β-Hydroxybutyrate Reduces Psoriasiform Dermatitis

Toshihiko Mochizuki, Tomomitsu Miyagaki, Mayumi Tamaki, Sora Takeuchi, and Takafumi Kadono

(Received for Publication: October 28, 2020)

Abstract

Background: Psoriasis, a chronic inflammatory skin disease, has a high prevalence of metabolic syndrome. The fat stored in obese people tends to be proinflammatory, however, certain fatty acids such as short-chain fatty acids are anti-inflammatory. Although short-chain fatty acids are known to attenuate inflammation in several diseases, the effect of short-chain fatty acids, such as β-hydroxybutyrate, on psoriasis remains unknown.

Objectives: To investigate the role of β-hydroxybutyrate in psoriasis.

Materials and Methods: Several short-chain fatty acids, such as acetic acid, propionic acid, butyric acid, and β-hydroxybutyrate were administered to murine imiquimod-induced psoriasis-like skin inflammation. The clinical score was evaluated through the course of this treatment and the number of infiltrating neutrophils was counted. The expression of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-17 in skin tissues was measured by quantitative reverse transcription polymerase chain reaction (RT-PCR). In addition, β-hydroxybutyrate was added to IL-17-stimulated HaCaT cells and the expression of inflammatory cytokines and receptors for β-hydroxybutyrate were measured.

Results: β-Hydroxybutyrate significantly improved skin symptoms and attenuated neutrophilic infiltration. β-Hydroxybutyrate also reduced the expression of inflammatory cytokines such as TNF-α, IL-1β, and IL-17. In addition, β-hydroxybutyrate reduced the expression of TNF-α and IL-1β in IL-17-stimulated HaCaT cells.

Conclusion: These results suggest that β-hydroxybutyrate reduces psoriasiform dermatitis and it may act directly on keratinocytes to reduce inflammation.

Key Words

β-Hydroxybutyrate, psoriasis, short-chain fatty acids, IL-1β

Introduction

Psoriasis is a chronic inflammatory skin disease with a prevalence of approximately 3% worldwide. The prevalence of metabolic syndrome is high in psoriasis, and a high-fat diet is known to exacerbate murine models of psoriasis. In obese individuals, adipose tissue produces large amounts of inflammatory cytokines and adipokines such as TNF-α, visfatin, and resistin are increased in psoriatic patients. In addition, saturated fatty acids such as palmitic acids directly activate keratinocytes and are thought to be involved in epidermal hyperplasia in psoriasis. Although saturated fatty acids abundant in obese individuals are proinflammatory, some fatty acids that include short-chain fatty acids are known to be anti-inflammatory.

Short-chain fatty acids such as acetic, propionic, and butyric acids consist of small hydrocarbon chains and carboxylic acids, and are generally produced by microbiota. Administration of short-chain fatty acids is known to reduce inflammation in certain settings. Butyric acid, which inhibits histone deacetylase, promotes the differentiation of naive T cells into regulatory T cells, and reduces enteritis. In addition, intraperitoneally injected acetate decreases hepatic lipid accumulation in high-fat diet mice.

Here, we investigated the effects of various...
short-chain fatty acids on psoriasis using imiquimod (IMQ)-induced murine psoriasiform dermatitis. Among them, we found that administration of β-hydroxybutyrate attenuated this form of psoriasis.

Materials & Methods

Mice
C57BL/-/- 6J female mice (5 to 8 weeks old) were obtained from Japan SLC, Inc. (Shizuoka, Japan) for this experiment. The experimental protocol was approved by the Animal Experiment Committee of the St. Marianna University School of Medicine, Kawasaki, Japan (Approval No. 2006013).

Induction of imiquimod-induced psoriasiform skin inflammation
The backs of each mouse was shaved and IMQ cream (Beselna cream: Mochida Pharmaceutical Co., Tokyo, Japan) was applied topically for 7 consecutive days. Mice received food and water ad libitum. In some mice, acetic acid, propionic acid, or butyric acid (FUJIFILM Wako Pure Chemical Co., Osaka, Japan) at a concentration of 0.2 mol/L was added to the drinking water for 14 days, or 500 mg/kg β-hydroxybutyrate (Alfa Aesar, Heysham, UK) was intraperitoneally administered for 7 consecutive days.

The clinical score was calculated on a total of 12 points, with the degree of erythema, scaling, and thickening in four stages of 0–4 (0, absent; 1, mild; 2, moderate; 3, severe; 4, very severe).

Histology and immunohistochemical staining
Seven days after the initiation of IMQ cream topical application, a skin sample from the back of each mouse was embedded in paraffin the skin sample was then stained with hematoxylin and eosin. Sections were incubated with mouse anti-Ly6G (1:500) (BioLegend Inc. San Diego, CA, USA) for 1 h and then incubated with Human Fine Simple Stain MAX-PO (Rat) (Nichirei Bio Science, Tokyo, Japan), and finally stained with a Vector ABC staining kit (Vector Laboratories, Burlingame, CA, USA).

Quantitative RT-PCR
Total RNA was extracted from either mouse skin samples or HaCaT cells using the Rneasy® Mini kit (Qiagen, Hilden, Germany). RNA was converted to cDNA using the ReverTra Ace® qPCR RT Master Mix (Toyobo Co., Ltd., Osaka, Japan).

Gene expression was confirmed using the mouse primers for TNFα, IL-1β, IL-12, IL-17, IL-22, and IL-23 and the human primers for TNF-α, IL-1β, IL-17, G-protein coupled receptors (GPR)109A (Thermo Fisher Scientific, Tokyo, Japan). All samples were analyzed in parallel with GAPDH gene expression as an internal control. The relative expression levels of each gene were determined by the $2^{-ΔΔCT}$ method.

Cell culture
HaCaT cells were maintained in Dulbecco’s Modified Eagle’s Medium (Sigma-Aldrich, Tokyo, Japan). When HaCaT cells reached semiconfluence, they were treated with recombinant IL-17A (100 ng/mL) with or without 10 mM β-hydroxybutyrate. After 24 h, total RNA from the cells was collected for quantitative RT-PCR.

Statistical analysis
The experimental results were obtained from three independent experiments. Statistical analysis was performed using the Kruskal-Wallis test to compare values. P-values less than 0.05 were considered statistically significant.

Results
Administration of β-hydroxybutyrate reduces psoriasis-like skin inflammation
To elucidate the role of short-chain fatty acids in psoriasis, we utilized IMQ-induced psoriasis-like skin inflammation in mice. Oral administration of acetic acid, butyric acid, and propionic acid did not significantly change the course of skin inflammation (data not shown). When β-hydroxybutyrate was intraperitoneally injected prior to IMQ treatment, psoriasis-like skin inflammation was clinically reduced (Figure 1a).

Histopathology also revealed that the administration of β-hydroxybutyrate reduced epidermal thickness and inflammatory cell infiltration (Figure 1b). In addition, clinical skin scores that consisted of erythema, scaling, and thickening were significantly lower in β-hydroxybutyrate-treated mice (Figure 1c).

β-Hydroxybutyrate reduces neutrophil infiltration
Next, we measured the extent of neutrophil infiltration. Mice treated with IMQ showed increased neutrophil infiltration throughout the skin compared to Vaseline_-treated mice (Figure 2a). When β-hydroxybutyrate was administered, neutrophil infiltrat-
Figure 1. Intraperitoneal injection of $\beta$-hydroxybutyrate reduced psoriasiform skin inflammation. (A) Clinical pictures at day 7 of imiquimod (IMQ) treatment. IMQ-treated mice showed psoriasiform skin inflammation compared to Vaseline®-treated, and the additional administration of $\beta$-hydroxybutyrate (IMQ+$\beta$-hydroxybutyrate) mitigated skin dermatitis. (B) Skin sections stained with hematoxylin-eosin at day 7 of IMQ treatment. Parakeratosis, acanthosis, and dermal leukocytic infiltration induced by imiquimod were attenuated by $\beta$-hydroxybutyrate. Bar=100μm. (C) Transition of cumulative disease severity scores. The addition of $\beta$-hydroxybutyrate significantly reduced the score of IMQ-treated mice from day 2 to day 7. *P<0.01, (n=10).
Figure 2. Neutrophil infiltration and the expression of proinflammatory cytokines were attenuated by β-hydroxybutyrate. (A) Immunohistochemical staining for Ly6G at day 7 of IMQ treatment. The infiltration of Ly6G neutrophils caused by IMQ treatment was decreased by β-hydroxybutyrate. Bar=100μm. (B) The number of Ly6G cells/mm² at day 7. β-hydroxybutyrate significantly reduced the number of Ly6G-cells induced by IMQ treatment. **P<0.01, ***P<0.001, (n=10). (C) Relative expression levels of inflammatory cytokines in mouse skin at day 7 of IMQ treatment. The expression levels of TNF-α, IL-1β, and IL-17 were increased by IMQ treatment and the addition of β-hydroxybutyrate significantly reduced them. *P<0.05, (n=5).
Relative levels of inflammatory cytokines in mouse skin

To examine the changes in cytokine expression in psoriatic skin after the administration of β-hydroxybutyric acid, quantitative real-time PCR was performed. The expression levels of inflammatory cytokines such as TNF-α and IL-1β were increased by IMQ treatment, and their expression was significantly decreased by the administration of β-hydroxybutyrate (P<0.05). In a similar manner, the expression levels of IL-17, which is an important cytokine in psoriasis, were also increased by treatment with IMQ, and its expression was significantly reduced by the administration of β-hydroxybutyrate (P<0.05) (Fig. 2c). These data collectively demonstrate that β-hydroxybutyrate reduced IMQ-induced psoriasiform skin inflammation by reducing TNF-α, IL-1β, and IL-17.

The expression levels of IL-12, IL-22, and IL-23 did not significantly change following β-hydroxybutyrate administration (data not shown).

Relative levels of inflammatory cytokines in HaCaT cells

The receptors of β-hydroxybutyrate include GPR109A. As these receptors are expressed on both leukocytes and keratinocytes, we examined the direct effect of β-hydroxybutyrate on keratinocytes. To this end, HaCaT cells stimulated with IL-17 were treated with β-hydroxybutyrate and the expression levels of TNF-α, IL-1β, and GPR109A were measured by quantitative real-time PCR. The expression levels of TNF-α and IL-1β were increased by IL-17 stimulation, and their expression was decreased by the administration of β-hydroxybutyrate (Fig. 3). The expression of GPR109A was decreased by IL-17 treatment, which was slightly increased by β-hydroxybutyrate.
Discussion

In this study, the administration of β-hydroxybutyrate, a butyric acid metabolite, significantly improved psoriasiform dermatitis and decreased neutrophil infiltration. β-Hydroxybutyrate also reduced the expression of inflammatory cytokines such as TNF-α IL-17, and IL-1β within the skin. In addition, β-hydroxybutyrate slightly reduced the expression and production of TNF-α and IL-1β in IL-17-treated HaCaT cells.

Although several short-chain fatty acids are known to exert anti-inflammatory effects, only β-hydroxybutyrate was able to attenuate IMQ-induced psoriasiform dermatitis by increasing the activation of nuclear factor-kappa B (NF-κB) and the expression of GPR109A in IL-17-treated HaCaT cells. Notably, IL-1β levels were also decreased in the skin.

Several cytokines within the skin were decreased in the current study. TNF-α and IL-17 are well-known targets for psoriasis, and the reduction of these cytokines is likely to be one of the causative factors for the attenuation of psoriasiform dermatitis. GPR109A is a G protein-coupled receptor and exhibits anti-inflammatory action by suppressing the activation of nuclear factor-kappa B (NF-κB) and the expression of inflammatory cytokines such as IL-1β. IL-1β suppresses transforming growth factor (TGF)-β-induced Foxp3 expression in CD4-positive T cells. IL-1β is also known to polarize CD4-positive T cells into Th17 cells and even redirect regulatory T cells into Th17 cells under certain conditions. In our study, β-hydroxybutyrate reduced IL-1β and elevated the expression of GPR109A. β-Hydroxybutyrate is also known to block the NLRP3 inflammasome, which is critical for IL-1β processing. Considering that a high-fat diet exacerbates murine psoriasiform dermatitis by increasing the activation of IL-1β, the balance of saturated fatty acids, which are mostly proinflammatory, and short-chain fatty acids, which are mostly anti-inflammatory, might affect the course of psoriasis.

Administration of short-chain fatty acids such as β-hydroxybutyrate is known to reduce several types of inflammation. Oral administration of β-hydroxybutyrate reduces inflammatory cytokines in human serum, and β-hydroxybutyrate inactivates the neutrophil NLRP3 inflammasome, thereby relieving gout flares. β-Hydroxybutyrate also attenuates stress-induced behavioral and inflammatory responses, and reduces inflammation in glioma. Tye et al. reported that butyric acid suppresses NLRP1 to alleviate inflammatory bowel diseases.

As the receptors of β-hydroxybutyrate are expressed on various cells, they exert anti-inflammatory effects in multiple ways. One of the targets is hematopoietic cells. β-Hydroxybutyrate is known to block the NLRP3 inflammasome and subsequent IL-1β production in peripheral blood monocytes. Goldberg et al. reported that administration of β-hydroxybutyrate reduced IL-1β in peripheral blood neutrophils. In our study, HaCaT cells were influenced by β-hydroxybutyrate to decrease the expression of inflammatory cytokines. Thus, β-hydroxybutyrate might act on both blood cells and keratinocytes to affect the course of inflammation.

In summary, our results suggest that β-hydroxybutyrate improves psoriasiform skin inflammation by reducing inflammatory cytokines and β-hydroxybutyrate may directly modulate cytokine expression in keratinocytes.

Acknowledgements

Financial support: The study was supported by JSPS KAKENHI Grant Number 16K10179.

Conflicts of Interest

The authors have nothing to disclose.

References

1) Gupta R, Debbaneh MG, Liao W. Genetic epidemiology of psoriasis. Curr Dermatol Rep 2014; 3: 61–78.
2) Gisondi P, Tessari G, Conti A, et al. Prevalence of metabolic syndrome in patients with psoriasis: a hospital-based case–control study. Br J Dermatol 2007; 157: 68–73.
3) Nakamizou S, Honda T, Adachi A, et al. High fat diet exacerbates murine psoriatic dermatitis by increasing the number of IL-17-producing γδ T cells. Sci Rep 2017; 7: 14076. doi:10.1038/s41598-017-14292-1.
4) Herbert D, Franz S, Popkova Y, et al. High-fat...
diet exacerbates early psoriatic skin inflammation independent of obesity: saturated fatty acids as key players. J Invest Dermatol 2018; 138: 1999–2009.

5) Higashi Y, Yamakuchi M, Fukushige T, et al. High-fat diet exacerbates imiquimod-induced psoriasis-like dermatitis in mice. Exp Dermatol 2018; 27: 178–184.

6) Ismail SA, Mohamed SA. Serum levels of visfatin and omentin-1 in patients with psoriasis and their relation to disease severity. Br J Dermatol 2012; 167: 436–439.

7) Johnston A, Arnadottir S, Gudjonsson JE, et al. Obesity in psoriasis: leptin and resistin as mediators of cutaneous inflammation. Br J Dermatol 2008; 159: 342–350.

8) Lai Y, Li D, Li C, et al. The antimicrobial protein REG3A regulates keratinocyte proliferation and differentiation after skin injury. Immunity 2012; 37: 74–84.

9) Corrêa-Oliveira R, Fachi JL, Vieira A, et al. Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunol 2016; 5: e73. doi: 10.1038/cti.2016.17.

10) Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 2013; 504: 446–450.

11) Sahuri-Arisoylu M, Brody LP, Parkinson JR, et al. Reprogramming of hepatic fat accumulation and ‘browning’ of adipose tissue by the short-chain fatty acid acetate. Int J Obes (Lond) 2016; 40: 955–963.

12) Kasubuchi M, Hasegawa S, Hiramatsu T, et al. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. Nutrients 2015; 7: 2839–2849.

13) Krejner A, Bruhs A, Mrowietz U, et al. Decreased expression of G-protein-coupled receptors GPR43 and GPR109a in psoriatic skin can be restored by topical application of sodium butyrate. Arch Dermatol Res 2018; 310: 751–758.

14) Boehncke WH, Schön MP. Psoriasis. Lancet 2015; 386: 983–994.

15) Fu SP, Wang JF, Xue WJ, et al. Anti-inflammatory effects of BHBA in both in vivo and in vitro Parkinson’s disease models are mediated by GPR109A-dependent mechanisms. J Neuroinflamm 2015; 12: 9. doi:10.1186/s12974-014-0230-3.

16) Bent R, Moll L, Grabbe S, et al. Interleukin-1 beta-a friend or foe in malignancies? Int J Mol Sci 2018; 19: 2155. doi:10.3390/ijms19082155.

17) Chung Y, Chang SH, Martinez GJ, et al. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity 2009; 30: 576–587.

18) Youm YH, Nguyen KY, Grant RW, et al. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med 2015; 21: 263–269.

19) Neudorf H, Myette-Côté É, P Little J. The impact of acute ingestion of a ketone monoester drink on LPS-stimulated NLRP3 activation in humans with obesity. Nutrients 2020; 12: 854. doi:10.3390/nu12030854.

20) Goldberg EL, Asher JL, Molony RD, et al. β-hydroxybutyrate deactivates neutrophil NLRP3 inflammasome to relieve gout flares. Cell Rep 2017; 18: 2077–2087.

21) Yamanashi T, Iwata M, Kamiya N, et al. Beta-hydroxybutyrate, an endogenic NLRP3 inflammasome inhibitor, attenuates stress-induced behavioral and inflammatory responses. Sci Rep 2017; 7: 7677. doi:10.1038/s41598-017-08055-1.

22) Shang S, Wang L, Zhang Y, et al. The Beta-hydroxybutyrate suppresses the migration of glioma cells by Inhibition of NLRP3 Inflammasome. Cell Mol Neurobiol 2018; 38: 1479–1489.

23) Tye H, Yu CH, Simms LA, et al. NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. Nat Commun 2018; 9: 3728. doi:10.1038/s41467-018-06125-0.