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The lipoxygenase-hepoxilin pathway is activated in cutaneous plaque lesions of psoriasis

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Abstract

Psoriasis is a common skin disorder that is characterized by hyperkeratosis, epidermal hyperplasia, T-cell and neutrophil recruitment, and changes in the vascular endothelial system. The several upregulated variable lipid mediators, including the eicosanoids, in psoriatic keratinocytes were described previously, although some results were contradictory. Since hepoxilin is less stable than other eicosanoids such as hydroxyeicosatetraenoic acid (HETE), the measurement of hepoxilin is difficult. In this study, to clarify the relationship between hepoxilin/hepoxilin-related fatty acid derivatives and psoriasis vulgaris, we evaluated the amount of production of lipid mediators including hepoxilins in skin tissues of patients with psoriasis vulgaris in comparison with normal controls by high-performance liquid chromatography/mass spectrometry. Most of the hepoxilins and related lipids evaluated in this study tended to be more abundant in the lesional skin from the psoriasis patient group (PPG) than in the skin from controls. Interestingly, 12*R*-HETE was markedly higher in the skin samples from the PPG. In addition, the lipids produced in the lipoxygenase pathway, including hepoxilin, were elevated in the skin lesions of the PPG compared with those of normal controls, whereas the lipids produced in the cyclooxygenase pathway were decreased in the skin lesions from the PPG. Our results suggest that an imbalance between the lipoxygenase and cyclooxygenase pathways may contribute to psoriatic pathomechanisms.

KEYWORDS
cyclooxygenase, hepoxilin, lipoxygenase, psoriasis vulgaris, psoriatic arthritis

1 | INTRODUCTION

There is increasing evidence that hepoxilins are novel inflammatory mediators. In vitro studies have shown that hepoxilins cause the mobilization of intracellular calcium in human neutrophils, induce plasma leakage, and potently stimulate the chemotaxis of human neutrophils. In vivo, the hepoxilin pathway is activated in conditions with inflammation, for example, pathogen infection, psoriasis, and arthritis, and hepoxilins promote inflammatory hyperalgesia and allodynia.[1] The "hepox" comes from hydroxy-epoxy, and the "ilin" comes from hepoxilins' activity as an insulin secretagogue (albeit an activity whose physiological significance remains unconfirmed).

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All fatty acid hydroperoxides readily give rise to epoxyalcohol derivatives, although strictly speaking, only derivatives of the arachidonic acid hydroperoxide 12*S*-hydroperoxyeicosatetraenoic acid (12*S*-HPETE) merit the designation of "hepoxilin." Nonetheless, all the others are regarded as hepoxilin-related or hepoxilin-type fatty acid derivatives.[2] In addition, a genetic defect linked to a deficiency in hepoxilin formation has been described and is believed to be responsible for the scaly skin observed in ichthyosis.[1] Mutations in the coding regions of *ALOX12B*, which encodes 12R-lipoxygenase (ALOX12), and *ALOXE3*, which encodes lipoxygenase-3 (ALOXE3), have been reported in patients with autosomal recessive congenital ichthyosis.[3] Pathogenic mechanisms of ichthyosis arising from *ALOX12B* and *ALOXE3* mutations are now thought to be the deficient oxidation of linoleic acids of EOS ceramides and the malformation of the corneocyte lipid envelope.[4] In addition, both enzymes convert arachidonic acids to the corresponding hepoxilin-like epoxyalcohols, 8*R*-hydroxy-11*R* and 12*R*-epoxyeicosatrienoic acid, via 12*R*-HPETE.[5] This sequence has been hypothesized to be a part of a novel lipoxygenase (LOX) pathway in skin that plays an important role in terminal differentiation.[5] Thus, the hepoxilins play an important role not only in inflammation but also in the regulation of proliferation and differentiation of epithelial cells, especially keratinocytes.

Psoriasis is a common skin disorder that is characterized by hyperkeratosis, epidermal hyperplasia, T-cell and neutrophil recruitment, and changes in the vascular endothelial system.[6,7] The etiology of psoriasis is thought to originate from an interplay of genetic, environmental, infectious, and lifestyle factors.[8,9] The manner in which genetic and environmental factors interact to contribute to the molecular disease mechanisms has remained elusive.[8] Upregulation of several variable lipid mediators, including the eicosanoids, in psoriatic keratinocytes was described previously, although some results were contradictory.[7] Since hepoxilin is less stable than other eicosanoids such as hydroxyeicosatetraenoic acid (HETE), the measurement of hepoxilin is difficult. In this study, to clarify the relationship between hepoxilin/hepoxilin-related fatty acid derivatives and the development of psoriatic skin, we determined the amount of production of lipid mediators including hepoxilins in plaque lesions of patients with psoriasis vulgaris (PV) and psoriatic arthritis (PsA) compared to normal controls.

2 | METHODS

2.1 | Human skin tissues

Patients with PV and PsA were enrolled at the outpatient clinic of the Department of Dermatology, Nagoya University Hospital. Psoriatic skin was obtained from psoriatic plaque lesions in eight patients with PV or PsA (the psoriasis patient group (PPG); median age of 65.5 years; range of 57-78). Three PsA patients were included in the PPG (37.5%). The diagnoses were based on the clinical and histological findings of PV and PsA. Skin lesion severity was evaluated by using the Psoriasis Area and Severity Index (PASI), whose scores cover the range from 0 to 72. Skin samples from ten healthy individuals were used as controls (median age of 66.5 years; range of 53-86). A group of healthy control subjects was selected from individuals who had no history of psoriasis or other chronic inflammatory systemic diseases. The detailed clinical features are described in Table 1. All skin tissue samples were taken after written informed consents had been obtained. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and was conducted according to the Declaration of Helsinki Principles.

2.2 | Materials for lipid analysis

Hepoxilin B_3, (10*R*,11*S*,12*S*)-trioxilin B_3, and (10*S*,11*S*,12*S*)-trioxilin B_3, were supplied by Ono Pharmaceutical Co., Ltd., Osaka, Japan 12-oxoETE, 12(*S*)-HETE, and 12(*S*)-HETE-d_8, prostaglandin D_2 (PGD_2)-d_4, arachidonic acid (AA)-d_8, and leukotriene B_4 (LTB_4)-d_4 were purchased from Cayman Chemical. Ethanol of high-performance

TABLE 1 Characteristics of patients with psoriasis and normal controls

	Normal		Psoriasis		
Variable	N	Value	N	Value	*P*-value*
Age (y)	10	66.5 (53-86)	8	65.5 (57-78)	0.929
Male, n (%)	10	4 (40%)	8	3 (37.5%)	1
Duration (mo)	-	-	7	96 (12-444)	-
PASI	-	-	8	6.35 (1.2-22.4)	-
Smoking, n (%)	8	2 (25%)	7	3 (43%)	0.608
Drinking, n (%)	8	4 (50%)	7	2 (29%)	0.608
Hyperlipidemia, n (%)	10	3 (30%)	8	4 (50%)	0.631
History of psoriatic arthritis, n (%)	-	-	8	3 (37.5%)	-

Abbreviations: PASI, Psoriasis Area and Severity Index.
Continuous variables are displayed as medians (minimum-maximum).
**P* values were calculated using Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables.

TABLE 2 Comparison of each hepoxilin and its related lipids between patients with psoriasis and normal controls

Target	Normal (N = 10) (10^2 ng/mg tissue)	Psoriasis (N = 8) (10^2 ng/mg tissue)	*P*-value*	FDR[a]
Hepoxilin B3	0.051 (0-2.85)	1.25 (0.26-5.62)	0.003	**0.007**
Hepoxilin A3	0 (0-0)	0 (0-0)	-	-
12-HPETE	0 (0-0)	0 (0-0)	-	-
12-HETE	3.56 (0.69-39.77)	19.20 (10.84-71.38)	0.008	**0.018**
12-KETE	0.031 (0-3.80)	2.32 (0.31-10.03)	0.002	**0.006**
Trioxilin B3-1	0 (0-0.88)	0.77 (0-6.52)	0.017	**0.026**
Trioxilin B3-2	0 (0-0)	0.23 (0-9.40)	0.015	**0.025**
5R	0 (0-0)	0 (0-2.28)	0.264	0.281
5S	0 (0-0)	0 (0-6.00)	0.104	0.134
8R	0 (0-0)	1.14 (0-4.36)	0.015	**0.025**
8S	0 (0-0)	2.24 (0-8.70)	0.015	**0.025**
9R	0 (0-0)	1.71 (0-6.48)	0.015	**0.025**
9S	0 (0-0)	0 (0-0)	-	-
11R	0 (0-4.43)	1.21 (0-7.59)	0.225	0.263
11S	0 (0-0)	0 (0-0)	-	-
12R	0 (0-8.55)	21.61 (9.53-125.78)	<0.001	**0.002**
12S	8.18 (0-96.02)	12.86 (6.23-34.94)	0.155	0.185
15R	0 (0-0)	0 (0-6.00)	0.104	0.134
15S	5.42 (0-12.29)	18.40 (6.01-116.97)	0.001	**0.005**
HepoA3R	0 (0-0)	0 (0-0)	-	-
HepoA3S	0 (0-0)	0 (0-4.37)	0.264	0.281
HepoB3R	0 (0-0)	0 (0-0)	-	-
HepoB3S	0 (0-0)	0 (0-3.81)	0.104	0.134

Abbreviations: HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; KETE, keto-eicosatetraenoic acid.
Data are displayed as medians (minimum-maximum).
Boldface indicates statistically significant results.
[a]FDR was calculated by Benjamini-Hochberg adjustment.
**P* values were calculated by Wilcoxon rank-sum test.

liquid chromatography (HPLC) grade was purchased from Wako Pure Chemical Industries (Osaka, Japan) or Kishida Chemical Co., Ltd. (Osaka, Japan) Acetonitrile of HPLC grade or LC/MS grade was from Wako Pure Chemical Industries. Methanol of HPLC grade or LC/MS grade was from Nacalai Tesque, Inc. (Kyoto, Japan) and Wako Pure Chemical Industries. Acetic acid of guaranteed grade or of liquid chromatography/mass spectrometry (LC/MS) grade was from Wako Pure Chemical Industries.

2.3 | Determination of epidermal concentrations of hepoxilin B_3, 12-oxoeicosatetraenoic acid (oxoETE), 12(*S*)-HETE, (10*R*,11*S*,12*S*)-trioxilin B_3, and (10*S*,11*S*,12*S*)-trioxilin B_3

Cryogenically crushed human epidermal fragments were suspended in 500 μL of ethanol. The epidermal suspension was centrifuged at 13 800 g at 4°C for 5 minutes. The entire volume of the resultant supernatant (epidermal extract) was collected. The deuterated compound 12(*S*)-HETE-d_8 was used as the internal standard (IS) for the quantification of hepoxilin B_3, 12-oxoETE, 12(*S*)-HETE, (10*R*,11*S*,12*S*)-trioxilin B_3, and (10*S*,11*S*,12*S*)-trioxilin B_3. An appropriate amount of IS was spiked in ethanol to make solutions of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL. For the quantification of hepoxilin B_3, 100 μL of the epidermal extract was spiked with 20 μL of IS solution of a specified concentration and diluted with 100 μL of water. For the quantification of the other analytes, 50 μL of the epidermal extract was spiked with 5 μL of the IS solution of a specified concentration and diluted with 50 μL of water.

For the quantification of hepoxilin B3, samples were analyzed by using a HPLC system Nexera-X2 (Shimadzu Corp.) coupled to electrospray ionization on a triple-quadrupole mass spectrometer API 5000 (AB Sciex Pte. Ltd., Framingham, MA, USA). Chromatographic separation was achieved on a Poroshell 120 SB-C18 column (2.7 μm, 2.1 × 100 mm, Agilent Technologies, Inc., Santa Clara, CA, USA) using a flow rate of 0.2 mL/min at 40°C. Two solvents were used for gradient elution: (A) 0.1% acetic acid aqueous solution and (B) acetonitrile/methanol/acetic acid (40/60/0.1, v/v/v). The gradient program was as follows: 0 minutes - 50% B; 10 minutes - 90% B.

TABLE 3 Comparison of each lipid mediator between patients with psoriasis and normal controls

Target	Normal (N = 10) (ng/mg tissue)	Psoriasis (N = 8) (ng/mg tissue)	*P*-value*	FDR[a]
PGE3	0 (0-1611)	0 (0-8980)	0.832	0.832
PGE1	1199.5 (125-2600)	0 (0-5031)	0.127	0.156
Tetranor-12-HETE	45 (0-779)	860 (267-1829)	0.007	**0.017**
12,13-diHOME	861 (559-3126)	1884.5 (1308-7467)	0.016	**0.025**
9-HOTrE	67 (0-461)	2883 (983-5663)	<0.001	**0.002**
13-HOTrE	0 (0-754)	3258 (1237-13 099)	<0.001	**0.002**
13(s)-HOTrEr	0 (0-1168)	0 (0-6879)	0.127	0.156
15-HEPE	893 (0-2919)	4560 (2440-58 842)	0.001	**0.005**
11-HEPE	0 (0-687)	1223.5 (0-4810)	0.010	**0.022**
12-HEPE	2093.5 (263-13 297)	7441.5 (4036-25 127)	0.021	**0.030**
13-HODE	12 240 (4812-53 576)	523 709 (83 956-1 164 152)	<0.001	**0.002**
13-oxoODE	610.5 (0-5029)	274 305 (31 177-5 066 353)	<0.001	**0.002**
17-HDoHE	956.5 (0-3954)	4339 (2412-52 905)	0.001	**0.005**
9-oxoODE	798.5 (396-4324)	34 992 (15 548-258 095)	<0.001	**0.002**
13-HDoHE	126 (0-2260)	4433 (1691-16 975)	0.001	**0.002**
14-HDoHE	1284 (424-5126)	4586.5 (2920-13 613)	0.006	**0.015**
10-HDoHE	0 (0-1535)	2860.5 (0-8246)	0.001	**0.004**
11-HDoHE	0 (0-4563)	9940.5 (4323-27 510)	<0.001	**0.002**
12-oxoETE	0 (0-22 158)	11 493 (0-65 199)	0.015	**0.025**
8-HDoHE	0 (0-0)	570.5 (0-2972)	0.005	**0.013**
15-HETrE	1273 (0-3664)	8109 (3083-46 134)	0.001	**0.002**
8-HETrE	428.5 (0-12 908)	6361 (0-47 638)	0.067	0.094
11-HEDE	1050 (45-4070)	4480 (1654-11 272)	0.010	**0.022**
15-HEDE	0 (0-557)	21 147 (1349-34 022)	<0.001	**0.002**
15-oxoEDE	75 (0-243)	8114 (199-166 387)	0.001	**0.004**
EPA	23 484.5 (7930-49 780)	61 649 (20 845-254 505)	0.013	**0.025**
DHA	49 010 (30 751-104 059)	179 498 (40 853-361 440)	0.016	**0.025**
Arachidonic acid	119 911 (67 807-181 169)	318 063.5 (81 083-632 217)	0.016	**0.025**
PGD2	2915 (0-38 340)	723 (0-11 356)	0.247	0.275
13,14-diH-15-oxo-PGE2	5693.5 (0-24 275)	3247 (0-7965)	0.247	0.275
PGA2	0 (0-10 823)	2296 (0-21 055)	0.306	0.319
PGE2	14 462 (3087-60 663)	8688 (1954-148 420)	0.722	0.737

Abbreviations: DHA, docosahexaenoic acid; diHOME, dihydrometabolites; EPA, eicosapentaenoic acid; HDoHE, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HETrE, hydroxyeicosatrienoic acid; HODE, hydroxyoctadecadienoic acid; ND, not detected; PG, prostaglandin.

Data are displayed as medians (minimum-maximum).

Boldface indicates statistically significant results.

[a]FDR was calculated by Benjamini-Hochberg adjustment.

**P* values were calculated by Wilcoxon rank-sum test.

Electrospray ionization was performed in the negative ion mode with a temperature of 400°C. The ion spray voltage was set at −4500 V.

For the quantification of the other analytes, the same HPLC and mass spectrometry (MS) conditions as above were applied, except that Qtrap 5500 (AB Sciex Pte. Ltd.) was used as the mass spectrometer and the length of the Poroshell 120 SB-C18 column was 150 mm. Multi-reaction monitoring (MRM) was used for quantification of the analytes. The mass transitions (Q1/Q3, *m/z*), declustering potential (DP, V), and collision energy (CE, V) for hepoxilin B_3 were, respectively, 335/183, −70, and −20, those for 12-oxoETE were 317/179, −115, and −22, those for 12(*S*)-HETE were 319/179, −110, and −18, and those for (10*R*,11*S*,12*S*)-trioxilin B_3 and (10*S*,11*S*,12*S*)-trioxilin B_3 were 353/153, −85, and −22. For the IS, the mass transition was 327/184, and DP and CE were,

TABLE 4 Comparison of each hepoxilin and its related lipids between patients with psoriasis and psoriatic arthritis

Target	PsA (N = 3) (10^2 ng/mg tissue)	PsV (N = 5) (10^2 ng/mg tissue)	*P*-value*	FDR[a]
Hepoxilin B3	0.76 (0.43-1.25)	1.34 (0.26-5.62)	0.297	0.661
Hepoxilin A3	0 (0-0)	0 (0-0)	-	-
12-HPETE	0 (0-0)	0 (0-0)	-	-
12-HETE	18.42 (11.91-19.99)	32.77 (10.84-71.38)	0.456	0.698
12-KETE	0.73 (0.69-1.67)	4.63 (0.31-10.03)	0.180	0.661
Trioxilin B3-1	0 (0-1.08)	1.05 (0-6.52)	0.285	0.661
Trioxilin B3-2	0 (0-0.75)	0.45 (0-9.40)	0.427	0.698
5R	0 (0-0)	0 (0-2.28)	0.439	0.698
5S	0 (0-0)	0 (0-6.00)	0.242	0.661
8R	0 (0-2.28)	2.57 (0-4.36)	0.266	0.661
8S	0 (0-4.47)	4.84 (0-8.70)	0.266	0.661
9R	0 (0-3.42)	3.84 (0-6.48)	0.266	0.661
9S	0 (0-0)	0 (0-0)	-	-
11R	0 (0-3.92)	2.41 (0-7.59)	0.427	0.698
11S	0 (0-0)	0 (0-0)	-	-
12R	13.46 (9.53-24.53)	50.99 (13.82-125.78)	0.101	0.661
12S	11.51 (6.23-34.94)	14.20 (6.36-18.47)	0.882	0.882
15R	0 (0-0)	0 (0-6.00)	0.242	0.661
15S	15.20 (12.36-19.44)	26.33 (6.01-116.97)	0.297	0.661
HepoA3R	0 (0-0)	0 (0-0)	-	-
HepoA3S	0 (0-0)	0 (0-4.37)	0.439	0.698
HepoB3R	0 (0-0)	0 (0-0)	-	-
HepoB3S	0 (0-0)	0 (0-3.81)	0.242	0.661

Abbreviations: HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; KETE, keto-eicosatetraenoic acid.
Data are displayed as medians (minimum – maximum).
[a]FDR was calculated by Benjamini-Hochberg adjustment.
**P* values were calculated by Wilcoxon rank-sum test.

respectively, set at −80 and −20 for the quantification of hepoxilin B_3 and at −110 and −18 for the quantification of the other analytes.

2.4 | Determination of epidermal concentrations of eicosanoid metabolites

Ethanol solutions of the deuterated compounds 12(*S*)-HETE-d_8, PGD_2-d_4, AA-d_8, and LTB_4-d_4 at 200 ng/mL were combined and used as the IS solution. To 100 μL of the epidermal extract were added 100 μL of water, 80 μL of 1% acetic acid aqueous solution, and 20 μL of methanol. Subsequently, 10 μL of the 200 ng/mL IS solution and 600 μL of ethyl acetate were spiked, and the mixture was centrifuged at 12 000 rpm at 4°C for 3 minutes for separation. The organic phase (550 μL) was collected in a tube, and 600 μL of ethyl acetate was added to the aqueous phase and further centrifuged under the same conditions. The resultant organic phase (550 μL) was combined with that collected previously and centrifugally concentrated. The resultant solid residue was reconstituted with 100 μL of ethanol.

Quantification was performed under the same HPLC and MS conditions described in the analytical method for the quantification of the other analytes. The gradient program was as follows: 0 minutes - 27% B; 5 minutes - 27% B; 15 minutes - 70% B; 25 minutes - 85% B; 30 minutes - 100% B. The applied MRM parameters are described elsewhere.[10]

2.5 | Statistical analysis

Variables are expressed as medians (range) or n (%), and they were compared between patients and normal controls using the Wilcoxon rank-sum test and Fisher's exact test. All *P*-values were two-tailed. A *P*-value of <0.05 was considered statistically significant. When the production of a lipid mediator was not detected, its amount was set as 0. Lipid mediators that were detected in at least one sample were subjected to further analysis. The amount of production of each lipid mediator was compared between patients and normal controls using the Wilcoxon rank-sum test. *P*-values for lipid mediators that were detected in at least one sample were adjusted for false discovery rate (FDR) based on the Benjamini and Hochberg method to correct for multiple testing. An FDR value of <0.05 was considered to be statistically significant. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

TABLE 5 Comparison of each lipid mediator of patients between psoriasis and psoriatic arthritis

Target	PsA (N = 3) (ng/mg tissue)	PsV (N = 5) (ng/mg tissue)	*P*-value*	FDR[a]
PGE3	5356 (0-8980)	0 (0-0)	0.051	0.661
PGE1	4908 (0-5031)	0 (0-203)	0.124	0.661
Tetranor-12-HETE	559 (267-1780)	988 (298-1829)	0.456	0.698
12,13-diHOME	2720 (1799-3140)	1810 (1308-7467)	0.456	0.698
9-HOTrE	2439 (1204-3019)	4425 (983-5663)	0.297	0.661
13-HOTrE	2650 (1246-4348)	3866 (1237-13 099)	0.655	0.823
13(s)-HOTrEr	0 (0-1567)	0 (0-6879)	0.608	0.823
15-HEPE	6077 (2902-6906)	3043 (2440-58 842)	0.882	0.882
11-HEPE	1287 (1160-1290)	1069 (0-4810)	0.653	0.823
12-HEPE	9263 (4512-12 565)	5620 (4036-25 127)	0.882	0.882
13-HODE	263 538 (108 382-626 569)	831 640 (83 956-1 164 152)	0.297	0.661
13-oxoODE	157 002 (31 177-459 830)	391 608 (100 598-5 066 353)	0.456	0.698
17-HDoHE	4560 (3110-8457)	4118 (2412-52 905)	0.882	0.882
9-oxoODE	32 748 (15 548-37 236)	52 074 (20 673-258 095)	0.297	0.661
13-HDoHE	3374 (1691-6869)	5170 (2789-16 975)	0.297	0.661
14-HDoHE	4401 (3619-6034)	4772 (2920-13 613)	0.882	0.882
10-HDoHE	3048 (0-3711)	2673 (1525-8246)	0.655	0.823
11-HDoHE	7585 (4323-16 264)	12 296 (5273-27 510)	0.456	0.698
12-oxoETE	6232 (0-6612)	27 649 (0-65 199)	0.134	0.661
8-HDoHE	449 (0-1210)	692 (0-2972)	0.647	0.823
15-HETrE	5521 (3083-19 641)	9279 (4306-46 134)	0.297	0.661
8-HETrE	5746 (0-16 409)	6976 (0-47 638)	0.764	0.882
11-HEDE	3060 (1685-11 272)	5900 (1654-7669)	0.882	0.882
15-HEDE	13 960 (1349-24 385)	24 074 (3104-34 022)	0.297	0.661
15-oxoEDE	7576 (199-15 791)	8652 (3977-166 387)	0.655	0.823
EPA	70 546 (20 845-126 615)	59847 (29 597-254 505)	0.882	0.882
DHA	200 907 (40 853-240 642)	158 089 (56 501-361 440)	0.882	0.882
Arachidonic acid	328 833 (81 083-494 579)	307 294 (122 432-632 217)	0.882	0.882
PGD2	389 (181-11 356)	1057 (0-2712)	0.653	0.823
13,14-diH-15-oxo-PGE2	2579 (0-3887)	3555 (0-7965)	0.368	0.698
PGA2	5074 (0-21 055)	2229 (0-3895)	0.285	0.661
PGE2	79 158 (3513-148 420)	7869 (1954-11 961)	0.297	0.661

DHA, docosahexaenoic acid; diHOME, dihydrometabolites; EPA, eicosapentaenoic acid; HDoHE, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HETrE, hydroxyeicosatrienoic acid; HODE, hydroxyoctadecadienoic acid; ND, not detected; PG, prostaglandin.

Data are displayed as medians (minimum-maximum).

[a]FDR was calculated by Benjamini-Hochberg adjustment.

**P* values were calculated by Wilcoxon rank-sum test.

3 | RESULTS

3.1 | Hepoxilin and its related lipids in psoriatic skin

First, we measured the content of hepoxilin B3, hepoxilin A3, 12-HPETE, 12-HETE, 12-keto-eicosatetraenoic acid (KETE), and trioxilin B3 in the skin specimens collected from the PPG and 10 normal controls. In the PPG, all hepoxilins and their related lipids were more abundant than in the normal controls (Table 2). Among these lipids, we could not detect (below the limit of quantification) hepoxilin A3 or 12-HPETE in the PPG or the control group, and trioxilin B3-2(*S*) was only detected in the PPG (Table 2).

3.2 | Each isomer of hepoxilin and HETE in psoriatic skin

Next, all of the hepoxilin and HETE contained in the skin samples were separated for each isomer, and the amount of each isomer

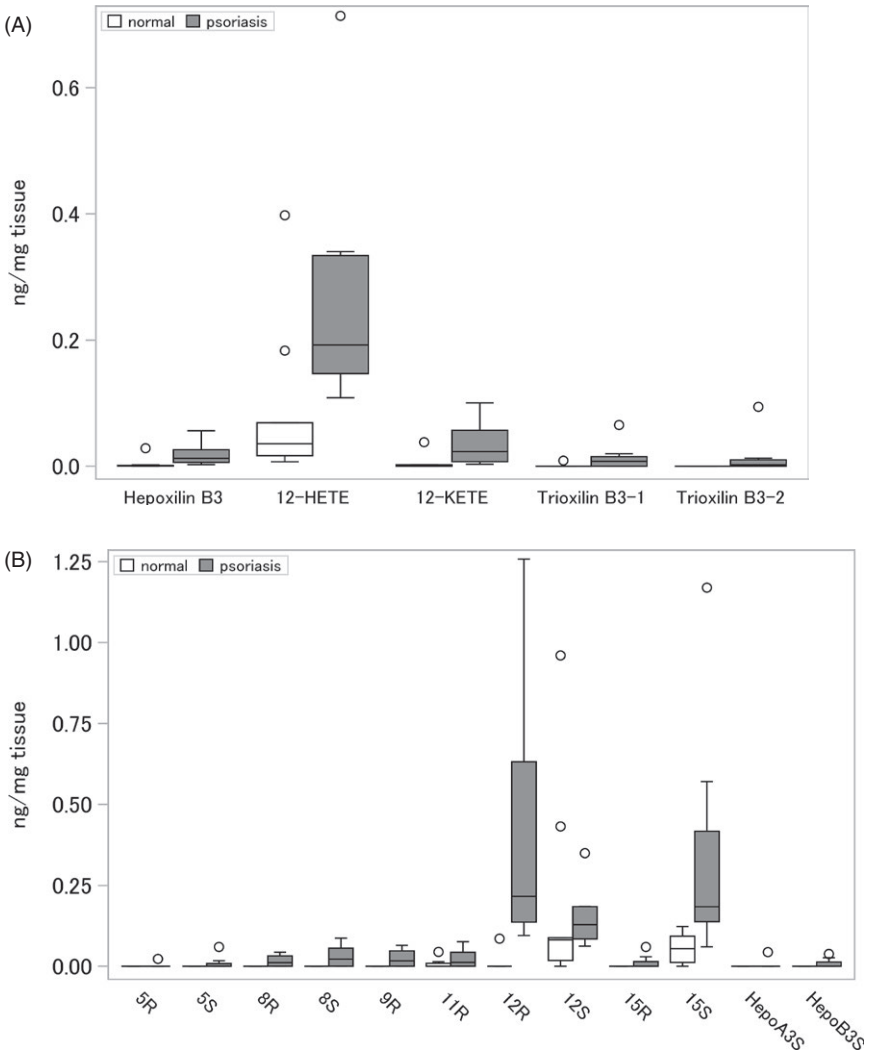

FIGURE 1 Comparisons of the amount of each hepoxilin-related lipid, each isomer of hepoxilin and HETE between psoriatic skin and normal skin, and of the amount of prostaglandin E_1, E_2, and E_3 in lesional skins between patients with psoriatic arthritis and those with psoriasis without arthritis. A, Box plot for the amount of each hepoxilin-related lipid extracted from the lesional skin samples of patients with psoriasis and normal control skins. B, Box plot for the amount of each isomer of hepoxilin and HETE extracted from the lesional skin samples of patients with psoriasis and normal control skins. *Significant difference ($P < 0.05$, Wilcoxon rank-sum test)

was measured. In the PPG, most of the isomers of hepoxilin and HETE were more abundant than those in controls. As for 12*R*-HETE, the PPG had significantly higher amounts than those of the controls. The ratio of 12*R*-HETE/12*S*-HETE differed between the PPG and the normal controls (PPG: 2.47 (0.39-8.86); controls: 0 (0-0.09)). The amount of 12*S*-HETE did not significantly differ between the PPG and the controls. Thus, the production of 12*R*-HETE was specifically increased in the PPG (Table 2).

3.3 | Each lipid mediator in psoriatic skin

To ascertain which pathway is important in the development of psoriatic skin, the amount of each metabolic product in arachidonic acid and eicosapentaenoic acid (EPA) systems contained in the skin samples from the PPG and the controls was measured. Median values for each lipid in skin tissues from the PPG and the controls are described in Table 3. Lipids produced in the LOX pathway were more abundant in the skin tissue from PPG than in the skin from controls, whereas the lipids produced by COX pathway tended to be less abundant in the psoriatic skin.

3.4 | Comparison of lipid production between psoriatic arthritis and psoriasis vulgaris (psoriasis without arthritis)

Furthermore, we compared the amounts of each lipid between PsA patients and PV patients (Tables 4 and 5). Within this analysis, prostaglandins (PGs) E_1, E_2, and E_3 extracted from lesional skin samples were more abundant for the PsA patients than for the PV patients, although statistical significance was not obtained. No significant difference was present between the two study groups in any hepoxilin derivatives or lipid mediators.

4 | DISCUSSION

To the best of our knowledge, no reports have given a comprehensive analysis of lipid mediators in psoriatic skin lesions. Most of the hepoxilins and their related lipids that were evaluated in this study tended to be more abundant in the lesional skin of the PPG than in skin from the controls (Tables 2 and 3). Hepoxilins are classified into type A and type B.[2] Because of the differences in chemical stability,

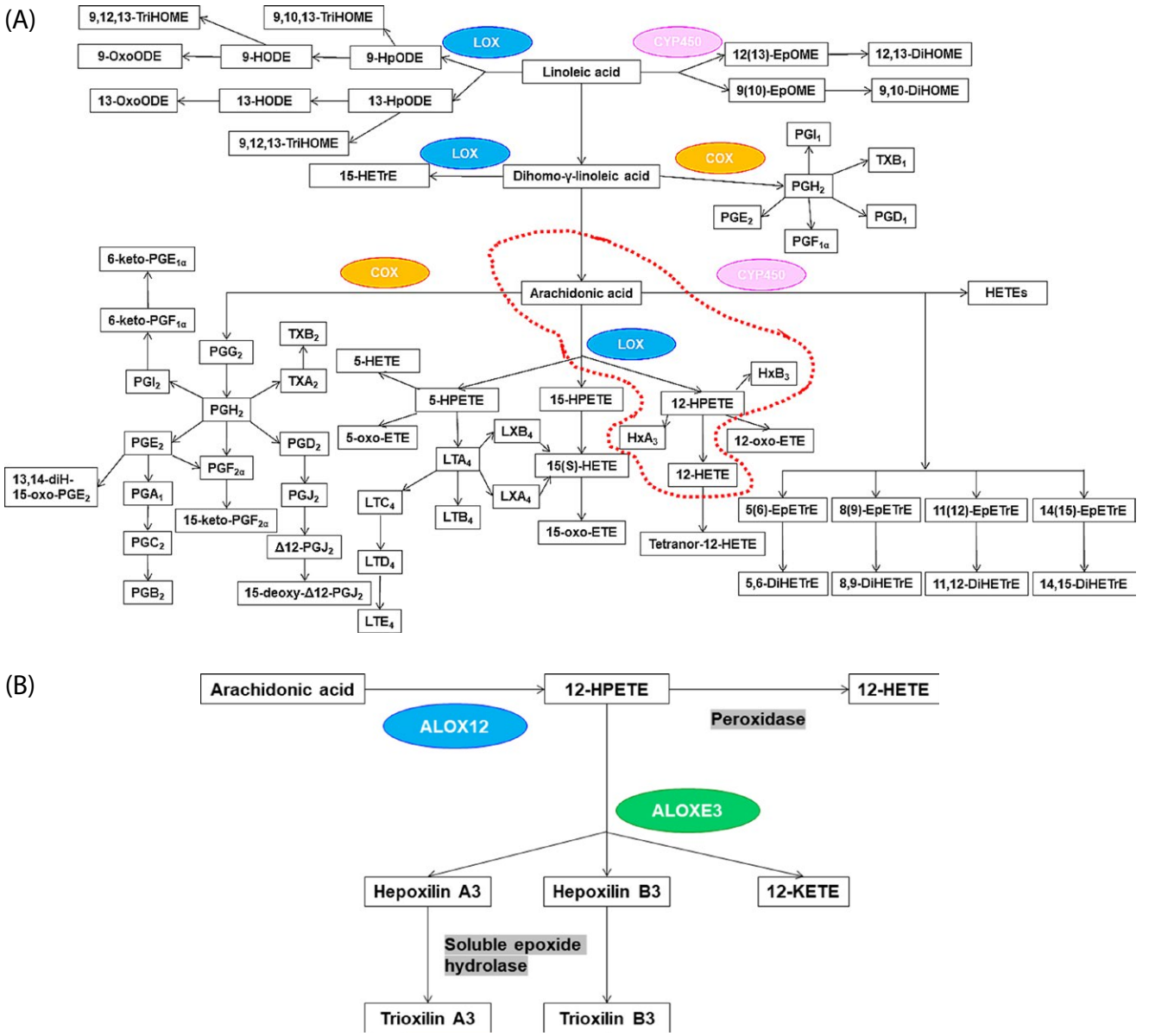

FIGURE 2 Spectra of metabolic pathways to and from arachidonic acids (eicosanoids). A, A comprehensive schematic of the arachidonic acid metabolic cascade. B, Detailed pathways and enzymes in the area marked with the red dotted line in (A). 12-HPETE produced via ALOX12 pathway is converted by ALOXE3 to hepoxilin A3 and hepoxilin B3, which are further metabolized to the corresponding trioxillions. Abbreviations: COX, cyclooxygenase; diHOME, dihydrometabolites; HDoHE, hydroxydocosahexaenoic acid; HETE, hydroxyeicosatetraenoic acid; HETrE, hydroxyeicosatrienoic acid; HODE, hydroxyoctadecadienoic acid; Hx, hepoxilin; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane

hepoxilin B3 is commonly detected in tissue extracts, whereas the hepoxilin A3 may appear as the corresponding hydrolysis product, trioxilin A3.[2] In the literature, the level of hepoxilin B3 was greatly increased in the lesional scales in psoriasis, along with the corresponding trioxilin B3.[11,12] Our results are consistent with these findings. However, the previous report used extracts from scales (not skin samples from biopsy) and used the different methods (gas chromatography-mass spectrometry). These studies were performed at least two decades earlier, and the clinical features of psoriasis and the number of patients were not included in the previous report.[11]

Interestingly, 12*R*-HETE was markedly increased in the skin samples of the PPG. This result is consistent with lipid analyses in previous reports.[7] The first indication of a role for oxidized polyunsaturated fatty acids in skin biology came in the mid-1970s, when an accumulation of 12-HETE and free arachidonic acid was discovered in psoriatic epidermis.[2] A decade later, 12-HETE in psoriatic skin lesions was shown to be mainly 12*R*-HETE.[2] The synthesis of 12*R*-HETE in psoriasis had been thought to be induced by inflammation in the epidermis. Yet 12*R*-HETE itself showed only modest proinflammatory activity. From a current perspective, the abundance of 12*R*-HETE in psoriasis may merely reflect the production of free

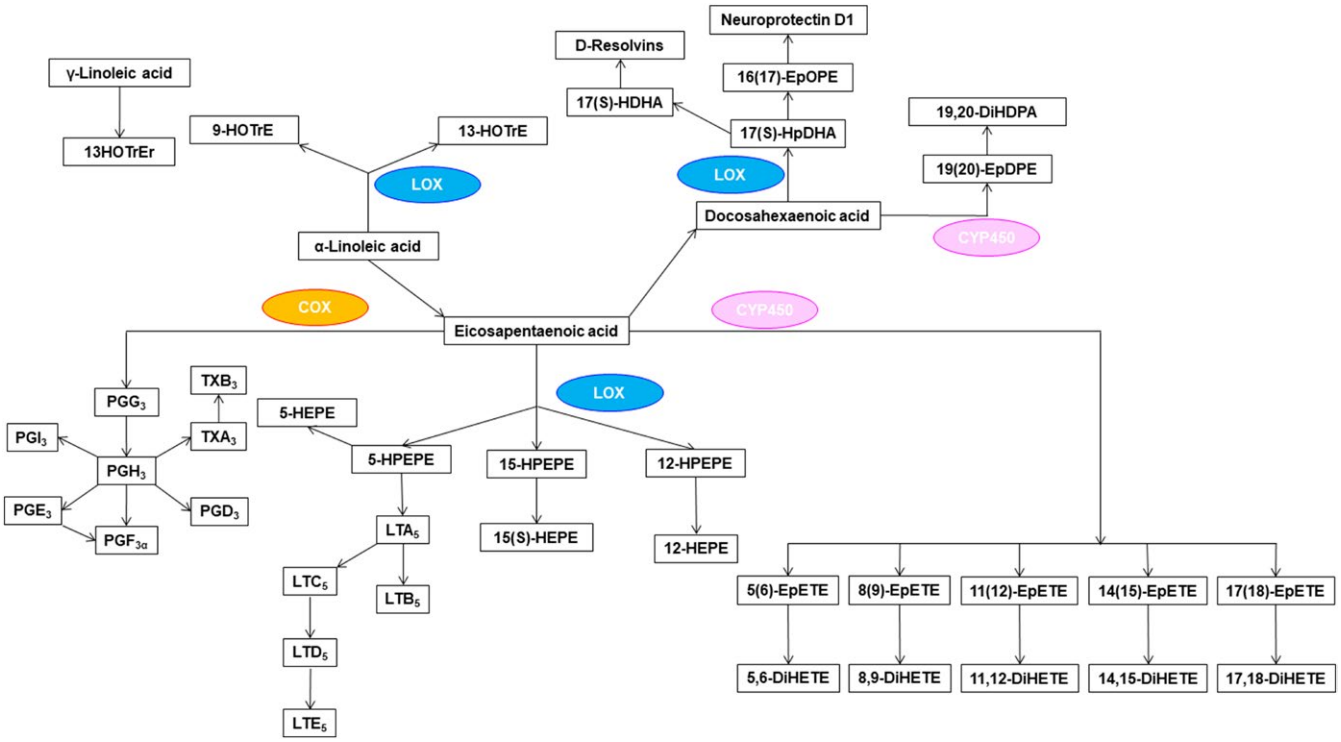

FIGURE 3 Spectra of metabolic pathways to and from eicosapentaenoic acids. Abbreviations: COX, cyclooxygenase; diHOME, dihydrometabolites; HDHA, hydroxy docosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HpETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane

arachidonic acids in psoriatic lesions.[2] Such acids are not present in normal skin but are present at the inflammation sites of psoriatic lesions. Sufficient amounts of arachidonic acid available as substrates together with the high expression of 12R-LOX may account for the excess of 12*R*-HETE in psoriatic epidermis.[2] 12*R*-HPETE is produced from arachidonic acid by the addition of a hydroxyperoxide group. This reaction is catalyzed by 12R-LOX. The amount of synthesized 12*R*-HETE is expected to depend on the amount of 12*R*-HPETE as a precursor of 12*R*-HETE. In this context, we speculate that the addition of a hydroxyperoxide group to arachidonic acid by 12R-LOX is abnormally upregulated in psoriatic skin lesions, resulting in the overproduction of 12*R*-HETE, which leads to the excessive 12*R*-HETE in psoriatic epidermis.

LTB_4 is a strong chemoattractant and chemoactivator of neutrophils.[13] LTB_4 synthesis from arachidonic acid is mediated by the activity of 5-LOX together with a 5-LOX-activating protein.[13] LTB_4 is produced from LTA_4 by LTA_4 hydrolase.[13] 12*R*-HETE can also activate the LTB_4 receptor, but with less efficiency.[13] Several LTB_4 antagonists are known, and their clinical evaluation is ongoing.[13] In vivo, SC53228, an LTB_4 antagonist, inhibits LTB_4-induced skin inflammation in guinea pigs and counteracts inflammation induced by 12*R*-HETE.[13] The compound has been selected for clinical trials for a number of diseases including psoriasis,[13] although no clinical benefit has been reported.

In the present study, the lipids produced in the LOX pathway, including hepoxilin, were more abundant in the skin lesions for the PPG than in the skin of the normal controls. In contrast, the lipids produced by the COX pathway were decreased in the skin lesions of the PPG (Figure 1B and Table 3). From these results, the LOX pathway was more predominant than the COX pathway in the lesional skin of the PPG. Therefore, our results suggest that an imbalance between LOX and COX pathways may contribute to the pathomechanisms of psoriasis.

None of our results correlated with the PASI scores for the PPG in the present study (data not shown). The absence of correlation might be due to the fact that the severity of the psoriatic changes and the levels of inflammation at the skin biopsy sites were not directly associated with the PASI scores of the patients. In addition, various treatments which the patients had might have affected both the PASI and the levels of lipid mediators.

The clinical course of psoriasis may be complicated by the concomitant joint disease PsA in 7%-40% of patients.[14] The relationship between skin and joint manifestations is not clear.[15] Although early diagnosis may lead to early treatment and benefits for PsA patients, the reliable prediction of PsA development in PV patients has not been established.[16] Recently, a group for the research and assessment of psoriasis and psoriatic arthritis proposed serum biomarkers including interleukin-6, matrix metalloproteinase-3, and others as markers to distinguish PsA from PV.[15,17] In the present study, interestingly, PGE_1 and PGE_3 were more highly expressed in the skin samples from PsA than in those from PV. Different from PGE_2, PGE_1 has anti-inflammatory effects on endothelial cells and certain subsets of leukocytes.[18] In addition, PGE_3 may have antiproliferative and anti-inflammatory activities.[19] In contrast,

in the case of rheumatoid arthritis, PGE_2 has been found in the synovial fluid and the roles of PGE_2 in inflammation was emphasized.[20] Perhaps the inflammation in the affected joints of patients with PsA might correlate with the production of PGE_1 and PGE_3 (Figures 2 and 3).

To ascertain whether there are any molecular differences between the psoriasis plaque lesions from PsA patients and those from PV patients, we analyzed the difference in amounts of each lipid in the two groups (Table 5). In the present study, no significant differences were found in the levels of hepoxilin derivatives and lipid mediators between the two groups, although the small sample size of our study for this analysis was a limitation of the present study. The further accumulation of samples from psoriasis cases with and without PsA is needed to confirm the molecular role of PGs in PsA.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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