Immunobiology 216 (2011) 497–504

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

The antimycotic drugs itraconazole and terbinafine hydrochloride induce the production of human β-defensin-3 in human keratinocytes

Naoko Kanda[a,*], Rui Kano[b], Takeko Ishikawa[a], Shinichi Watanabe[a,c]

[a] Department of Dermatology, Teikyo University, School of Medicine, 11-1, Kaga-2, Itabashi-Ku, Tokyo, Japan
[b] Department of Pathobiology, Nihon University, School of Veterinary Medicine, Kanagawa, Japan
[c] Teikyo University School of Medical Mycology, Tokyo, Japan

ARTICLE INFO

Article history:
Received 30 March 2010
Received in revised form 29 August 2010
Accepted 29 August 2010

Keywords:
Activator protein-1
c-Fos
Itraconazole
Prostaglandin D2
Terbinafine hydrochloride

ABSTRACT

The antimicrobial peptide human β-defensin-3 (hBD-3) is produced by epidermal keratinocytes, and exhibits broad killing activity against bacteria or fungi. Prostaglandin D_2 enhances hBD-3 production in human keratinocytes by stimulating a transcription factor, activator protein-1 via chemoattractant receptor-homologous molecule expressed on T helper 2 cells (CRTH2). Prostaglandin H_2, a precursor of prostaglandin D_2 can be converted to thromboxane A_2. Certain antimycotic drugs act on keratinocytes and modulate their production of chemokines. In this *in vitro* study, we examined the effects of antimycotics on hBD-3 production in human keratinocytes. Antimycotics itraconazole and terbinafine hydrochloride increased hBD-3 secretion and mRNA levels in parallel to the enhanced activity of activator protein-1, expression and phosphorylation of activator protein-1 component, c-Fos, but fluconazole was ineffective. These effects were abrogated by CRTH2 antagonist. Itraconazole and terbinafine hydrochloride increased prostaglandin D_2 release from keratinocytes and reduced the release of thromboxane B_2, a thromboxane A_2 metabolite. The conditioned medium from itraconazole or terbinafine hydrochloride-treated keratinocytes inhibited the growth of *Candida albicans* dependently on hBD-3. These results suggest that itraconazole and terbinafine hydrochloride may enhance c-Fos expression and phosphorylation, activator protein-1 activity and hBD-3 production by increasing prostaglandin D_2 release from keratinocytes. These antimycotic drugs may suppress thromboxane A_2 synthesis and redirect the conversion of prostaglandin H_2 towards prostaglandin D_2. The induction of hBD-3 in keratinocytes is another possible mechanism for the antimycrobial effects of these drugs, which may augment the cutaneous defense activity against infection.

© 2010 Elsevier GmbH. All rights reserved.

Introduction

The azole or allylamine antimycotic drugs are therapeutically effective for cutaneous infection with fungi, such as *Trichophyton*, *Malassezia*, or *Candida* (Borgers et al. 2005). An allylamine, terbinafine hydrochloride inhibits the fungal squalene epoxidase enzyme (Ryder 1985); an azole itraconazole inhibits fungal lanosterol 14α-demethylase (Borgers et al. 2005). It is also reported that these antimycotics *in vitro* inhibit the growth of gram-positive bacteria, such as *Propionebacterium acnes* or *Staphylococcus aureus* (Sugita et al. 2010). In addition to dermatomycoses, the topical or oral administration of these antimycotics is therapeutically effective for the other dermatoses, like atopic dermatitis (Broberg and Faergemann 1995; Darabi et al. 2009; Gupta et al. 2004a; Nikkels and Pierard 2003; Sugita et al. 2005) or seborrheic dermatitis (Gupta et al. 2004b). Several *Malassezia* species have been isolated from these skin lesions, and abnormal or inflammatory immune responses to the species may be involved in their pathogeneses (Darabi et al. 2009; Gupta et al. 2004a,b; Sugita et al. 2005). Treatment with antimycotics, itraconazole, ketoconazole, or terbinafine hydrochloride reduces *Malassezia* colonization in the lesions with atopic dermatitis or seborrheic dermatitis and alleviates the symptoms (Darabi et al. 2009; Sugita et al. 2005; Gupta et al. 2004b). Besides their direct effects on fungi, these antimycotics act on lymphocytes or keratinocytes and manifest immunomodulatory effects. Ketoconazole and terbinafine hydrochloride suppress

Abbreviations: ANOVA, analysis of variance; AP-1, activator protein-1; COX, cyclooxygenase; CRTH2, chemoattractant receptor-homologous molecule expressed on T helper 2 cells; DMSO, dimethylsulfoxide; DP, D prostanoid receptor; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; h, haematopoietic; hBD-3, human β-defensin-3; IL-4, interleun-4; KBM, keratinocyte basal medium; KGM, keratinocyte growth medium; PGD_2, prostaglandin D_2; PGDS, prostaglandin D_2 synthase; PMA, phorbol myristate acetate; pRLtk, herpes simplex virus thymidine kinase promoter-linked Renilla luciferase vector; SEM, standard error of mean; TXA_2, thromboxane A_2; TXAS, thromboxane A2 synthase.

* Corresponding author. Fax: +81 3 5375 5314.
E-mail address: nmk@med.teikyo-u.ac.jp (N. Kanda).

0171-2985/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2010.08.008

interleukin-4 (IL-4) and IL-5 production in anti-CD3-plus anti-CD28-stimulated T cells from patients with atopic dermatitis (Kanda et al. 2001). Ketoconazole also suppresses IL-4 plus anti-CD40-induced IgE class switching of surface IgE-negative B cells from patients with atopic dermatitis (Kanda and Watanabe 2002). Topical application of an azole antimycotic miconazole on guinea pig skin reduces the proliferation of epidermal keratinocytes (Niczyporuk et al. 1996). We previously found that ketoconazole and terbinafine hydrochloride *in vitro* suppress the production of chemokines CCL27, CCL2 and CCL5 in human keratinocytes (Kanda and Watanabe 2006).

An antimicrobial peptide, human β-defensin-3 (hBD-3) is produced by epidermal keratinocytes (Kisich et al. 2007), and exhibits broad killing activity against gram-positive and gram-negative bacteria, fungi or viruses (Kisich et al. 2007; Schneider et al. 2005). This peptide kills *Candida albicans* in an energy-dependent and salt-sensitive manner without causing membrane disruption though it causes membrane permeabilization (Vylkova et al. 2007). We recently found that lipid mediator prostaglandin D_2 (PGD_2) *in vitro* enhances hBD-3 production in human keratinocytes (Kanda et al. 2009a). PGD_2 binds to the G protein-coupled receptors D prostanoid receptor (DP) (Boie et al. 1995) and chemoattractant receptor-homologous molecule expressed on T helper 2 cells (CRTH2) (Sawyer et al. 2002), both of which are detectable on keratinocytes (Colombe et al. 2008). The binding of PGD_2 to CRTH2 receptors on human keratinocytes stimulates the activity of transcription factor activator protein-1 (AP-1), leading to AP-1-dependent hBD-3 expression (Kanda et al. 2009a). AP-1 is composed of dimers of Jun family proteins or those of Fos and Jun family proteins. PGD_2 may promote the AP-1-mediated expression of the *hBD-3* gene in human keratinocytes by inducing the expression and phosphorylation of AP-1 component c-Fos, and thus alter the AP-1 composition from c-Jun/c-Jun homodimers to c-Fos/c-Jun heterodimers (Kanda et al. 2009a). PGD_2 in the skin is mainly released from mast cells by degranulation; however, it is also released from keratinocytes (Henke et al. 1986). PGD_2 is intracellularly produced from arachidonic acid through two steps (Rosenbach et al. 1990): (1) arachidonic acid is converted to PGH_2 by cyclooxygenase (COX; EC 1.14.99.1); and (2) PGH_2 is converted to PGD_2 by PGD_2 synthase (PGDS; EC 5.3.99.2). PGH_2 is also converted to thromboxane A_2 (TXA_2) by TXA_2 synthase (TXAS; EC 5.3.99.5).

Epidermal keratinocytes can be target cells of antimycotic drugs and these drugs improve the symptoms of dermatomycoses. It is thus plausible that these drugs may act on keratinocytes and enhance their production of an antimicrobial peptide hBD-3, and thus augment the antifungal activity of the skin. However, their effects on hBD-3 production have not been studied yet. In this *in vitro* study, we investigated if azole or allylamine antimycotics alter the hBD-3 production in human keratinocytes.

Materials and methods

Reagents

Itraconazole was donated by Janssen Pharmaceutica NV (Beerse, Belgium). Terbinafine hydrochloride, fluconazole and voriconazole were purchased from Novartis Pharma (Tokyo, Japan), Wako Pure Chemicals (Osaka, Japan), and Tocris Bioscience (Bristol, UK), respectively. The antimycotics were dissolved in dimethylsulfoxide (DMSO) at 100 mM to create solutions and subsequently diluted in the experimental media to yield the final concentrations. The DMSO concentration as a vehicle control was 0.1% (vol/vol). Phorbol myristate acetate (PMA) was purchased from Calbiochem (San Diego, CA, USA), and DP antagonist, MK0524 was purchased from Cayman Chemical (Ann Arbor, MI, USA). CRTH2 antagonist, BAY U3405 was donated by Bayer Yakuhin Ltd. (Osaka, Japan).

Keratinocyte culture

Human neonatal foreskin keratinocytes were purchased from Clonetics (Walkersville, MD, USA). They were cultured in serum-free keratinocyte growth medium (KGM; Clonetics, Walkersville, MD, USA) containing keratinocyte basal medium (KBM) supplemented with 0.5 μg/mL hydrocortisone, 5 ng/mL epidermal growth factor, 5 μg/mL insulin and 0.5% bovine pituitary extract. Cells in the third passage were used. Each experiment was performed four times using the same lot of keratinocytes.

Secretion of hBD-3

The keratinocytes (5×10^4 cells per well) were seeded onto 24-well plates containing 0.4 mL KGM, adhered overnight, washed and incubated with KBM for 24 h. The cells were washed and treated for 48 h with the vehicle (DMSO) or indicated concentrations of the antimycotics in KBM. Each treatment was performed in triplicate. The hBD-3 concentration in the culture supernatants was measured using an ELISA kit (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) according to the manufacturer's instruction. The cell number was counted at the end of the incubation, and the amounts of hBD-3 were standardized to the cell number and expressed as nanograms per 10^6 viable cells.

Real-time PCR

In order to measure the mRNA levels of hBD-3, the keratinocytes (seeded at 5×10^4 cells per well on 24-well plates) were incubated with the vehicle (DMSO) or indicated concentrations of the antimycotics for 12 h. Total cellular RNA was extracted and reverse-transcribed to produce cDNA. Real-time PCR was performed in a fluorescence temperature cycler (LightCycler; Roche Diagnostics GmbH, Mannheim, Germany), using specific forward and reverse primers as described previously (Harder et al. 2001). A SYBR Green I system (Roche Diagnostics GmbH, Mannheim, Germany) was utilized in the reaction. The hBD-3 mRNA expression was normalized to that of β-actin and shown as a fold induction relative to control keratinocytes treated with the vehicle.

Plasmid and transfection

Plasmid pAP-1-luc contains seven copies of AP-1-like sequences (5′-TGACTTCA-3′) in the hBD-3 promoter at position −1258 (Menzies and Kenoyer 2006) in front of the TATA box, upstream of the firefly luciferase reporter gene. Transient transfection was performed using Fugene 6 (Roche Diagnostics GmbH, Mannheim, Germany) as previously described (Kanda et al. 2009a). Keratinocytes were seeded on 24-well plates and grown to approximately 60% confluence. Plasmids pAP-1-luc and herpes simplex virus thymidine kinase promoter-linked Renilla luciferase vector (pRLtk) (Promega, Madison, WI, USA) were premixed with Fugene 6 and added to the keratinocytes. After 6 h, the cells were washed and incubated in KBM for 24 h and treated with the vehicle or indicated concentrations of the antimycotics. After 18 h, the activities of the firefly luciferase and Renilla luciferase in the cell extracts were measured using a dual luciferase assay system (Promega, Madison, WI, USA). The transcriptional activity of AP-1 was expressed as the ratio of the firefly luciferase activity to the Renilla luciferase activity.

Download English Version:

https://daneshyari.com/en/article/2183133

Download Persian Version:

https://daneshyari.com/article/2183133

Daneshyari.com