including M ϕ against H22 cells, serum IgG and the plaque forming cells and tumor necrosis factor (TNF-α) content in Balb/C mice with experimentally implanted tumor of H22 were investigated.

MATERIALS AND METHODS

Materials

Resveratrol, MTT, IPS and dimethylsulfoxide (DMSO) were purchased from SGMA Co. Mouse hepatocellular carcinoma cells H22, L929 and sheep red blood cell (SRBC) were kindly supplied by Cheng Wei (Center of Molecular Biology, First Affiliated Hospital, Xi' an Jiaotong University). Cells were subcultured in RPMI 1640 (Gibco) which was supplemented with 10 % fetal bovine serum, penicillin (100 IU·ml^{-1}) and streptomycin (100 mg·l^{-1}). Stock solution of resveratrol was made in dimethylsulfoxide (DMSO) at a concentration of 10 g·L^{-1}. Working dilutions were directly made in the tissue culture medium. [^{3}H]TdR was purchased from Shanghai Nuclear Research Institute. IL test kit and LPS were purchased from Gibco Co. Balb/C mice, 2.5 month old, weighing 20±2 g, were purchased from the Animal Center, Xi' an Jiaotong University.

Methods

Effect of resveratrol on cytotoxicity of peritoneal macrophages (M ϕ) against H22 cells M ϕ was collected from the peritoneal cavity of Balb/c mice 3 days after ip 10 % sheep red blood cells (SRBC,1 ml/mouse). The cells were washed twice and resuspended in RPMI 1640 culture medium. H22 cells were cultured for 12 h, and 100 μl M ϕ suspension and different concentrations of resveratrol were added to each well of 96-well plates at a ratio of effectors: target (E:T) cell 10:1 or 25:1. After cultured for 24 h, each well was added with [^{3}H]TdR (9. 3 kBq/well), and then was incubated for another 6 h. Cells were placed onto the glass fiber filter and [^{3}H]TdR incorporation was determined by liquid scintillation. The cytotoxicity was calculated with the following formula: the cytotoxicity of M ϕ =(control-treatment)/control×100 % (dpm).
Anti-tumor activity of resveratrol and its effect on serum antibody IgG, plaque forming cells (PFC) in Balb/C mice with implanted tumor of H22 Mouse ascites (including 2×10^5 cells) were injected into the right axilla of 40 Balb/c mice. On the second day, 40 Balb/c mice were divided into 4 groups randomly, and then were injected with resveratrol at a dose of 500 mg·kg^{-1},1000 mg·kg^{-1}, 1 500 mg·kg^{-1} and normal saline for 10 d. Mice were sensitized to ip SRBC (3×10^7 cells). After 4 d, the mice were bled to obtain serum for IgG investigation. At the same time, spleens were excised for PFC counting. IgG contents were determined by single immunodiffusion method. PFC was measured by modified Cunningham method.
Effect of resveratrol on serum tumor necrosis factor alpha

(TNF-α) production induced by LPS in Balb/c mice Ascites cells of 2×10^5 were injected into the Balb/c mice. Resveratrol at a dose a 500 mg· kg^{-1}, 1 000 mg· kg^{-1} and 1 500 mg· kg^{-1} was injected for 10 d, and BCG of 200 mg· kg^{-1} as a positive control agent was injected ip on d 1.On d 11, 90 minutes after ip LPS of 0.1 mg· kg^{-1}, the mice were exsanguinated. Blood was centrifuged (400×g,10 min). The levels of serum TNF-α were measured by cytotoxic assay against L929 cells as described previously. The TNF-α activity was calculated with the following formula: cytotoxicity=$(A_{control}-A_{test})/A_{control}\times100$ %.

RESULTS

Effect of resveratrol on cytotoxicity of peritoneal macrophages (M φ) against H22 cells

Resveratrol at 2.5 mg· l^{-1} could decrease the cytotoxicity of M φ against H22 cells (P>0.05). Resveratrol at l2.5 mg· l^{-1}, 5.0 mg· l^{-1}, 10.0 mg· l^{-1} could enhance insignificantly the cytotoxicity of M φ against H22 cells (P>0.05) concentration-dependently, However, resveratrol at 20.0 mg· l^{-1} could raise significantly the cytotoxicity of M φ against H22 cells (P<0.05) (Table 1).

Table 1 Effect of resveratrol on cytotoxicity of peritoneal macrophages (M φ) against H22 cells *in vitro* (n=3)

Resveratrol (mg·l^{-1})	Cytotoxicity of M φ: H22 (10:1)	φ(%) M φ: H22 (25:1)
Control	12.6±7.9	15.6±6.0
1.25	10.9±2.9[a]	10.6±5.4[a]
2.50	12.5±3.2[a]	16.4±1.8[a]
5.0	13.4±2.8[a]	27.6±2.6[a]
10.0	14.6±3.7[a]	18.3±4.2[a]
20.0	16.7±4.7[b]	20.2±3.1[b]

[a]P>0.05, [b]P<0.05 *vs* control.

Anti-tumor activity of resveratrol and its effect on serum antibody IgG, plaque forming cells(PFC) in Balb/c mice with implanted tumor of H22

Resveratrol ip at a dose 500 mg· kg^{-1}, 1 000 mg· kg^{-1} and 1 500 mg· kg^{-1} could significantly curb the growth of implanted tumor of H22 in mice. The inhibitory rates were 31.5 %, 45.6 % and 48.7 %,respectively (P<0.05, Table 2).

Table 2 Inhibitory rates of resveratrol on H22 in mice *in vivo*

Group	Dose mg.kg^{-1}	Route	Mice begin/end	Tumor weight (x±s) (g)	Inhibitory rate (%)	P value
Control		ip	10/9[b]	1.81±0.62		
Resveratrol 1	500	ip	10/8[b]	1.24±0.40	31.5	<0.05[a]
Resveratrol 2	1000	ip	10/9[b]	0.99±0.35	45.6	<0.05[a]
Resveratrol 3	1500	ip	10/10	0.93±0.25	48.7	<0.05[a]

[a]P<0.05 *vs* control; [b]killed by other mice.

The result also showed that the immunity of mice with tumor could be more significantly inhibited than that of normal mice, and resveratrol ip could raise the level of serum LgG and number of PFC in Balb/c mice with implanted tumor of H22 *in vivo*. Resveratrol, however, could insignificantly increase the immunity of mice with tumor (P>0.05, Table 3).

Effect of resveratrol on serum tumor necrosis factor alpha (TNF-α) production induced by LPS in Balb/c mice

The ability of TNF-α production of mice with H22 tumor was significantly stronger than that of normal mice. Furthermore, the group of control and BCG at 200 mg· kg^{-1} ip had an increase in the at production of serum TNF-α in mice with H22 tumor (P<0.05), but resveratrol at a dose of 500 mg· kg^{-1}, 1 000 mg· kg^{-1} and 1 500 mg· kg^{-1} had less effect on mice with H22 tumor (P>0.05, Table 4).

Table 3 Effects of resveratrol on serum antibody IgG, plaque forming cells (PFC) in Balb/c mice with implanted tumor of H22 *in vivo*

Group	Dose mg.kg^{-1}	Route	IgG/g·L^{-1}	PFC/10^6 cells
Normal mice	NS	ip	27±8	441±32
Control	NS	ip	19±6[a]	297±57[a]
Resveratrol 1	500	ip	20±8[a]	305±53[a]
Resveratrol 2	1000	ip	23±6[a]	328±49[a]
Resveratrol 3	1500	ip	24±5[a]	348±46[a]

[a]P>0.05 *vs* normal mice or control.

Table 4 Effect of resveratrol on TNF-α production induced by LPS in Balb/c mice

Group	Dose (mg· kg^{-1})	Route	TNF-α activity specific lysis
Normal mice	NS	ip	7.1±3.2
Control	NS	ip	16.3±2.3[a]
Resveratrol 1	500	ip	15.8±2.0[ab]
Resveratrol 2	1000	ip	17.7±2.9[ab]
Resveratrol 3	1500	ip	19.5±3.1[ab]
Control+BCG	200	ip	29.8±3.7[ab]

[a]P<0.05 *vs* normal mice; [b]P>0.05 *vs* control.

DISCUSSION

Resveratrol is a phytopolyphenol isolated from the seeds and skin of grapes. Recent studies have indicated that resveratrol can block the process of multistage carcinogenesis, namely, tumor initiation, promotion and progression. Resveratrol can also reduce the risk of cardiovascular diseases in man. These activities have been identified by some authors[8-13]. Roberto *et al*[14] have shown that PBMC exposure to resveratrol produced a biphasic effect on the anti-CD3/anti-CD28-induced development of both IFN-y-IL-2 and IL4-producing CD8+ and CD4$^+$T cells, with stimulation at low resveratrol concentrations and suppression at high concentrations. Similarly, the compound was found to induce a significant enhancement at low concentrations and suppression at high concentrations of both CTL and NK cell cytotoxic activity. Gao *et al*[15] found that mitogen-, IL-2- or alloantigen-induced proliferation of splenic lymphocytes, induction of cytotoxic T lymphocytes (CTLs) and lymphokine activated killer (LAK) cells, and production of the cytokine interferon (IFN)-y, interleukin (IL)-2, tumor necrosis factor(TNF)-α were significantly suppressed at 25-50 μM resveratrol, but in some cases mitogen/IL-2-induced production and CTL generation were enhanced following pretreatment of cells with resveratrol. The effects of resveratrol on immune function of mice *in vivo* have not been reported yet.

Our results indicate that resveratrol of 2.5 mg· l^{-1} could decrease the cytotoxicity of M φ against H22 cells (P>0.05). Resveratrol of 2.5 mg· l^{-1}, 5.0 mg· l^{-1} and 10.0 mg· l^{-1} could insignificantly enhance the cytotoxicity of M φ against H22 cells concentration-dependently (P>0.05). However, resveratrol of 20.0 mg· l^{-1} could raise significantly the cytotoxicity of M φ against H22 cells (P<0.05). So, resveratrol could alone affect the [^{3}H]TDR uptake by H22 cells *in vitro*,

suggesting that the antitumor action of resveratrol had a direct cytotoxic effect. This result is coincident with the previous studies[16-18]. Resveratrol ip could insignificantly increase the host nonspecific immunological defense of mice with H22 tumor, by raising the level of serum IgG and TNF-α and the number of PFC (P>0.05). *In vivo* resveratrol could also augment the cytotoxicity of peritoneal macrophages against H22 cells, and there was an insignificant difference compared with the control group (P>0.05). Therefore, resveratrol could inhibit the growth of H22 cells *in vivo*, but it could not significantly enhance the host immune defense against tumor. Based on the results of the present study, it can be suggested that the antitumor activity of resveratrol might be due to direct cytotoxic/antiproliferative activity against tumor cells, but not to the augmentation of immune response against tumors. It has demonstrated that resveratrol inhibits cell proliferation, cell-mediated cytotoxicity, and cytokine production, at least in part through the inhibition of NF-kappa B activation. But the molecular mechanism by which resveratrol imparts cancer chemopreventive effects has not been clearly defined and further studies are needed.

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