Bioorganic & Medicinal Chemistry Letters 22 (2012) 3177–3180

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The effects of diketopiperazines from *Callyspongia* sp. on release of cytokines and chemokines in cultured J774A.1 macrophages

Jin-Hui Chen [c,†], Xiang-Peng Lan [a,†], Yonghong Liu [b,*], Ai-Qun Jia [a,*]

[a] School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Xiaolingwei 200, Nanjing 210094, China
[b] Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, China
[c] Key Laboratory of Forest Genetics and Biotechnology of the Ministry of Education of China, Nanjing Forestry University, Nanjing 210037, China

ARTICLE INFO

Article history:
Received 18 October 2011
Revised 17 January 2012
Accepted 12 March 2012
Available online 16 March 2012

Keywords:
Cytokine
Chemokine
Diketopiperazines
J774A.1 macrophage
Structure–activity relationships

ABSTRACT

Diketopiperazines (DKPs) are a class of secondary metabolites that result from peptide bonds between two amino acids to form a lactam. Due to their rigid structure, chiral nature, and varied side chains, DKPs have been of research interest for their diverse bioactivities. However, little is known about whether DPKs stimulate the release of cytokine and chemokines in macrophage cells. The present aim was to study the effect of DKPs firstly isolated from sponge *Callyspongia* sp. on the release of several cytokines in murine macrophage-like cell line J774A.1 after stimulation in vitro, and their potential structure–activity relationship of five natural DKPs on four representative cytokines, interferon-γ (IFN-γ), pro-inflammatory (tumor necrosis factor, TNF-α), anti-inflammatory cytokine (interleukin-10, IL-10), and chemokine (monocyte chemoattractant protein-1, MCP-1). Results suggested that these five DKPs, especially DKP **1** bearing 3-hydroxyl-L-proline (L-Hyp), might be useful as a promising macrophage cytokines stimulator.

Macrophages, within the cytokines network, are major sources of many cytokines that are involved in immune response, hematopoiesis, inflammation and many other homeostatic processes.[1] Upon stimulation by micro-organisms, exogenous molecules, or endogenous factors including cytokines, macrophage can de novo synthesize and release a large variety of cytokines, such as IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-α, IFN-γ, MCP-1, MCP-3, MIF, and M-CSF et al. Some cytokines can up-regulate the production of cytokines in macrophages (IL-3, GM-CSF, and IFN-γ), while others can inhibit the production (IL-4, IL-10, IL-13, and TGF-β). Certain cytokines (the chemokines such as MCP-1, 2, 3, MIP-1, 2, and RANTES) contribute to the recruitment of circulating monocytes within tissues. More importantly, these cytokines can modulate most of the macrophage functions after they are regulated by internal/external stimulators. So it is worth noting that these macrophages can be their own source of regulatory cytokines after stimulated by environmental factors. Macrophage via their production of cytokines actively participates in the immune response, the hematopoietic process, and inflammation.

There is an increasing interest in using natural products to modulate immune responses and neutralize inflammatory processes by stimulate cytokines in immune cells such as macrophages or T cells. Some natural products have been suggested to be promising anti-inflammatory agents, immune responders, or even tumoral cytotoxic agents.[2] Given the important functions of cytokines in many immune cells, examining whether natural products can be used as cytokine stimulators is particularly valuable for developing new drugs.

Diketopiperazines (DKPs) are a class of smallest cyclic peptides which are of significant interest in bioactivities or drug discovery.[3] Some DPKs have diverse pharmaceutical effects including antimicrobial, antitumor, and antiviral activities, and diverse bioactivities including quorum-sensing signaling, plant-growth promotion, and inhibition against aflatoxin production, especially some DKPs bearing proline residues or unusual amino acidic residues which isolated from marine organisms or microorganisms were found to have diverse bioactivities.[4]

A few studies have indicated that linear peptides stimulate production of cytokines in human monocytes, T cells, and rat spleen cells,[5–7] few paper reported cyclopeptides as cytokine modulators.[8,9] However, whether the smallest cyclic peptides, DKPs, have any effects on the releases of cytokines in the murine macrophage-like cell line J774A.1 was unknown. Here we show that applying 5 DKPs isolated from sponge *Callyspongia* sp. to J774A.1 cells induced release of cytokines such as IL-10, IFN-γ, TNF-α, and MCP-1. The

* Corresponding authors. Tel: +86 20 89023244; fax: +86 20 84451672 (Y.L.); tel./fax: +86 25 84315512 (A-Q.J.).
E-mail addresses: yonghongliu@scsio.ac.cn (Y. Liu), jiaaiqun@gmail.com (A.-Q. Jia).
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.03.045

Table 1
NMR data for DKPs at 500/125 MHz, rep. in CD_3OD except that DKP **5** was in $CDCl_3$ (δ in ppm, J in Hz)

Carbon no.	DKP **1**		DKP **2**		DKP **5**	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
2		169.2 (s)		166.5 (s)		166.2 (s)
3	4.25 (q, J = 7.0)	52.1 (d)	4.12 (d, J = 16.8, H-3a), 3.75 (d, J = 16.8, H-3b)	47.0 (t)	4.09	45.5 (d)
5		172.9 (s)		172.0 (s)		170.0 (s)
6	4.48 (t, J = 4.0)	58.9 (d)	4.26 (t, J = 8.75)	59.9 (d)	4.13	59.4 (d)
7	2.30 (dd, J = 6.5, 6.0, H-7a),2.10 (m, H-7b)	38.3 (t)	2.34 (m, H-7a), 2.06 (m, H-7b)	29.4 (t)	2.37 (H-7a), 2.16 (H-7b)	28.2 (t)
8	4.54	69.1 (d)	1.93	23.3 (t)	2.02 (H-8a), 1.92 (H-8b)	22.8 (t)
9	3.69 (dd, J = 4.5, 4.5, H-9a),3.47 (d, J = 12.5, H-9b)	55.2 (t)	3.54	46.4 (t)	3.63 (H-9a), 3.54 (H-9b)	51.3 (t)
10	1.41 (d, J = 7.0)	15.7 (q)			1.46 (d, J = 6.8)	16.2 (q)
	DKP **3**		DKP **4**			
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C		
2		169.2 (s)		166.0 (s)		
3	3.68 (dd, J = 4.0)	57.7 (d)	4.46	59.8 (d)		
5		170.9 (s)		170.0 (s)		
6	3.23 (dd, J = 3.5)	54.2 (d)	4.09	59.2 (d)		
7	2.85 (dd, J = 4.5)	45.3 (t)	1.26	29.4 (t)		
8	1.45	24.7 (d)	2.11 (H-8a), 1.83 (H-8b),	22.8 (t)		
9	0.76	23.4 (q)	3.18	46.0 (t)		
10	0.76	21.5 (q)	3.56 (H-10a), 3.40 (H-10b)	38.2 (t)		
11	4.25 (d, J = 3.5)	39.5 (t)		137.4 (s)		
12		127.2 (s)	7.29	129.6 (d)		
13	7.01 (d, J = 8.5)	132.8 (d)	7.29	131.3 (d)		
14	6.73 (d, J = 8.5)	116.5 (d)	7.29	128.1 (d)		
15		158.1 (s)	7.29	131.3 (d)		
16	6.73 (d, J = 8.5)	116.5 (d)	7.29	129.6 (d)		
17	7.01 (d, J = 8.5)	132.8 (d)				

potential structure–activity relationship between the five natural DKPs and cytokines was also discussed.

Five DKPs were extracted, isolated, and purified from sponge *Callyspongia* sp. according to the literature previously described.[10,11] The detection methods of cyclopeptides on TLC followed Zhou and Tan.[12] ^{1}H NMR and ^{13}C NMR data of all these five DKPs were listed in Table 1 consistent with the data reported for cyclo(L-Hyp-L-Ala) (DKP **1**),[13] cyclo(L-Pro-Gly) (DKP **2**),[14,15] cyclo(L-Tyr-L-Leu) (DKP **3**),[16] cyclo(L-Pro-L-Phe) (DKP **4**),[17,18] and cyclo(L-Pro-L-Ala) (DKP **5**).[14] To our knowledge, this is the first report on the isolation of these DKPs from sponge *Callyspongia* sp. The structures of these DKPs are shown in Figure 1.

The bioactive assay of investigating five DKPs on release of cytokines and chemokines in cultured J774A.1 was described in the literature with some modifications.[6,19]

IL-10, an anti-inflammatory cytokine, has pleiotropic effects on immuno-regulation and inflammation. It inhibits synthesis of pro-inflammatory cytokines like IFN-γ, IL-2, IL-3, TNF-α, and granulocyte macrophage colony stimulating factor (GM-CSF) from the cells such as macrophages and regulatory T-cells.[20] We found that DKP **1**, **2**, **4**, and **5** induced 1.65, 1.29, 1.54 and 1.56 fold increased secretion of IL-10 from J774A.1 cells, respectively. DKP 3, however, had no effect on IL-10 secretion. (Fig. 2A).

IFN-γ has antiviral, immuno-regulatory, and anti-tumor properties.[21] All these five DKPs induced 4.49, 3.50, 1.82, 1.62 and 1.36 fold greater levels of IFN-γ secretion from J774A.1 cells, respectively (Fig. 2B).

MCP-1 recruits monocytes, memory T cells, and dendritic cells to sites of tissue injury, infection, and inflammation.[22,23] Recent data indicated an important role for MCP-1 in the neuro-inflammatory processes that take place in various central nervous system (CNS) diseases characterized by neuronal degeneration.[24] In this study, all these five DKPs induced 3.09, 1.86, 2.24, 2.05 and 1.47 fold higher levels of MCP-1 secretion from J774A.1 cells from J774A.1 cells, respectively (Fig. 2C).

The primary role of TNF-α is to regulate immune cells.[1] TNF-α is able to induce apoptotic cell death, to induce inflammation, and to inhibit tumorigenesis and viral replication. DKP **1** and DPK**5** induced 1.12 fold greater levels of TNF-α secretion; in contrast, application of DKP **5** reduced 88.3% secreted TNF-α from J774A.1 cells (Fig. 2D). DKP **2**, **3** and **4** showed no effect on TNF-α secretion.

Recognition of invasive microbial pathogens is an essential function of innate immunity.[25] Positioned at the front-line of the antimicrobial host defenses, macrophages are pivotal cells of the innate immune system.[26] Activated by the binding of microbial products (internal/external cytokine stimulators) to pathogen-recognition receptors, macrophages and other innate immune cells release, within a few hours, a myriad of polydunctional cytokines, such as TNFs, ILs, chemokines (for example MCP-1), and IFNs,[27–29] then these stimulated and produced cytokines and chemokines act in concert with other mediators to orchestrate the innate and adaptive immune responses that serve to eliminate or wall-off invasive pathogens.[30]

In contrast to linear peptides which showed broad bioactivities, such as promutoxin being the stimulators of cytokines from human monocytes or T cells,[5,6] DKPs are very stable to proteolysis, which are considered to be important backbones in drug designing.[31] More interestingly, since L-Pro being conservative amino acidic residue, L-Pro-based DKPs for the core of several interesting natural product classes, such as marcfortines, brevianamides, and tryprostatines, showed promising bio-activities, such as anti-inflammatory agents, immune modulators, tumoral cytotoxic agents.[25,32]

When we investigate the potential antimicrobial/antitumor activities of natural DKPs, we found some enriched DKP fractions from some biological tissues showed modulation effects on cytokines in macrophage cells (Data not shown here). Here, we discussed five DKPs from sponge *Callyspongia* sp. regulate the cytokines in macrophage cells.