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Neochromine S5 improves contact hypersensitivity through a selective effect on activated T lymphocytes

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ABSTRACT

Strategy on activated T cells is an effective treatment for T cell mediated diseases. By using a synthesized chromone derivative, we examined its effects on the activated T cells. This compound, (Z)-1,3-dihydroxy-9-methyl-13H-benzo[b]chromeno[3,2-f][1,4]oxazepin-13-one (neochromine S5), exhibited immunosuppressive activity in vitro and in vivo. Interestingly, neochromine S5 selectively inhibited proliferation and induced apoptosis in T lymphocytes activated by concanavalin A (Con A) in a dose-dependent manner but not in naïve T lymphocytes, distinct from quercetin. This compound triggered mitochondrial apoptotic pathway including cleavage of caspase 3, caspase 9 and PARP, downregulation of bcl-2 and release of cytochrome c in activated T cells, but did not affect ER stress or Fas signals. In addition, neochromine S5 downregulated the expression of CD25 and CD69 and the production of inflammatory cytokines, including TNFα, IFNγ and IL-2, improved ear swelling in mice with contact hypersensitivity, reduced $CD4^+$ T cells infiltration, and increased apoptosis of isolated T lymphocytes from peripheral lymph nodes. Moreover, neochromine S5 showed no effect on the weight of mice and their immune organs, while dexamethasone caused a significant weight loss. Taken together, our results suggest that neochromine S5 exerts a unique anti-inflammatory activity mainly through a selective effect on activated T cells, which is different from the current immunosuppressant, dexamethasone.

1. Introduction

During immune response, T lymphocytes will be activated and develop effector functions that help to kill the pathogens. However, the effector functions are also harmful to healthy tissue. To limit the damage, the host will evolve some mechanisms to shut down immune responses, including causing cell inactivation and inducing apoptosis of activated cells [1–3]. As to the mechanisms that participate in the progress of apoptosis, there include the direct activation of caspase cascade (mitochondrial pathway) [4], the activation of the Fas death receptor/Fas ligand (FasL) complex (death receptors) [5,6], and the ER stress-mediated pathway [7]. The activated T cells express several cell surface markers such as CD25 and CD69, and produce specific cytokines such as TNFα, IFNγ, IL-2, and so on. In addition, a number of studies have illustrated that the increased expression of CD25, CD69 and production of inflammatory cytokines correlates with autoimmune and inflammatory diseases [8–11]. By means of such mechanisms, the apoptosis induction in such as activated T cells as well as the inhibition on inflammatory cytokines or T cell activation has been used as an effective strategy in the drug discovery for various immune diseases [12,13].

However, there is still lack of drugs selectively targeting the effector population such as activated T cells to avoid the injury of irrelevant cells. In previous studies, we have reported that several herbal extracts showed a selective inhibition on activated T lymphocytes [12–14]. These are quite different from the commonly used immunosuppressants, which have severe side effects primarily owing to their poor selectivity, including glucocorticoids, cyclophosphamide and even cyclosporine A [15–17].

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In the present study, we studied their effect on activated and non-activated T cells and found that (Z)-1,3-dihydroxy-9-methyl-13H-benzo[b]chromeno[3,2-f][1,4]oxazepin-13-oneF (neochromine S5), a synthesized chromone derivative, inhibited the proliferation and increased apoptosis of activated T cells, but had little effect on non-activated cells. In contact hypersensitivity, neochromine S5 prevented ear swelling and inhibited $CD4^+$ T cells infiltration. Such selective effect of neochromine S5 was further confirmed in a murine model of T cell-mediated contact hypersensitivity.

2. Materials and methods

2.1. Synthesis of neochromine S5

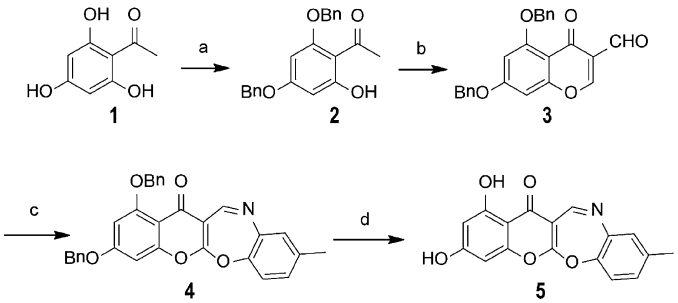
All reagents and solvents for the synthesis of neochromine S5 were commercially available and used without further purification. Melting points were determined on a Taike X-4 digital micromelting point apparatus and uncorrected. ^1H and ^{13}C NMR spectra were taken on a Bruker DPX-300 spectrometer, using TMS as an internal standard (chemical shifts in δ). ESI-HR-MS were obtained on Esquire 4000 mass spectrometer.

To a solution of 1-(2,4,6-trihydroxyphenyl)ethanone (**1**) (60 g, 0.36 mol) in HMPA (300 mL) was added K_2CO_3 (148 g, 1.07 mol) and BnCl (86.3 mL, 0.75 mol), and the suspension was stirred at 90 °C for 3 h. The solid was filtered, and the filtrate was poured into ice-water. The pH of the solution was adjusted to 2 by adding diluted hydrochloric acid. The resulting solid was filtered and recrystallized in CH_2Cl_2/MeOH to give 1-(2,4-bis(benzyloxy)-6-hydroxyphenyl)ethanone (**2**). Yield: 75%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.56 (3H, s) 5.06 (4H, s), 6.11 (1H, s), 6.17 (1H, s), 7.41 (10H, m), 14.04 (1H, s).

To a solution of **2** (15.0 g, 43 mmol) in DMF (100 mL) was added $POCl_3$ (19.5 mL, 213 mmol) dropwise at 0 °C and the solution was stirred at room temperature for 12 h. The reaction mixture was poured into ice water, and resulting solid was dissolved in CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified through chromatography eluting with CH_2Cl_2/EtOAc (25/1) to give 5,7-bis(benzyloxy)-4-oxo-4H-chromene-3-carbaldehyde (**3**). Yield: 70%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.11 (2H, s), 5.22 (2H, s), 6.56 (1H, d, J = 2.1 Hz), 6.58 (1H, d, J = 2.1 Hz), 7.42 (8H, m), 7.59 (2H, d, J = 7.5 Hz), 8.32 (1H, s), 10.38 (1H, s); ESI-MS m/z: 387 $[M+H]^+$.

To a solution of **3** (386 mg, 1 mmol) in toluene (10 mL) was added *o*-aminophenol (1 mmol) and *p*TSA (cat. amount), and the reaction mixture was stirred at 80 °C for 3 h and concentrated in vacuo. The residue was dispersed in xylene (10 mL) and DDQ (271 mg, 1.2 mmol) was added. The suspension was stirred at 130 °C for 3 h. The reaction mixture was cooled and poured into cold petroleum ether. The resulting solid was filtered and purified through chromatography to yield 1,3-bis(benzyloxy)-9-methyl-13H-benzo[b]chromeno[3,2-f][1,4]oxazepin-13-one (**4**).

To a solution compound **4** (0.5 mmol) in anhydrous CH_2Cl_2 at −78 °C was added a solution of BBr_3 in CH_2Cl_2 (1.1 mL, 1 M), and the suspension was stirred at room temperature for 8 h. The reaction was quenched by the addition of water (1 mL), and the resulting solid was filtered, washed with water and recrystallized in acetone/CH_2Cl_2 to afford (Z)-1,3-dihydroxy-9-methyl-13H-benzo[b]chromeno[3,2-f][1,4]oxazepin-13-one (**5**), which was named as neochromine S5. Yield: 52% (3 steps); mp 289–291 °C; ^1H NMR (C_5D_5N, 300 MHz) δ: 2.34 (3H, s), 6.69 (1H, s), 6.76 (1H, s), 7.15 (1H, d, J = 7.7 Hz), 7.55 (1H, d, J = 7.7 Hz), 7.70 (1H, s), 9.04 (1H, s), 13.34 (1H, br); ^{13}C NMR (C_5D_5N, 75 MHz) δ: 22.41, 96.68, 102.35, 106.61, 111.83, 113.90, 121.60, 128.10, 135.94, 143.43, 159.30, 161.14, 164.81, 167.89, 179.46; HRMS-ESI (m/z): calcd for $C_{17}H_{12}NO_5$ $[M+H]^+$: 310.0715 found: 310.0709.



Reagents and conditions: (a) BnCl, K_2CO_3, HMPA, 90 °C, 3 h; (b) $POCl_3$, DMF, 0 °C to rt, 12 h; (c) (i) *o*-aminophenol, *p*-TsOH, toluene, 80 °C, 3 h; (ii) DDQ, xylene, 130 °C, 3 h; (d) BBr_3, CH_2Cl_2, −78 °C to room temperature, 8 h.

2.2. Animals and cells

Female BALB/c mice (6–8 weeks of age, 18–22 g) were obtained from the Experimental Animal Center of Yangzhou University (Yangzhou, Jiangsu, China). They were maintained with free access to pellet food and water in plastic cages at 21 ± 2 °C and kept on a 12 h light–dark cycle. Animal welfare and experiments were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and the related ethical regulations of our university. All efforts were made to minimize animals' suffering and to reduce the number of animals used. Jurkat cell was purchased from the Shanghai Institute of Cell Biology (Shanghai, China).

2.3. Reagents and antibodies

CFSE cell proliferation kit was from Thermo Fisher Scientific Inc. (Waltham, MA). Annexin V-FITC/propidiumiodide (AV/PI) assay kit for flow cytometry was from Jingmei Biotech Co., Ltd. (Shenzhen, China). Cyclosporin A (CsA), thapsigargin (TG), concanavalin A (Con A), dimethylsulfoxide (DMSO), 3-(4, 5-dimethylthylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT), 2,4,6-trinitrobenzene-sulphonic acid (TNBS) and quercetin were obtained from Sigma (St. Louis, MO). Picryl chloride (PCl) was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Mouse anti-CD25-APC, mouse anti-CD69-FITC, mouse anti-CD44-FITC, mouse anti-CD62L-PE and mouse anti-CD4-FITC were from ebioscience Inc. (San Diego, CA). Mitochondrial membrane potential ($\Delta\Psi$m) assay kit with JC-1 was from Beyotime Institute of Biotechnology (Shanghai, China). Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) Kit was from Calbiochem (La Jolla, CA). TNFα, IFNγ, and IL-2 ELISA kits were from Dakewe Biotech Co., Ltd. (Beijing, China). Dexamethasone (DEX) was from Xianju Pharmaceutical Co., Ltd. (Zhejiang, China). Fetal bovine serum (FBS) and RPMI 1640 medium were purchased from Thermo Fisher Scientific Inc. (Waltham, MA). PCl was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Glycylglycin and all other chemicals were obtained from Nanjing Sunshine Biotechnology Co., Ltd. (Nanjing, China). The antibodies used in this study were as follows: anti-α-tubulin, anti-GADD153, anti-p-PERK (Santa Cruz Biotechnology, Santa Cruz, CA), anti-XBP-1, anti-PARP, anti-cleaved caspase 3, anti-cleaved caspase 9, anti-Bad, anti-Bcl-2 and anti-COX-IV (Cell Signaling Technology, Boston, MA), anti-cytochrome *c* (BD Pharmigen, San Diego, CA), peroxidase-labeled anti-mouse and anti-rabbit antibody (KPL, Gaithersburg, MD).