sorbed amount of diazepam was not sufficient to counteract the elimination of the drug from blood, thus the plasma concentration declined steadily without the second peak.

The results of this study suggested a general implication for the drugs with biochemical characteristics similar to those of diazepam, such as temazepam and veralipride, which also exhibited double peaks of their blood concentration-time curves following a single dose[9,10].

Enterogastric circulation, like enterohepatic circulation, may exert some influence on the pharmacokinetic behavior of certain drugs. Its theoretical and practical significance calls for further investigation.

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地西泮血浆浓度的第二峰与肠胃循环

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摘要 兔 iv 地西泮 5 mg·kg^{-1}，随即经胃管灌食物可使血药浓度出现明显的第二峰. 对兔行胃造瘘术和胆总管造瘘术后，第二峰消失. 从胃液中检测到大量地西泮，同期只有很少量的药物出现在胆汁中. 结果提示，地西泮在体内除肠肝循环外，还可进行肠胃循环，而第二峰的出现主要与后者有关.

关键词 地西泮；药物动力学；胃液；胆汁；胃造瘘术；胆总管造瘘术

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Effects of 4-[4″-(2″,2″,6″,6″-tetramethyl-1″-piperidinyloxy)amino]-4′-demethylepipodophyllotoxin on immune function in mice

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ABSTRACT 4-[4″-(2″,2″,6″,6″-Tetramethyl-1″-piperidinyloxy)amino]-4′-demethylepipodophyllotoxin (GP-7) 10—40 mg·kg^{-1} ip daily for 7 d reduced the specific antibody formation of splenocytes, serum

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agglutinin titer, and hemolysin HC_{50} in mice immunized with SRBC. GP-7 inhibited the footpad delayed hypersensitivity reaction and decreased the weights of spleen and thymus, but did not affect the phagocytic function of the peritoneal macrophages. *In vitro* the proliferation of mouse splenic lymphocytes activated by Con A was markedly inhibited by GP-7 in a concentration- dependent manner. At concentrations of 0.05 — 5 mg · L^{-1}, the inhibition rates were 24 — 96%. These results suggested that GP-7 was an immunosuppressive agent.

KEY WORDS podophyllotoxin; immunosuppression; delayed hypersensitivity; hemolysins; T-lymphocytes; phagocytosis

Introduction of nitroxy radical moiety into some antitumor drugs, such as thiotepa, could result in compounds with pharmacological properties superior to those of the parent compounds[1,2]. A new spin-labeled derivative of podophyllotoxin, 4-[4″- (2″, 2″, 6″, 6″-tetramethyl-1 ″-piperidinyloxy) amino]-4 ′-demethylepipodophyllotoxin (GP-7), was synthesized in Lanzhou University, China[3]. In our previous works[4—6], it was found that GP-7 markedly inhibited the growth of the transplanted mouse tumors sarcoma 180, solid carcinoma of ascitic hepatoma, leukemia P388 and Lewis lung carcinoma. *In vitro* it inhibited the proliferation of human gastric adenocarcinoma SGC-7901 and mouse leukemia L-1210 cells. Our recent works showed that GP-7 had a remarkable antitumor activity on bladder transitional cell carcinoma in patients by intratumor injection (data to be published). This experiment was to study the effects of GP-7 on immune function in mice.

MATERIALS

Drugs and chemicals GP-7 was synthesized by the Department of Chemistry, Lanzhou University. It was dissolved in 5% Tween-80 for ip in mice and in 20% Me_2SO (Demasorb) to a final concentration of 0.4% for the experiment *in vitro*. RPMI-1640 culture medium (Gibco) containing 15% calf serum, penicillin 100 IU · ml^{-1} (Suzhou Second Pharmaceutical Factory) and streptomycin 100 μg · ml^{-1} (Shanghai №4 Pharmaceutical Factory) was used as culture medium. Concanavalin A (Con A, Sigma).

Mice BALB c mice (either sex, aged 6—8 wk, weighing 20 ± *s* 2 g) were bred in the Animal Center of Lanzhou Institute of Biological Products.

METHODS AND RESULTS

Effects of GP-7 on serum agglutinin and hemolysin in mice Forty BALB c mice randomized into 4 groups were injected ip with GP-7 0, 10, 20, and 40 mg · kg^{-1} daily for 7 d, and immunized with 3×10^8 sheep red blood cells (SRBC) ip on d 3. The serum samples from mice were collected on d 8. Agglutinin titer and hemolysin HC_{50} were measured by agglutination test and spectrophotometry[7]. Following the above treatment the body weights of mice in groups of GP-7 0, 10, 20, and 40 mg · kg^{-1} were 25.1 ± 2.1, 25.4 ± 1.8, 23.9 ± 2.3, and 21.7 ± 2.3 ($P < 0.01$ *vs* 0 mg · kg^{-1}). GP-7 decreased the serum agglutinin titer and hemolysin HC_{50} in mice in a dose-dependent manner (Tab 1).

Tab 1. Effects of GP-7 ip 7 d on serum agglutinin and hemolysin in mice immunized with SRBC. *n* = 8. $\bar{x} \pm s$. $^{***}P < 0.01$ *vs* vehicle.

GP-7/ mg · kg^{-1} · d^{-1}	Agglutinin titer	Hemolysin HC_{50}
0 (vehicle)	508.3 ± 1.4	580 ± 93
10	381.2 ± 1.4	441 ± 58***
20	195.0 ± 1.9***	319 ± 46***
40	40.9 ± 1.4***	148 ± 5***

Effect of GP-7 on specific antibody formation of splenocytes in mice The mice were treated with the same program. On d 8 the spleens were excised after cervical dislocation, and the single cell suspensions were prepared by grinding gently against sterile stainless

less steel mesh. The specific antibody formation of splenocytes in mice was assayed by spectrophotometry[7]. Results showed that GP-7 inhibited the specific antibody formation of splenocytes in mice. The absorbances (at 540 nm) in the groups of GP-7 0, 10, 20, and 40 mg·kg^{-1} were 0.83±0.17, 0.72±0.14, 0.53±0.17, and 0.49±0.12 (n=10, P <0.01 *vs* 0 mg·kg^{-1}).

Effect of GP-7 on delayed hypersensitivity (DH) in mice Four groups of BALB c mice were treated ip with GP-7 0, 10, 20, and 40 mg·kg^{-1} daily for 7 d (d 1－7), immunized with 2×10^8 SRBC sc on d 3, challenged with 1×10^8 SRBC injected sc to the right footpad on d 8; a same volume of saline was injected sc to left footpad as control. DH was assayed by measuring the thickness difference between right and left footpads with a micrometer 24 h after challenge. Results showed that the DH induced with SRBC was inhibited by GP-7 10－40 mg·kg^{-1} ip for 7 d. The thickness differences between their right and left footpads decreased by 37－70% (Tab 2).

Tab 2. Effect of GP-7 ip 7 d on footpad delayed hypersensitivity (DH) in BALB c mice immunized with SRBC. n=10, $\bar{x}\pm s$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* vehicle.

GP-7/ mg·kg^{-1}·d^{-1}	Thickness of pad/mm Right	Left	Thickness difference/mm
0 (vehicle)	2.8±0.7	2.3±0.6	0.54±0.18
10	2.7±0.7	2.3±0.5	0.34±0.15**
20	2.5±0.5	2.3±0.6	0.23±0.11***
40	2.3±0.5	2.2±0.5	0.16±0.12***

Effect of GP-7 on proliferation of mouse splenic lymphocytes activated by Con A *in vitro* Splenocytes of 10 mice were prepared on ice and suspended in RPMI-1640 culture medium (2×10^8 cells·ml^{-1}). the suspension was put into the wells of 24-wells culture plates at 1 ml/well. GP-7 and Con A were added. Following the incubation in a 5% CO_2 incubator 37℃ for 56 h, [^{3}H]deoxythymidine ([^{3}H]TdR) was added into the wells to a final concentration of 37 kBq·ml^{-1}. After incubation in a CO_2 incubator for another 16 h, the cells were harvested on glass fiber filters, and the incorporated radioactivity was determined by a FJ-2100 liquid scintillation counter. The proliferation of mouse splenic lymphocytes activated by Con A *in vitro* was inhibited 24－96% by GP-7 0.05－5 mg·L^{-1} in a concentration- dependent manner (Tab 3).

Tab 3. Effect of GP-7 on proliferation of mouse splenic lymphocytes activated by Con A *in vitro*. n=6, $\bar{x}\pm s$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control (GP-7 0 mg·L^{-1}, Con A 3 mg·L^{-1}).**

GP-7/ mg·L^{-1}	Con A/ mg·L^{-1}	Radioactivity of [^{3}H]TdR in DNA/dpm
0	0	1 663±468
0	3	19 802±3721
0.05	3	15 114±2990**
0.5	3	6 087±932***
5	3	698±189***

Effect of GP-7 on peritoneal macrophage phagocytosis in mice After GP-7 ip for 7 d, the mouse was killed 1 h after ip 2% chicken RBC 1 ml. The cells in the peritoneal exudate were collected and examined (Giemsa stain, ×1000). No significant effect of GP-7 on the phagocytosis of peritoneal macrophages in mice was seen (Tab 4).

Effects of GP-7 on spleen and thymus weights in mice Forty BALB c mice randomized into 4 groups were injected ip with GP-7 0, 10, 20, and 40 mg·kg^{-1} for 7 d. Their spleens and thymuses were excised after cervical dislocation and weighed. The results showed that the weights of spleen and thymus decreased by 17－55% and 34－85%, respectively after GP-7 (Tab 5).

Tab 4. Effect of GP-7 ip 7 d on peritoneal macrophage phagocytosis in mice. $n=10$, $\bar{x}\pm s$, $^{*}P>0.05$ *vs* vehicle.

GP-7/ mg·kg^{-1}·d^{-1}	Phagocytosis/%	Phagocytic index
0 (vehicle)	46±6	0.86±0.12
10	48±6*	0.92±0.11*
20	39±8*	0.91±0.18*
40	42±7*	0.87±0.13*

Tab 5. Effects of GP-7 ip 7 d on spleen and thymus weights in mice. $n=10$, $\bar{x}\pm s$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* vehicle.

GP-7/ mg·kg^{-1}·d^{-1}	Spleen wt/mg	Thymus wt/mg
0 (vehicle)	183±28	68±17
10	152±30**	45±16***
20	108±31***	21±6***
40	82±16***	10±3***

DISCUSSION

Our results showed that the new podophyllotoxin derivative, GP-7 markedly suppressed the humoral and cell-mediated immunities in mice, suggesting that podophyllotoxin and its congeners may turn out to be a new series in the immunosuppressive agents. It was known that most of cytotoxic antitumor agents, such as cyclophosphamide, had an immunosuppressive activity to a certain extent. The inhibitory effects of GP-7 on immune function may also be attributed to its cytotoxic effect. However, the exact mechanism remained to be further investigated.

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4-[4″-(2″,2″,6″,6″-四甲基哌啶氮氧自由基氨基]-4′-去甲表鬼臼毒素对小鼠免疫功能影响

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摘要 4-[4″-(2″,2″,6″,6″-四甲基哌啶氮氧自由基氨基]-4′-去甲表鬼臼毒素(GP-7) 10－40 mg·kg^{-1} ip 7 d，降低小鼠脾细胞特异抗体的产生、血清凝集滴度和溶血素 HC_{50} 值，抑制小鼠足垫迟发型超敏反应，减轻小鼠脾和胸腺重量. 在体外，GP-7 0.5－5 mg·L^{-1}，处理72 h，Con A 刺激的小鼠脾淋巴细胞增殖率降低24－96%. GP-7对小鼠腹腔巨噬细胞功能无影响.

关键词 鬼臼毒素；免疫抑制；迟发型超敏感性；血清素类；T－淋巴细胞；吞噬作用