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Original Research Article

Immunosuppressive effect of voacamine from *Voacanga africana* Stapf based on SPRi experiment

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Abstract

Purpose: *To investigate the affinity of a bis-indole alkaloid - voacamine from Voacanga Africana Stapf for IL-2Rα - and its immunosuppressive effect on concanavalin A-induced T cell proliferation and lipopolysaccharide -induced B cell proliferation in vitro.*
Methods: *Surface plasmon resonance imaging (SPRi) was used to screen the target protein of voacamine, while CCK-8 kit was used to evaluate cytotoxicity. Mitogen-induced proliferation assay was carried out to assess the inhibitory effect of voacamine on Con A-induced T cell proliferation and LPS-induced B cell proliferation. The binding characteristics of voacamine were investigated using a binding model with IL-2Rα constructed based on molecular docking simulation.*
Results: *Voacamine had a high-affinity for IL-2Rα with an equilibrium dissociation constant (K_D) of 1.85×10^{-8} M. Cytotoxicity data showed that voacamine did not exhibit cytotoxicity at concentrations lower than 0.32 μM. However, it exerted significant immunosuppressive effect on B cells at a lower concentration, but had no influence on proliferation of T cells. Autodock results indicate that voacamine has a good interaction with the enzyme active site.*
Conclusion: *Voacamine and its analogues exert influence on the immune system.*

Keywords: *Immunosuppressive, IL-2Rα, SPRi, Voacanga africana Stapf Voacamine*

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INTRODUCTION

Interleukin-2 (IL-2) is a member of a cytokine family involved in the immune system. It mediates its effects by binding to three different cell surface receptors: IL-2Rα chain, IL-2Rβ chain and IL-2Rγ chain [1]. The receptor IL-2α is expressed by T cells after activation of T cell receptors. The interaction of IL-2 with its receptors leads to proliferation of T cell, B cell, and natural killer (NK) cell, and clonal expansion [2].

Voacanga africana Stapf ex Scott-Elliot is a small tropical tree found mainly in West Africa [3]. The crude extract of its stem bark exerts a variety of pharmacological effects, and it has been used for the treatment of leprosy, diarrhea, generalized oedema, convulsions in children, and mental problems [3-5]. Previous studies showed that

alkaloids from this plant are bioactive compounds [6,7]. Voacamine (Figure 1) is one of the major alkaloids with strong antitumor effects [8]. It is also used with chloroquine and artemisinin for malaria treatment *in vivo* [9].

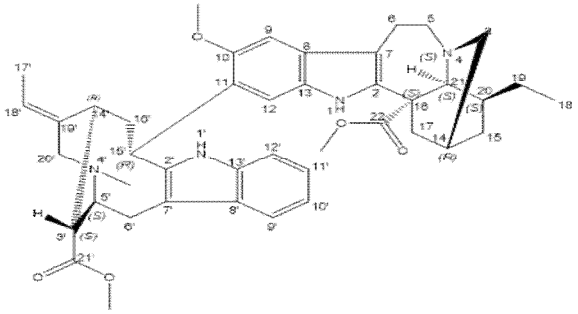

Figure 1: Structure of voacamine

EXPERIMENTAL

Plant material

The bark of *V. africana* Staph. was purchased from Ghana, Africa in April 2012 and was identified by Dr Hai-Feng Liu, Sichuan University. A voucher specimen (no. FKS-20120421-JN) was deposited in the herbarium of Laboratory of Chinese Medicinal Chemistry, Pharmaceutical College, Chengdu University of Traditional Chinese Medicine.

Measurement of SPRi

The measurement of SPRi was carried out in line with the procedure described previously [10]. Proteins were diluted to a final concentration of 0.5 mg/mL with PBS and applied onto bare gold-coated (thickness 47 nm) PlexArray Nanocapture Sensor Chip (Plexera Bioscience, seattle, WA, USA) at 40% humidity, using PDMS. The surface of the chips was first activated with sulfo-NHS/EDC (0.1 and 0.4 M, in Milli-Q water). The chip was blocked with 1 M ethanolamine (pH 8.5) for 30 min. All measurements were performed at the wavelength of 660 nm. Then, the sample (20 μM) and positive control (rapamycin; 10 μM) were injected into the 30 μL flow cell at a flow rate of 1 μL sec^{-1} using a non-pulsatile piston pump. The surface was washed with PBS at 1 μL sec^{-1}, and regenerated with 0.5 % (v/v) H_3PO_4 at 1 μL sec^{-1} for 300 sec. All measurements were performed at 4 ℃. The signal changes after binding and washing were recorded, and selected protein-grafted regions in the SPRi were analysed. Real-time binding signals were recorded and analysed with Data Analysis Module (DAM, Plexera Bioscience, Seattle, WA, USA).

Assay of biological activity

The biological activity was assayed as described in a previous study [11,12].

Reagents

Concanavalin A (Con A), lipopolysaccharide (LPS, *Escherichia coli 055*: B5), CCK-8; WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, monosodium salt], and RPMI 1640 medium were purchased from GibcoBRL, Life Technologies (USA). Fetal bovine serum (FBS) was product of HyClone Laboratories (Utah, USA). [^{3}H]-Thymidine (10 μCi/mL) was obtained from the Shanghai Institute of Atomic Energy (SIAE).

Experimental animals

BALB/c mice were purchased from Shanghai Experimental Animal Center and were housed in a controlled environment (12-h light/12-h dark photoperiod at a temperature of 22±1℃ and relative humidity of 55 ± 5 %. All animal experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals [13] and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica (nos. SCXK 2002-0010; SCXK 2003-0029).

Preparation of spleen cell suspensions

Mice were sacrificed by cervical dislocation, and mononuclear cell suspensions of spleens were prepared after the removal of cell debris and clumps. The erythrocytes were lysed with Tris-buffered ammonium chloride. Lymphocytes were washed and re-suspended in RPMI 1640 medium containing 10 % fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 mg/mL).

Cytotoxicity assay

The mice spleen cells were seeded into 96-well plates at a density of 1×10^{-6} cells per well in the presence or absence of various concentrations of voacamine solution, and incubated in humidified incubator at 37℃ and 5 % CO_2 for 48 h. Then, a certain amount of CCK-8 was added to every well after 8 - 10 h of culture, and absorbance was read at a wavelength of 450 nm in a microplate reader (Bio-Rad 650).

Mitogen-induced proliferation assay

Mice spleen cells were seeded into 96-well plates at a density of 5×10^5 cells per well, and 5

μg/mL of Con A or 10 μg/mL of LPS, and various concentrations of samples were added simultaneously in order to induce T cell or B cell proliferative responses. The mixtures were incubated at 37 ℃ and 5 % CO_2 for 48 h. Proliferation was assessed in terms of uptake of [^{3}H]-thymidine within 8 h after addition of 25 μL [^{3}H]-thymidine. Then, the cells were harvested onto glass fiber filters, and the incorporated radioactivity was counted using a Beta Scintillation Counter (MicroBeta Trilux, PerkinElMER Life Sciences). Lymphocyte proliferation was calculated in terms of ±% by subtracting the CPM value of positive control. In the absence of cytotoxicity, proliferation value of more than ± 15 % indicated that the sample had a strong enhancement/inhibitory effect on lymphocytes.

Molecular docking simulation

The crystal structure of IL-2Rα derived from Protein Data Bank [14] was used as a target for molecular docking simulation, and the 3D structure of voacamine was generated using Chembio3D Utra 14.0, followed by energy minimization molecular docking simulation performed with Autodock 4.0 software. The Autodock program operates with pre-generated grid maps in a way that conformational flexibility of the IL-2Rα is not considered during the docking process. Ligand and water molecules in crystal structure were removed. The affinity grids were centered on the pre-defined active site of protein with dimensions of 50 × 60 × 50 Å. As a docking algorithm, a Lamarckian genetic algorithm (LGA) [15-17] was adopted in the molecular docking simulation, and the number of individuals for the population was set as 100. The docking models were analyzed and represented using ADT and Pymol.

RESULTS

Based on SPRi test, data for each imprint spot was obtained. These data showed that IL-2Rα had a much higher binding signals than that of bovine serum albumin (BSA). Voacamine was able to bind to IL-2Rα with an equilibrium dissociation constant (K_D) of 1.85 × 10^{-8} M, as shown in Figure 2.

Due to the fact that IL-2Rα is involved in lymphocyte cell proliferation, the immune-suppressive effect of voacamine was investigated using a previously reported method [18], with cyclosporine A (CsA) as the positive control. The pharmacological results are summarized in Table 1. Voacamine did not exhibit cytotoxicity at a lower concentration (0.32 μM), but when this concentration was exceeded, it exhibited cytotoxic effect against lymphocytes, and even killed lymphocytes completely. Within non-noxious concentration range, voacamine exhibited significant inhibitory bioactivity against LPS-induced B lymphocyte cell proliferation. However, it had no significant inhibitory effect on Con A-induced T lymphocyte cell proliferation, and there was no dose-dependent relationship.

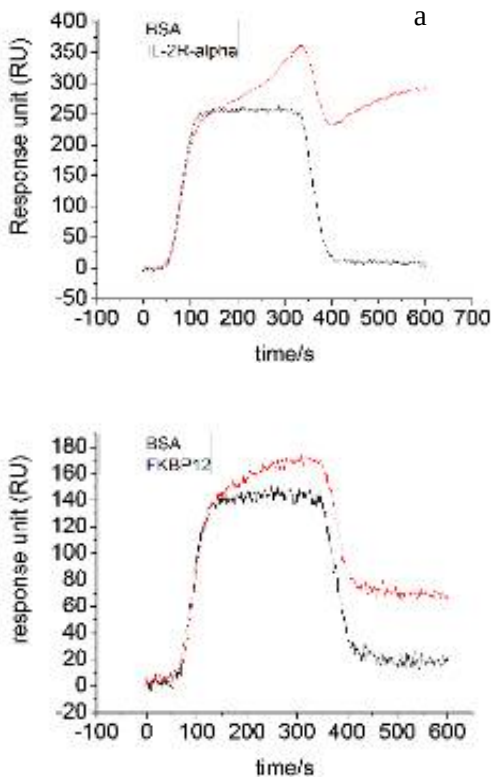

Figure 2: a: Binding signal from IL-2Rα to voacamine. b: The binding signal from FKBP12 to rapamycin (rapamycin and FKBP12 used as positive control); K_D = 2 × 10^{-8} M

For the analysis of complexed structures of voacamine with IL-2Rα obtained by molecular docking simulation, the best docking mode was determined by choosing the poses with the lowest energy for voacamine, as shown in Figure 3. Results revealed that voacamine was bound to the active site of IL-2Rα protein at the surface through Met25, Asn37, Cys28, Glu29, Cys30, Arg36, Leu42, Tyr43 and His120 residues. The binding between voacamine and IL-2Rα protein involved Van der Waals interactions, hydrophobic interactions and hydrogen bonds. The ketone group at C-21' played an important role and formed H-bonds with Arg36 (1.8 Å).

Table 1: Effect of voacamine on murine lymphocyte proliferation induced by Concanavalin A (ConA) (5 µg/mL) or lipopolysaccharide (LPS)

Compound	Concentration (µM)	Cytotoxicity	Inhibition (%)	
			CoA-induced T cell proliferation	LPS-induced B cell proliferation
Voacamine	20.000	+	-100	-100
	4.000	+	-100	-100
	0.800	+	-5	-27
	0.160	-	6	-26
	0.032	-	-3	-22

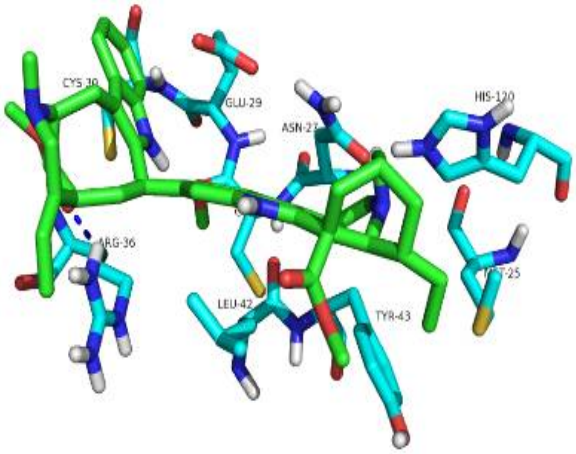

Figure 3: The binding mode of voacamine to IL-2Rα as predicted with molecular docking simulation. The dashed line denotes H-bond

DISCUSSION

Surface plasmon resonance imaging (SPRi) is a real-time, label-free, and high-throughput sensor technique widely used to study biomolecular interactions based on detection of changes in refractive index due to molecular binding. In this study, this high-throughput method was used for simultaneous monitoring of interactions between voacamine and different proteins. The results revealed that voacamine binds to IL-2Rα. This implies that the biological effect of voacamine may affect the immune system. Typically, a protein that binds to a drug not indicated exist biological activity, because SPRi experiment is not able to predict binding sites of the protein. The immunosuppressive effect of voacamine on lymphocyte cell proliferation was evaluated. Moreover, molecular docking simulation was used for analysis of complexed structures of voacamine with IL-2Rα. The autodock results indicated that voacamine had a good interaction with the enzyme active site. The molecular docking simulation and SPRi experiment indicate that voacamine has binding interaction with IL-2Rα. These results also suggest that voacamine may exert an influence on the immune system when developed into a drug.

CONCLUSION

The findings of this study show that voacamine has a high affinity for IL-2Rα, indicating that the bioactivity of voacamine is probably relevant to the immune system. At certain concentrations, voacamine exhibits *in vitro* cytotoxic effects on lymphocytes. Within non-noxious concentration range, it suppresses LPS-induced B lymphocyte cell proliferation, but has no inhibitory effect on Con A-induced T cell proliferation. Molecular docking analysis has provided insight into the mode of interaction of voacamine with IL-2R receptor.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Hong Xiang Li and Yan Qiu Wang contributed equally.

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