Met is independent of lower doses, but rested upon higher dosages.

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537—542 (16)

剂量对美托洛尔离体大鼠肝灌流消除的影响

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A **摘要** 用0.2，0.5，1.0和2.0 mg 美托洛尔进行离体大鼠肝灌流时，消除呈一级动力学. $T_{\frac{1}{2}}$分别为8.3，8.8，9.6和10.6 min. 用4.0，8.0和12.0 mg 美托洛尔灌流时，消除呈零级动力学. V_m 分别为0.98，1.05和0.94 $\mu g \cdot min^{-1} \cdot ml^{-1}$. K_m 分别为15.5，16.9和14.6 $\mu g \cdot ml^{-1}$. 提示低剂量美托洛尔肝消除无剂量依赖性，较高剂量呈剂量依赖性.

关键词 美托洛尔；肝脏；灌流；药物动力学 R96

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Nov, 14 (6) : 542—545

Antitumor activity and tumor necrosis factor production of *Phytolacca acinosa* polysaccharides I in mice[1]

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ABSTRACT The antitumor activities of *Phytolacca acinosa* polysaccharides I (PAP-I) and its effects on the induction of tumor necrosis factor (TNF) and immunological cytotoxicity of peritoneal macrophages were studied. PAP-I was given ip 5—20 mg·kg^{-1}·d^{-1}×7 d to ICR mice as priming agent with subsequent lipopolysaccharides (10 μg/mouse) iv for TNF production. TNF activity was measured by crystal violet staining assay using L929 cells. PAP-I showed priming activity for TNF production with hepto-splenic hyperplasia in a dose-dependent manner. The peritoneal macrophages treated with PAP-I 10 and 20 mg·kg^{-1} showed 67 and 74%, respectively, cytotoxicity (the control 34% cytotoxicity) against Meth A cells at effector:target = 40:1. PAP-I 10 and 20 mg·kg^{-1} prolonged the survival time of mice bearing ascites Meth A tumor from 21±4 to 32±10 and 38±8 d and inhibited the solid Meth A tumor growth with inhibition rate of 28.5 and 55.7%, respectively. These results sug-

Received 1991-08-30 Accepted 1993-06-24

[1] Project supported by the National Natural Science Foundation of China, № 3880748.

gested that the antitumor activities of PAP-I were based on the activation of macrophages and induction of TNF.

KEY WORDS *Phytolacca acinosa*; polysaccharides; tumor necrosis factor; macrophages; immunological cytotoxicity

The tumor necrosis factor (TNF) is considered to be one of the most promosing anticancer cytokines because of its potent and very specific antitumor effect on target cells[1,2]. There are two possible ways for the clinical application of TNF; one is to use inducer of intrinsic TNF and the other is to use extrinsic TNF. Ever since TNF is identified with cachectin and is known to have many side effects, great efforts have been made to search for non-toxic inducers of intrinsic TNF[3-7].

Phytolacca acinosa polysaccharides I (PAP-I), one of the major ingredients extracted from the root of traditional antitumor herb *Phytolacca acinosa* Roxb, was an acidic polysaccharide of 10 kDa with low toxicity. We found that PAP-I activated macrophages and enhanced TNF and interleukin-1 production by peritoneal macrophages in mice[7]. Here we studied the antitumor activity of PAP-I on TNF production and immunologic cytotoxicity of macrophages.

MATERIALS AND METHODS

Agents Lipopolysaccharides (LPS) from Coli 0111 : B4 was purchased from Sigma. Dactinomycin (Dac) was purchased from Xinya Pharmaceutical Co, Shanghai. *Corynebacterium parvum* (*CP*) was purchased from Lanzhou Biological Products Research Institute.

Cells and tumor L929 cells, fibroblasts derived from C3H/HeN mice, were used for the assay of TNF activity. They were maintained in the culture medium composed of RPMI 1640 medium (Sigma), 10% heat-inactivated newborn calf serum, 2-mercaptoethanol 10 $\mu mol \cdot L^{-1}$, $NaHCO_3$ 2 $mg \cdot ml^{-1}$, penicillin 3.0 $\mu g \cdot ml^{-1}$, and streptomycin 25 $\mu g \cdot ml^{-1}$ at 37℃ in a humidified atmosphere of 5% CO_2 in air.

Syngeneic Meth A fibrosacoma, originating from a BALB/c mouse, was used. For antitumor test, Meth A maintained in the peritoneal cavity of BALB/c mice by weekly sequential transplantations was used. For cytotoxicity test *in vitro*, Meth A cells were maintained in suspension culture in RPMI 1640 culture medium.

Mice and drug treatment BALB/c mice, ♀, 6—8 wk old, weighing 19.5±*s* 0.4 g were used for Meth A tumor transplantation. ICR mice, ♀, 8—10 wk, weighing 26.3±*s* 3.1 g were used for macrophage immunologic cytotoxicity test and induction of TNF.

Mice were injected ip PAP-I 5—20 $mg \cdot kg^{-1} \cdot d^{-1} \times 7$ d. Control mice were injected with equal volume of saline without PAP-I. In some experiments mice were injected a single dose of *CP*.

Antitumor activity of PAP-I against Meth A tumors Meth A cells (1×10^6) suspended in sterile saline were inoculated ip or sc into mice which were then treated with PAP-I. The solid tumor weight (W, mg) was estimated from the linear dimensions of width (A, mm) and length (B, mm) of tumor on d 8 using the formula: $W = 0.5 \times A^2/B$. The inhibition rate of tumor growth (IR, %) was calculated. The survival time of mice bearing ascites tumors was recorded.

Production and quantification of TNF Mice were given PAP-I for priming and 7 d later received an iv injection of LPS 10 μg/mouse. Blood, liver, and spleen were taken 2 h after LPS injection.

TNF activity in serum was evaluated by cristal violet staining assay[8] *in vitro*. Serially diluted sera were incubated with 5×10^4 L929 cells/well in 96 flat microplate in the presence of Dac ($1 \mu g \cdot ml^{-1}$) for 18 h at 37℃. Recombinant TNF alpha was included as an internal standard of TNF in each assay. Units of TNF were defined as the reciprocals of the dilution necessary to lyse 50% L929 target cells.

Macrophage immunologic cytotoxicity test The effector cells were obtained from mice treated with PAP-I on d 8. Cytotoxicity of peritoneal macrophages to tumor cells were assessed by [^{3}H]TdR incorporation assay[7].

Statistical analysis was made by *t* test.

RESULTS

Antitumor effects of PAP-I against Meth

A tumors The development of solid tumors was inhibited in a dose-dependent manner. The best effect was seen at PAP-I 20 mg $\cdot kg^{-1}$, having IR 55.7%. the survival time of mice bearing ascites tumor were prolonged by PAP-I. In the group of PAP-I 20 mg $\cdot kg^{-1}$ the prolonged lifespan of mice was comparable to that of *CP* treatment (Tab 1).

Tab 1. Antitumor effects of *Phytolacca acinosa* polysaccharides I (PAP-I) and *Corynebacterium parvum* (CP) on Meth A tumors. BALB/c mice inoculated sc or ip Meth A cells (1×10^6) on d 0 were given ip×7 or a single dose of 0.5 ml *CP* ip. The tumor weight was estimated from the linear dimensions of tumor on d 8. IR (%) was the inhibition rate of tumor growth. T/C (%) was the ratio of median survival days of treatment group to that of control. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Treatment	Dose/ mg·kg^{-1}	Tumor weight/g	IR/%	Survival/ days	T/C, %
Control		1.4±0.4	—	21±4	100
CP	0.5 ml	0.78±0.16	44.3^c	41±8	195^c
PAP-I	5	1.3±0.5	7.1^a	23±4	110^a
	10	1.0±0.3	28.5^a	32±10	152^c
	20	0.62±0.25	55.7^c	39±8	186^c

Effects of PAP-I on peritoneal macrophages cytotoxicity Macrophages from PAP-I treated mice had significantly higher cytotoxicity than the control (Tab 2).

Tab 2. Effects of PAP-I and *CP* on *in vitro* cytotoxicity of macrophages against Meth A cells. Macrophages were obtained on d 8 from ICR mice treated with PAP-I ip×7 or a single dose of 0.5 ml *CP* ip. $n=3$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Treatment	Dose/ mg·kg^{-1}	Cytotoxicity$^+$ at effector:target 10:1	20:1	40:1
Control		19±7	22.0±2.1	34±11
CP	0.5 ml	47±10^c	53±4^c	72±3^c
PAP-I	10	31±11^b	52±8^c	67.3±2.5^c
	20	21±9^a	63±12^c	74±11^c

$^+\%\text{cytotoxicity}=(1-\frac{\text{dpm in treatment sample}}{\text{dpm in control}})\times100$

Priming activity of PAP-I for TNF production TNF activities in the sera from mice treated with PAP-I were significantly higher than that of control and exhibited in a dose-dependent manner. A significant decrease in body weight/organ weight of liver and spleen was seen (Tab 3).

Tab 3. Effects of PAP-I and *CP* on TNF production, liver and spleen weights in ICR mice. TNF activity was assessed by crystal violet staining assay using L929 cells in the presence of dactinomycin (1 μg · ml^{-1}). $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Treatment	Dose/ mg·kg^{-1}	Body weight/ organ weight Liver	Spleen	TNF activity/ U·ml^{-1}
Control		18.5±2.9	131±24	68±51
CP	0.5 ml	11.4±1.0^c	73±14^c	8 337±988^c
PAP-I	5	14.7±0.8^b	117±25^a	113±37^a
	10	13.1±1.9^c	97±18^c	269±125^c
	20	12.9±2.7^c	129±50^a	1 461±1 096^b

DISCUSSION

We demonstrated previously that *Phytolacca acinosa* polysaccharides I exerted no direct cytotoxic effect on tumor cells and showed some immunological regulating activities[7]. In the present study, treatment with PAP-I was effective in inhibiting the solid Meth A tumor growth and prolonging the survival time of mice bearing ascites tumors. These results indicates that antitumor activities of PAP-I are likely mediated by host immune defense mechanism.

Macrophage-mediated immune defense has been well documented to be an important host mechanism for dealing with tumors. Macrophages displayed an antitumor activity through not only a direct tumoricidal action but also by production of soluble tumoricidal factor such as TNF[1,8]. The present results demonstrated that PAP-I activated *in vivo*

murine macrophages capable of inhibiting the proliferation of tumor cells *in vitro*. In addition to the cytotoxic activity, these macrophages were also shown to release interleukin 1 and TNF in acid of LPS[7]. These would seen to qualify PAP-I as an useful stimulant for macrophages. On the other hand, TNF activity was observed in the sera of mice treated with PAP-I as a priming agent (Tab 3). Taken together, these results suggest that antitumor activities of PAP-I may be related to the stimulation of macrophages and induction of endogenous antitumor factor like TNF and may be used as an inducer of TNF in the clinical treatment of neoplastic diseases.

Large differences in the capacity for TNF production were found among individuls. This phenomenon was also seen in other research group[8]. The reason for the difference remains unknown and needs to be investigated.

ACKNOWLEDGMENTS We acknowledge Dr Y SOHMURA (Dainippon Pharmaceutical Co Ltd, Osaka, Japan) for generously providing recombinant human TNF and Meth A cells and Mr CHEN Hai-Sheng for PAP-I.

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商陆多糖I抗瘤活性及对小鼠产生肿瘤坏死因子的作用[1]

R965.2

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A 摘要 小鼠ip商陆多糖I(PAP-I)5—20mg·kg^{-1}·d^{-1}×7 d，在脂多糖辅助下呈剂量依赖地诱生肿瘤坏死因子(TNF)。10和20 mg·kg^{-1}组的腹腔巨噬细胞(MΦ)对Meth A细胞毒%分别为67%和74%，显著高于生理盐水组(34%)。两组对Meth A实体瘤的抑瘤率分别为28.5%和55.7%；对腹水型小鼠存活期从21±4延长到32±10和38±8 d。提示PAP-I激活MΦ和启动诱生TNF是其抗瘤作用机制之一。

关键词 商陆，多糖，肿瘤坏死因子，巨噬细胞，免疫细胞毒性