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Effects of tubeimoside—1 on HIV core protein p24 and cytopathogenesis *in vitro*[1]

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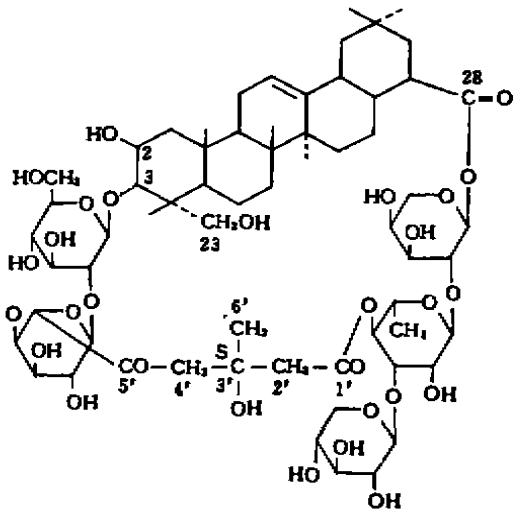
ABSTRACT Tubeimoside-1 (Tub) is a triterpenoid saponin first isolated in China from *Bolbostemma paniculatum* (Maxim) Franquet, Cucurbitaceae. To find out whether Tub has any anti-infective acitivity on human immunodeficiency virus (HIV), we tested its effects on HIV core protein p24 (with an ELISA) and HIV-mediated cytopathogenesis (using a colorimetric assay). The results showed that Tub inhibited both p24 production and cytopathogenesis mediated by HTLV-III_B, and the median effective concentrations (EC_{50}) were 24.1 and 22.9 $\mu g \cdot ml^{-1}$, respectively. Tub also effectively neutralized the infection of 2 other isolates, HTLV-III_{RF} and HTLV-III_{MN}. It is concluded that Tub had an inhibitory action on the infection of HIV-1 isolates and would be a promising candidate for treatment of AIDS.

KEY WORDS *Bolbostemma paniculatum*; tubeimoside-1; saponins; HIV-1; HIV core protein p24; viral cytopathogenic effect; T-lymphocytes; cultured cells

Chemotherapy for the acquired immunodeficiency syndrome (AIDS), which is caused by human immunodeficiency virus type 1, has been rapidly developed. 3'-Azido-3'-deoxythymidine (zidovudine) improves the clinical and immunologic status of patients with AIDS and AIDS-related complex[1,2], but suppresses the proliferation of erythroid, granulocyte, macrophage, and primitive hematopoietic stem cells[3], resulting in adverse effects like anemia and neutropenia[1]. Long-term treatment with zidovudine induces drug-resistance. Therefore, more effective and less toxic antiviral agents are urgently needed.

Bolbostemma paniculatum (Maxim) Franquet, Cucurbitaceae, has been used as a traditional Chinese medicinal plant for centuries. Tubeimoside-1 isolated from this plant[4] is a triterpenoid saponin with an inter-saccharide chain bridged by dicrotalic acid to form a unique macrocyclic structure[5].



Tubeimoside-1

We demonstrated the inhibitory effects of Tub on inflammatory mouse ear edema and on

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^{3}H-incorporation into phospholipids of cultured cells induced by 12-O-tetradecanoylphorbol-13-acetate[6]. Tub exhibited potent antitumor activity[7] and antitumor promoting activity on skin tumor formation induced by phorbol ester tumor promoter in 7,12-dimethylbenz(α)anthracene-treated mice by topical application[8]. Since Tub is effective to neutralize the activity of herpes simplex virus type 1[9], it is of great interest to study whether it is also has effect on HIV-1 infection.

MATERIALS AND METHODS

The plant was collected in Shaanxi Province (China) and identified by Associate Professor QUAN Yi-Shu (Department of Botany, Shaanxi Provincial Academy of Traditional Chinese Medicine and Pharmacology), and a voucher specimen was deposited in the Department of Biochemistry, Shaanxi Provincial Academy of Traditional Chinese Medicine and Pharmacology, Xi-an 710003, China.

Tub was isolated from the stem tuber by a modification of the method we reported previously[4]. Briefly, the dried and powdered tubers (500 g) were extracted with hot MeOH and then hot 50 % MeOH. After removal of the solvents by evaporation, the combined extracts were chromatographed on highly porous polymer, DIAION HP-20 (Mitsubish Chem Ind, Tokyo). The MeOH eluate was separated by column chromatography (CC) on silica gel and then reversed-phase silica gel, LiChroprep RP-8 (65 % aq. MeOH and 58 % aq. MeOH). This new compound: white needles, mp 252 — 2 (; yield 9.25 g (1.85 %); molecular formula $C_{55}H_{98}O_{29}$; M_r 1318. The purified Tub was dissolved in distilled water and stored at 4 (.

The T-lymphoblastoid cell line MT-2 cells (from Dr A K Fowler, SRA Technologies Inc, Alexandria VA, USA) were grown in RPMI-1640 (Gibco, Grand Island NY) with 10 % heat-inactivated fetal bovine serum (FBS, Gibco) and penicilin 100 units · ml^{-1} and streptomycin 100 μg · ml^{-1} (Gibco). The HTLV-III$_B$, HTLV-III$_{RF}$, and HTLV-III$_{MN}$ isolates of HIV-1 were cultivated in H$_9$ and MT-2 cells. Virus was obtained from producer cell culture supernatants by centrifugation (150 × g) followed by 0.8— μm filtration. The titers of the virus stocks were $1 \times 10^5 - 10 \times 10^5$ 50 % tissue culture infective doses ($TCID_{50}$) per ml of MT-2 cells.

Virus-neutralization assay The neutralizing activity of Tub on HIV-1 infection was determined by 2 methods: inhibition of synthesis of core protein p21 (nucleocapsid antigen of HIV-1) and protection of cells against the cytopathic effect of HIV-1 (colorimetric method). Tub was serially diluted in RPMI-1640 medium without phenol red (Gibco) containing 10 % FBS. The diluted solution was added to 96-well plates and mixed with an equal volume of diluted HIV-1 with multiplicity of infection (MOI) = 0.0015. After incubation for 1 h at 37 (, 25 μl of polybrene (1 μg · ml^{-1})-treated MT-2 cells (1×10^5 cells · ml^{-1}) were added. The mixture was incubated at 37 (, 100 μl of culture supernatants were collected from each well, and equal volumes of fresh medium were added to the wells. The supernatants were assayed for p21 using a kit from Coulter Immunology (Hialeah FL, USA). On d 6, an XTT tetrazolium dye indicator (1 mg · ml^{-1}; 50 μl/well; PolySciences Inc, Warrington PA, USA) was added to the wells. After 4 h, intracellular formazan was determined colorimetrically at 450 nm. The % of inhibition of HIV replication or cytopathogenesis was calculated: (C-E)/C × 100. E and C represent the amount of p21 or the % of cytotoxicity in the presence and absence of Tub, respectively. The median effective dose (ED_{50}) was calculated with a computer program for analysis of dose effect[10].

Cytotoxic assay Tub was serially diluted in RPMI-1640 with 10 % FBS and mixed with equal volume of MT-2 cells (1×10^5 · ml^{-1}). After 1 h, fresh culture medium was supplied to a total volume of 200 μl. On d 6 of incubation at 37 (, XTT was added and the optical density (OD) was measured as described above. Cytotoxity % was calculated: (OD_{450} in blank — OD_{450} in tested)/(OD_{450} in blank — OD_{450} in positive control) × 100. The cells were mixed with culture medium in the blank or with 5 % Triton X-100 in positive control. The median cytotoxic concentration (CC_{50}) was calculated using the same computer program[10].

RESULTS

Tubeimoside-1-mediated inhibition of HTLV-III$_B$ replication The virus core protein p24 was determined with ELISA. Tub

inhibited the production of p24 in a concentration-dependent manner (Fig 1). The EC_{50} of Tub for inhibition of $HTLV\text{-}III_B$ replication was 24.1 (94 % confidence limits 10.7 — 53.7) $\mu g \cdot ml^{-1}$.

Inhibition of HIV-mediated cytopathogenesis by Tub Tub markedly inhibited the cytopathogenesis mediated by $HTLV\text{-}III_B$ (Fig 1). The datum point at the dose of 8.0 $\mu g \cdot ml^{-1}$ of Tub was dispelled since most cells were lysed by Tub at this concentration. The EC_{50} was 22.9 (95 % confidence limits 9.6 — 54.9) $\mu g \cdot ml^{-1}$.

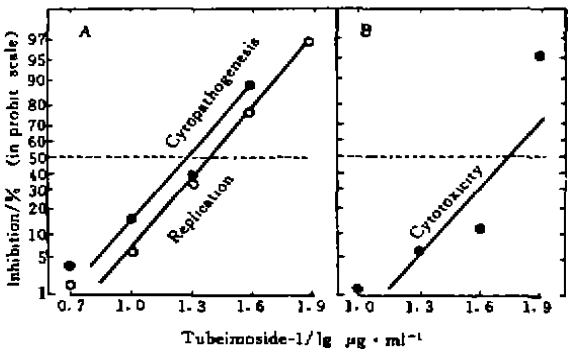

Fig 1. Inhibition of $HTLV\text{-}III_B$ replication and HIV-mediated cytopathogenesis by tubeimoside-1 (A) and cytotoxicity of tubeimoside-1 (B) *in vitro*. $n=3$.

Inhibiting replication of other HIV-1 isolates $HTLV\text{-}III_B$ is one of the HIV-1 isolated most used in the laboratories. Tub also effectively inhibited $HTLV\text{-}III_{MS}$ and $HTLV\text{-}III_{RF}$, and the EC_{50} were 24.9 and 19.7 $\mu g \cdot ml^{-1}$.

Cytotoxicity mediated by Tub Tub-mediated cytotoxicity was rapidly decreased when its concentration was diluted from 80 to 40 $\mu g \cdot ml^{-1}$. The cytotoxicity CC_{50} by Tub was 59 (95 % confidence limits 29.5 — 117.5) $\mu g \cdot ml^{-1}$.

DISCUSSION

Our results clearly showed that Tub inhibit HIV-1 replication and HIV-1-mediated cytopathogenesis *in vitro* and was effective in neutralizing the HIV-1 isolates tested. Aurintricarboxylic acid (ATA), the anionic triphenylmethane derivative, inhibits HIV-1 infection by targeting to both the reverse transcriptase and the V3 hypervariable loop of gp 120[11]. Tub did not target to the V3 hypervariable loop of HIV envelope glycoprotein, unlike ATA, since Tub did not inhibit the binding of antibody directed against V3 hypervariable loop of gp 120 to the V3 loop peptides which were coated on plate (data not shown). It is concluded that Tub is a candidate anti-HIV agent for future study.

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REFERENCES

1 Yarchoan R, Klecker RW, Weinhold KJ, Markham PD, Lyerly HK, Durack DT, *et al*. Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. Lancet 1986; (1): 575—80.

2 Fischl MA, Richman DD, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, *et al*. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N Engl J Med 1987; **317**: 185—91.

3 Damak N, Worthington M, Riordan MA, Kreczko S, Goldman L. 3'-Azido-3'-deoxythymidine (AZT) inhibits proliferation *in vitro* of human haematopoietic progenitor cells. Br J Haematol 1988; **69**: 299—304.

4 Wang YQ, Yu LJ, Zhu JL, Yang SY. Studies on anti-

tumor action of extracts of Bolbostemma paniculatum (Maxim) Franquet (short communication). Shaanxi Med J 1981; 10: 55—6.

5 Kong FH, Zhu DY, Xu RS, Fu ZC, Zhou LY, Iwashita T, *et al*. Structural study of tubeimoside 1, a constituent of tu-bei-mu. Terrahedron Lett 1986; 27: 5765—9.

6 Ma RD, Yu LJ, Wang YQ, Nishino H, Takayasu J. Potent inhibitory effects of tubeimoside 1 isolated from the bulb of *Bolbostemma paniculatum* (Maxim) Franquet on inflammatory ear oedema and tumor promotion in mice. Chin Sci Bull 1992; 37: 602—6.

7 Yu LJ, Ma RD, Wang YQ, Nishino H. Potent anti-tumor activity and low toxicity of tubeimoside 1 isolated from *Bolbostemma paniculatum* (Maxim) Franquet Cucurbitaceae. Planta Med, in press.

8 Yu LJ, Ma RD, Wang YQ, Nishino H, Takayasu J, He WZ, *et al*. Potent anti-tumorigenic effect of tubeimoside 1 isolated from the bulb of *Bolbostemma paniculatum* (Maxim) Franquet. Int J Cancer 1992; 50: 635—8.

9 Fu ZC, Zhao GS, Fang YL. Studies on pharmacology of tubeimosides. Shaanxi Med J 1985; 14: 49—53.

10 Chou J, Chou T-C. Dose-effect analysis with microcomputers; Quantitation of ED_{50}, LD_{50}, Synergism, antagonism, Low-dose risk, receptor ligand binding and enzyme kinetics. Cambridge UK; IBM-PC-Series Elsvier-Biosoft Science Publishers. 1987.

11 Cushman M, Wang P, Chang SH, Wild C, De Clercq E, Schols D, *et al*. Preparation and anti-HIV activities of aurintricarboxylic acid fractions and analogoues; direct correlation of antiviral potency with molecular weight. J Med Chem 1991; 34: 329—37.

土贝母苷甲在体外对人免疫缺陷病毒核心蛋白p24的产生和细胞病变的作用

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摘要 土贝母苷甲是首先在中国从土贝母中分离出的一种三萜皂苷。本文研究了它对人免疫缺陷病毒核心蛋白p24的产生和人免疫缺陷病毒介导的细胞病变的影响。结果表明土贝母苷甲既抑制p24的产生，也抑制细胞病变，其中位有效浓度(EC_{50})分别为24.1和22.9 $\mu g \cdot ml^{-1}$。土贝母苷甲也有效地中和另外2种分离株(HTLV-III_{RF}和HTLV-III_{MN})的感染。因此，土贝母苷甲可能是一种有希望的治疗艾滋病的药物。

关键词 土贝母；土贝母苷甲；皂苷类；人免疫缺陷病毒1型；人免疫缺陷病毒核心蛋白p24；病毒细胞病变效果；T-淋巴细胞，培养的细胞