Case Report

A case series of the dynamics of lipid mediators in patients with sepsis

Mitsuhide Hamaguchi,[1] Heng Ning Wu,[2] Masahiro Tanaka,[3] Noriko Tsuda,[1] Ourlad Alzeus Gaddi Tantengco,[2,4] Tomohide Matsushima,[1] Takami Nakao,[1] Takuya Ishibe,[1] Ikuhiro Sakata,[5] and Itaru Yanagihara[2]

[1]Department of Emergency and Critical Care Medicine, Kindai University Faculty of Medicine, Osakasayama, [2]Department of Developmental Medicine, Research Institute, Osaka Women's and Children's Hospital, Izumi, [3]Nagayama Hospital, Osaka, Japan, [4]College of Medicine, University of the Philippines Manila, Manila, Philippines, and [5]Bell land General Hospital, Sakai, Osaka, Japan

Background: Bioactive lipid mediators play a crucial role during infection. Previously, we showed the expression level of *FAAH* mRNA in septic patients was lower than in healthy controls.

Case Presentation: Four patients with a Sequential Organ Failure Assessment (SOFA) score of <7 recovered from sepsis. One patient with SOFA score of 12 on day 7 died on day 21. In the fatal case, eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid, and linoleic acid-derived lipid mediators, including 9-hydroxyoctadecadienoic acid (9-HODE), 13-HODE, 9,10-dihydroxy-12-octadecenoic acid (9,10-DiHOME), and 12,13-DiHOME, were elevated on day 1. Increase in anti-inflammatory prostaglandin E_1 ethanolamide together with persistently lower transcription level of *FAAH* mRNA was detected on day 7 in the fatal case.

Conclusion: Lipidomic analysis on day 1 revealed elevated linoleic acid metabolites, whereas on day 7, elevated prostaglandin E_1 ethanolamide and low level of *FAAH* mRNA transcription were observed in the fatal case of sepsis.

Key words: Anandamide, fatty acid amide hydrolase, linoleic acid, lipid mediator, sepsis

INTRODUCTION

ONE OF THE endocannabinoids, N-arachidonoyl ethanolamide (anandamide, AEA), is a lipid transmitter that has been implicated in the hypotension of septic shock. Anandamide is hydrolyzed to arachidonic acid (AA) and ethanolamine by fatty acid amide hydrolase (FAAH).[1] Previously, we examined the expression of *FAAH* mRNA between septic patients and healthy controls. The expression of *FAAH* mRNA in septic patients was significantly lower than in healthy controls, and the expression level of *FAAH* mRNA remained at low levels in two non-survivor cases, suggesting that the synthesis of FAAH, which is the lipid-degrading enzyme of AEA, might presumably be involved in sepsis.

In this study, we analyzed the profiles of several fatty acids and their metabolites as well as the expression level of *FAAH* mRNA from patients during their septic status. The data obtained during the first week of sepsis revealed high levels of 9-hydroxyoctadecadienoic acid (9-HODE), 13-HODE, 9,10-dihydroxy-12-octadecenoic acid (9,10-DiHOME), and 12,13-DiHOME on day 1, and elevated prostaglandin E_1 ethanolamide (PGE_1-EA) and decreased *FAAH* mRNA transcription level on day 7.

CASE

FIVE PATIENTS WERE admitted into the intensive care unit of Kindai University (Osakasayama, Japan) and treated before 2015; all patients met the diagnostic criteria of Sepsis-3. All five patients were treated with polymyxin B-immobilized fiber column-direct hemoperfusion (PMX-DHP) within 24 h of their admission followed by dialysis with continuous hemodiafiltration (CHDF) for >7 days.

Corresponding: Itaru Yanagihara, MD, PhD, Department of Developmental Medicine, Research Institute, Osaka Women's and Children's Hospital, Izumi, Osaka, Japan. E-mail: itaruy@wch.opho.jp.

Received 18 Feb, 2019; accepted 19 Jun, 2019; online publication 18 Jul, 2019

Funding information

This work was supported by research grants from the JSPS KAKENHI, Grant Numbers JP15K20353 (M.H.) and JP17H04237 (I.Y.); the Japan Agency for Medical Research and Development (AMED), JP17fk0108210 (I.Y.).

Various bioactive lipids in the plasma were measured using the LC/MS/MS Method Package for Lipid Mediators at Shimadzu Techno-Research, Kyoto, Japan (https://www.shimadzu.com/an/lcms/lipid_mediators.html). In total, 158 lipid mediators and internal standards were measured on days 1 and 7. The cut-off value of each compound was a peak area of 3,000.

Whole blood RNA was used to quantify FAAH mRNA according to our previous report.[2]

RESULTS

THE patient demographic data, the Sequential Organ Failure Assessment (SOFA) score, quick SOFA score, levels of *FAAH* mRNA expression, and AEA are listed in Table 1. Only case 5 died on day 21. The expression level of *FAAH* mRNA ratio (*FAAH* mRNA day 7/day 1) was 0.80 in case 5. The ratio in the fatal case 5 was lower compared to that of the survivor cases 1, 2, 3, and 4 with the ratios of 1.41, 5.64, 2.05, and 2.61, respectively. This indicates that the *FAAH* mRNA expression increased after 7 days among the survivors only.

The detected lipid mediators are described in Table 2 and summarized in Figure 1. The selected lipid mediators met the criteria that the peak area observed in the fatal case was at least larger than the maximum peak area among the survivors. As a result, the following 12 lipid mediators were selected from the 158 compounds in the fatal case, as their peak area was higher compared to the other four survivor cases. On day 1, we selected: eicosapentaenoic acid (EPA) pathway, EPA (peak area of case 5 vs. maximum peak area of cases 1–4: 7,303 vs. ≤3,000); docosahexaenoic acid (DHA) pathway, DHA (245,904 vs. 87,971); linoleic acid (LA) pathway; 9-HODE (110,625 vs. ≤3,000), 13-HODE (329,746 vs. 6,806), 9,10-DiHOME (229,402 vs. 7,738), and 12,13-DiHOME (100,736 vs. 8,431); ALA pathway; 9-HOTrE (10,263 vs. ≤3,000); AA pathway, AA (180,523 vs. 89,132), 5-iPF2α-VI (7,588,581 vs. 64,887); 11,12-DHET (4,351 vs. ≤3,000), 20-carboxy-AA (17,650 vs. 6,047); ethanolamide; and PGE_1-EA (7,782 vs. ≤3,000). In contrast, on day 7, ethanolamide and PGE_1-EA were specifically detected in the fatal case (23,434 vs. ≤3,000); however, almost all of the lipid mediators were not specifically detected after PMX-DHP and CHDF treatment. The levels of AEA on days 1 and 7 did not show any association with the septic status of the patients. In the fatal case, the peak area of AEA was 16,493, and 7,598 on days 1 and 7, respectively. In contrast, the peak area of the four survivors ranged from 5,368 to 41,893 and ≤3,000 to 12,369 on days 1 and 7, respectively.

Table 1. Patient characteristics, severity, *FAAH* mRNA, and anandamide (AEA) level in five patients with sepsis

Case	Gender	Age, years	Quick SOFA			SOFA score (excluding GCS)		*FAAH* mRNA			AEA		Cause of sepsis
			Respiratory rate ≥22/min	Altered mentation	Systolic blood pressure ≤100 mmHg	Day 1	Day 7	Day 1	Day 7	Day 7/day 1	Day 1	Day 7	
1	Male	47	○	○		2	5	2.26E-01	3.18E-01	1.41	5,368	3,000↓	Severe acute pancreatitis
2	Male	56	○			9	6	6.49E-02	3.66E-01	5.64	41,893	4,662	Severe acute pancreatitis
3	Male	66	○			7	7	1.33E-01	2.73E-01	2.05	9,749	12,369	Wound infection
4	Male	57				10	4	2.28E-01	5.95E-01	2.61	14,981	7,542	Unknown
5	Female	72	○		○	10	12	4.80E-01	3.83E-01	0.8	16,493	7,598	Gynaecological infection

GCS, Glasgow Coma Scale; SOFA, Sequential Organ Failure Assessment.

Table 2. Changes in lipid mediators on days 1 and 7 in five patients with sepsis

		Survivors												Fatal case		
		Case 1			Case 2			Case 3			Case 4			Case 5		
		Day 1	Day 7	TR	Day 1	Day 7	TR	Day 1	Day 7	TR	Day 1	Day 7	TR	Day 1	Day 7	TR
1	EPA	–	–		–	–		–	–		–	–		7,303	3,076	0.322
2	DHA	68,411	7,514	0.119	50,793	31,394	0.634	51,276	42,693	0.907	87,971	39,705	0.435	245,904	64,138	0.199
3	9-HODE	–	3,512		–	13,439	↑	–	15,135	↑	–	–		110,625	4,866	0.04
4	13-HODE	–	16,603	↑	3,301	36,772	13.194	4,668	47,239	10.821	6,806	5,403	0.741	329,746	4,905	0.014
5	9,10-DiHOME	–	6,957	↑	6,511	13,302	2.112	4,980	42,426	10.168	7,738	5,408	0.681	229,402	4,279	0.016
6	12,13-DiHOME	–	–		8,431	7,333	0.899	6,634	24,178	4.35	6,585	5,411	0.8	100,736	5,233	0.043
7	9-HOTrE	–	–		–	–		–	–		–	–		10,263	–	↓
8	AA	71,045	–	↓	80,723	92,128	1.17	89,132	83,886	1.026	51,319	80,990	1.52	180,523	95,488	0.404
9	5-iPF2α-VI	–	1,871,405	↑	–	5,583,998	↑	4,481	6,322,343	1,674.8	64,887	27,432	0.439	7,588,581	519,173	0.067
10	11,12-DHET	–	–		–	–		–	–		–	–		4,351	–	↓
11	20-carboxy-AA	–	–		6,047	–	–	–	–		–	–		17,650	4,475	0.211
12	PGE1-EA	–	–		–	–		–	–		–	–		7,782	23,434	2.155
13	PGD2	–	–		–	–		–	–		–	3,769	↑	–	6,156	↑
14	PGF2a	–	–		–	–		4,046	–	↓	–	6,317	↑	–	4,623	↑
15	PGE2	–	–		–	3,099	↑	–	–		3,215	14,025	4.489	–	6,349	↑
16	12-HETE	–	3,465	↑	5,382	96,102	21.162	4,019	29,268	7.874	23,701	154,556	5.920	25,393	45,915	1.444
17	15-HETE	–	–	–	–	–	–	–	–	–	–	7,455	↑	–	–	–

↑Data only observed in day 7; ↓Data only observed in day 1; –, not detected; 5-iPF2α-VI, 5-iso Prostaglandin F2α-VI; 9-HOTrE, 9-hydroxyoctadecatrienoic acid; 11,12-DHET, 11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; DiHOME, dihydroxy-12-octadecenoic acid; EPA, eicosapentaenoic acid; HETE, HODE, hydroxyoctadecadienoic acid; PGD2, prostaglandin D2; PGE1-EA, prostaglandin E1 ethanolamide; PGE2, prostaglandin E2; PGF2a, prostaglandin F2a; TR, tendency ratio after compensation with internal standard (day 7 / day 1).

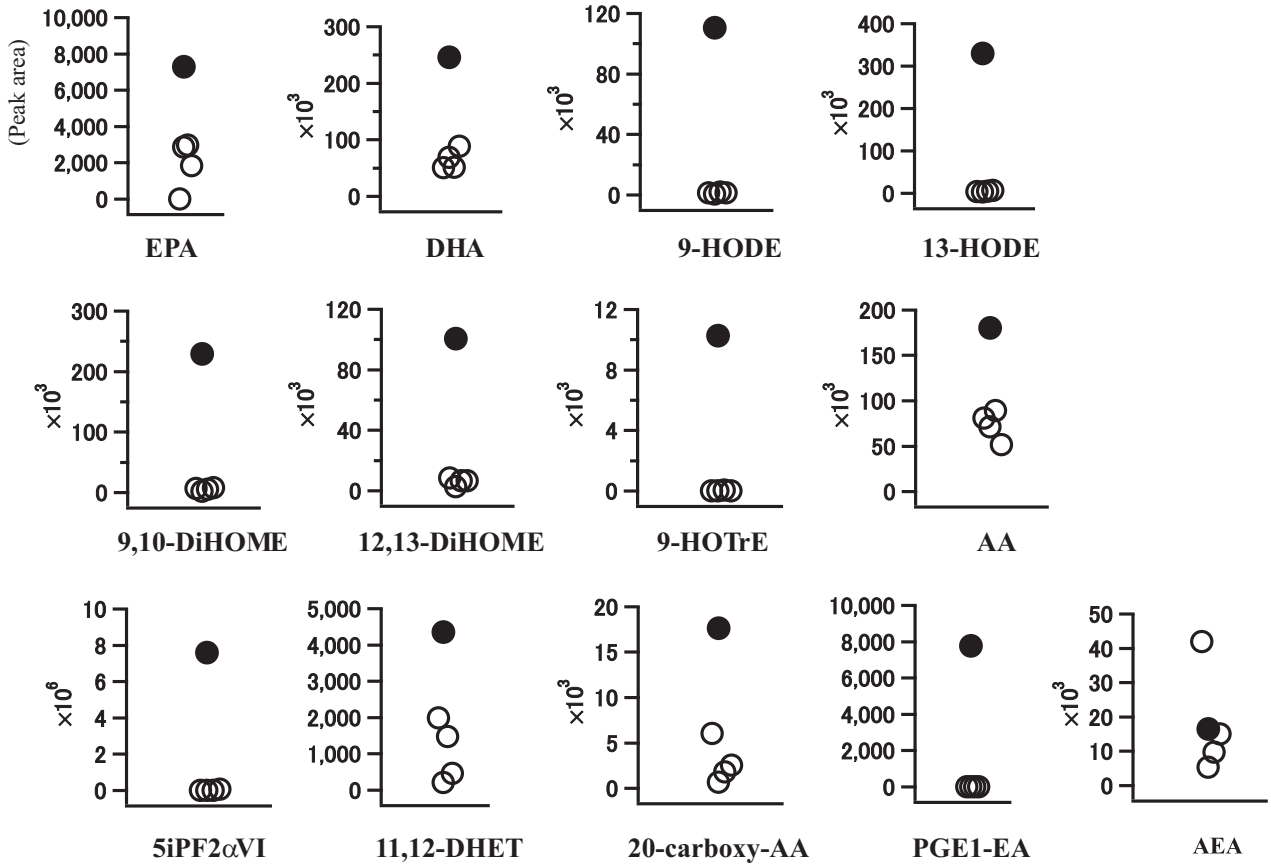

Fig. 1. Comparison of lipid mediators of the fatal case (black circle) and survivors (white circles) of sepsis on day 1. 5-iPF2α-VI, 5-iso Prostaglandin F2α-VI; 9-HOTrE, 9-hydroxyoctadecatrienoic acid; 11,12-DHET, 11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid; AA, arachidonic acid; AEA, N-arachidonoyl ethanolamide; DHA, docosahexaenoic acid; DiHOME, dihydroxy-12-octadecenoic acid; EPA, eicosapentaenoic acid; HODE, hydroxyoctadecadienoic acid; PGE1-EA, prostaglandin E1 ethanolamide.

DISCUSSION

BIOACTIVE LIPID MEDIATORS play a crucial role in the induction and resolution of various pathophysiological states during infection. Among the various lipid mediators, LA and its metabolites are considered as important mediators during an infection. The HODEs are stable oxidation products of LA. Higher HODE concentrations are an indication of oxidative stress. Both 9-HODE and 13-HODE are known to possess the potential to bind to the peroxisome proliferator-activated receptor-γ,[3] whereas 9-HODE imparts a pro-inflammatory effect through the G protein-coupled receptor 132 (GPR132). In contrast, 13-HODE cannot bind to GPR132 and its effects appear to be protective against inflammation.[4,5] Furthermore, the ratio of 13- to 9-HODE, hydroxylated metabolites of LA, has been identified as a biomarker of immune status during influenza infection in mice.[6]

The DiHOMEs are synthesized by soluble epoxide hydrolase from LA CYP450 metabolite epoxyoctadecanoic acids (EpOMEs). The EpOME levels are associated with acute respiratory distress syndrome, and DiHOMEs suppress the neutrophil respiratory burst.[7],13-DiHOME is a lipokine linked to metabolic homeostasis that increases the fatty acid uptake into the brown adipocytes through cold-stress exposure.[8]

In general, the dialysis membranes used in CHDF are not aimed to remove lipid mediators. The membrane pore size of the hemofilter used in CHDF only allows passage of substances with the molecular mass of 30,000–50,000.[9] From the speculation from the aspect of molecular masses of LA metabolites (i.e. 9-HODE is 296.44 and AEA is 347.53), LA metabolites and AEA can pass through the dialysis membrane and be removed as filtrate. However, if the lipid metabolites form a complex with the binding partners to become larger size than the pore, the lipid mediator complex

cannot pass through the filter. It is reported that hemodialysis alters lipidomic profiles, including significant decreases of phosphatidylcholine, phosphatidylinositol, and high-density lipoprotein cholesterol level, and the increase of sphingomyelin and diphosphatidylglycerol, of end-stage renal failure patients.[10] These analyses led us to anticipate that the hemodialysis might preferentially filter the lipid mediators. In our data, Table 2 shows that, in the fatal case, various lipid mediators decreased, except for PGE_1-EA, whereas in survivor cases the ratios of some of the lipid mediators increased and became detectable on day 7. These data seemed to imply that the decrease of the LA metabolites in the fatal case is not the natural course of CHDF, but it might reflect the vital metabolic phenomenon. In order to elucidate the dynamics of hemofiltration on lipid mediators, further study will be needed.

As a consequence of the reduced expression of *FAAH* mRNA, the level of plasma AEA was supposed to be higher in the fatal case; however, the blood AEA level was not raised (Table 1). It is known that enzymes other than FAAH can metabolize AEA. Anandamide is a substrate for cyclooxygenase-2 (COX-2), lipoxygenases (12-LOX and 15-LOX), and P450, resulting in the formation of prostaglandin-like compounds, prostamides.[11] We analyzed prostaglandin D2, prostaglandin F2a, prostaglandin E2, and 12-hydroxyeicosatetraenoic acid (HETE) and we found out that these analytes were elevated in the fatal case on day 7 (Table 2). In contrast, 15-hydroxy-eicosatetraenoic acid (15-HETE), 5,6-epoxyeicosatrienoic acid (5,6-EET), 8,9-epoxyeicosatrienoic acid (8,9-EET), 11,10-epoxyeicosatrienoic acid (11,10-EET), and 14,15-epoxyeicosatrienoic acid (14,15-EET) were below the detection limit (data not shown). Based on these data, at least, COX-2- and lipoxygenase-mediated AEA degradation might account for the discrepancy of the *FAAH* mRNA and AEA level.

A systematic review and meta-analysis of the randomized clinical trials revealed that nutritional supplementation with omega-3 fatty acids (n-3 FA) in septic patients reduced the length of stay in intensive care and the duration of mechanical ventilation; however, it did not affect the mortality.[12] In our fatal case, the EPA and DHA levels were much higher than those of the four survivor cases. In this case, with the high level of n-3 FA in the circulation system, the supplementation of n-3 FA might not be effective in controlling the excessive immune responses.

There is a concept that the elimination of inflammatory substances, such as pro-inflammatory cytokines and inflammatory lipid mediators, could be effective in sepsis treatment; however, the orchestration of each individual lipid mediator for its timing, duration, and magnitude is essential for homeostasis. A growing body of research has indicated that the convergence phase of inflammation needs anti-inflammatory and proresolving lipid mediators as well as anti-inflammatory cytokines.

This study is limited by its small sample size of only five Japanese cases. Further study with a larger sample size is needed to clarify the importance of LA metabolite profiling in the early stage of sepsis patients in Japan.

ACKNOWLEDGMENTS

THIS WORK WAS supported by research grants from the Japan Society for the Promotion of Science KAKENHI (Grant Nos. JP15K20353 to MH and JP17H04237 to IY) and the Japan Agency for Medical Research and Development (Grant No. JP17fk0108210 IY).

DISCLOSURE

Approval of the research protocol: The proposal for this research project was approved by the Ethics Committee of Kindai University Hospital, Approval No. 15-19. It conforms to the provisions of the Declaration of Helsinki.
Informed consent: Yes.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.
Conflict of interest: None.

REFERENCES

1 Izzo AA, Deutsch DG. Unique pathway for anandamide synthesis and liver regeneration. Proc. Natl. Acad. Sci. USA 2011; 108: 6339–40.
2 Tanaka M, Yanagihara I, Takahashi H, Hamaguchi M, Nakahira K, Sakata I. The mRNA expression of fatty acid amide hydrolase in human whole blood correlates with sepsis. J. Endotoxin Res. 2007; 13: 35–8.
3 Itoh T, Fairall L, Amin K *et al*. Structural basis for the activation of PPARgamma by oxidized fatty acids. Nat. Struct. Mol. Biol. 2008; 15: 924–31.
4 Hattori T, Obinata H, Ogawa A *et al*. G2A plays proinflammatory roles in human keratinocytes under oxidative stress as a receptor for 9-hydroxyoctadecadienoic acid. J. Invest. Dermatol. 2008; 128: 1123–33.
5 Vangaveti V, Shashidhar V, Collier F *et al*. 9- and 13-HODE regulate fatty acid binding protein-4 in human macrophages, but does not involve HODE/GPR132 axis in PPAR-gamma regulation of FABP4. Ther. Adv. Endocrinol. Metab. 2018; 9: 137–50.
6 Tam VC, Quehenberger O, Oshansky CM *et al*. Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. Cell 2013; 154: 213–27.

7 Thompson DA, Hammock BD. Dihydroxyoctadecamonoenoate esters inhibit the neutrophil respiratory burst. J. Biosci. 2007; 32: 279–91.
8 Lynes MD, Leiria LO, Lundh M *et al*. The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue. Nat. Med. 2017; 23: 631–7.
9 Oda S, Hirasawa H, Shiga H, Nakanishi K, Matsuda K, Nakamura M. Continuous hemofiltration/hemodiafiltration in critical care. Ther. Apher. 2002; 6: 193–8.
10 Piperi C, Kalofoutis C, Tzivras M, Troupis T, Skenderis A, Kalofoutis A. Effects of hemodialysis on serum lipids and phospholipids of end-stage renal failure patients. Mol. Cell. Biochem. 2004; 265: 57–61.
11 Giuffrida A, McMahon LR. In vivo pharmacology of endocannabinoids and their metabolic inhibitors: therapeutic implications in Parkinson's disease and abuse liability. Prostaglandins Other Lipid Mediat. 2010; 91: 90–103.
12 Lu C, Sharma S, McIntyre L *et al*. Omega-3 supplementation in patients with sepsis: a systematic review and meta-analysis of randomized trials. Ann. Intensive Care 2017; 7: 58.