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Lipidomes of brain from rats acutely intoxicated with diisopropylfluorophosphate identifies potential therapeutic targets

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ABSTRACT

Organophosphates (OPs), a class of phosphorus-containing chemicals that act by disrupting cholinergic transmission, include both toxic and fast-acting chemical warfare agents as well as less toxic but more easily accessible OP pesticides. The classical atropine/2-PAM antidote fails to protect against long-term symptoms following acute intoxication with OPs at levels that trigger *status epilepticus*. Acute OP intoxication also causes a robust neuroinflammatory response, which is implicated in the pathogenesis of long-term effects. In this study, we characterized the profiles of lipid mediators, important players in neuroinflammation, in the rat model of acute DFP intoxication. The profiles of lipid mediators were monitored in three different regions of the brain (cortex, hippocampus, and cerebellum) at 0, 1, 3, 7, 14, and 28 days post-exposure. The distribution pattern of lipid mediators was distinct in the three brain regions. In the cerebellum, the profile is dominated by LOX metabolites, while the lipid mediator profiles in cortex and hippocampus are dominated by COX metabolites followed by LOX and CYP 450 metabolites. Following acute DFP intoxication, most of the pro-inflammatory lipid mediators (*e.g.*, PGD2 and PGE2) increased rapidly from day 1, while the concentrations of some anti-inflammatory lipid mediators (*e.g.* 14,15 EpETrE) decreased after DFP intoxication but recovered by day 14 post-exposure. The lipidomics results suggest two potential treatment targets: blocking the formation of prostaglandins by inhibiting COX and stabilizing the anti-inflammatory lipid mediators containing epoxides by inhibiting the enzyme soluble epoxide hydrolase (sEH).

1. Introduction

Organophosphates (OPs) are a class of phosphorus-containing chemicals that disrupt cholinergic transmission by inhibiting acetylcholinesterase (AChE), an enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) and terminates impulse transmission at cholinergic nerve synapses (Li et al., 2011; Liu et al., 2012; Hobson et al., 2018). This group of chemicals includes the OP warfare agents, which are both toxic and fast acting as well as the generally less toxic OP pesticides. Acute intoxication with the OP warfare agents and a subset of the OP pesticides can trigger convulsions that progress to life-threatening *status epilepticus* (SE), and survivors face significant, long-term morbidity including mild-to-severe memory loss, affective disorders and recurrent seizures (de Vries et al., 2003; Pereira et al., 2014). Current medical countermeasures (atropine, 2-PAM, and high dose diazepam or midazolam) can reduce mortality in exposed individuals, but they do so with significant side effects and are maximally effective only if administered within minutes of exposure (Masson,

Abbreviation: DFP, diisopropylfluorophosphate; LOX, lipoxygenase; COX, cyclooxygenase; CYP450, cytochrome P450; PGD2, Prostaglandin D2; PGE2, Prostaglandin E2; EpETrE, epoxy eicosatrienoic acid; sEH, soluble epoxide hydrolase; AChE, acetylcholinesterase; ACh, acetylcholine; PGF1α, Prostaglandin F1α; TXB2, Thromboxane B2; LTB4, Leukotriene B4; DiHETrE, Dihydroxy eicosatrienoic acid; HETE, Hydroxyeicosatetraenoic acid; HODE, Hydroxyoctadecadienoic acid; BHT, butylate hydroxytoluene; UHPLC, ultra high-performance liquid chromatography; MRM, multiple reaction monitor; PLS-DA, partial least square discriminant analysis; ARA, arachidonic acid; DHA, docosahexaenoic acid; LA, linoleic acid; EpDPE, epoxydocosapentaenoic acid; EKODE, epoxy-keto-octadecenoic acids; HCA, Hierarchy Cluster Analysis; EpOME, epoxy octadecenoic acid; VIP, variable importance in projection; GFAP, glial fibrillary acidic acid; TSPO, translocator protein; NSAID, Nonsteroidal anti-inflammatory drug

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2011; Shih, 1990; Weissman and Raveh, 2008).

The OP diisopropylfluorophosphate (DFP) is toxicologically and structurally similar to the OP warfare agents soman and sarin and is considered a credible threat agent (Jett and Spriggs, 2018). Previously we have shown that rats acutely intoxicated with DFP at levels that triggered SE displayed significant neuroinflammation including astrogliosis and microglial activation that was evident within hours following administration and still persisted at 2 months post-exposure (Li et al., 2011; Liu et al., 2012; Flannery et al., 2016; Siso et al., 2017). Neuroinflammation has been implicated in the pathogenesis of persistent neurologic deficits associated with OP-induced SE (Collombet, 2011; de Araujo Furtado et al., 2012; Guignet and Lein, 2018). Regulatory lipid mediators are a class of lipid mediators derived from polyunsaturated fatty acids through three major metabolic pathways mediated by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 enzymes (Yang et al., 2011). These lipid mediators regulate many important pathophysiological processes, including inflammation (Yang et al., 2011). The characterization of these lipid mediators provides insight at the molecular level to understand how DFP induces neuroinflammation through changes in lipid mediators (Yang et al., 2011; Liu et al., 2010; Wang et al., 2017; Yang et al., 2009).

In this study, we profiled lipid mediators in the cortex, hippocampus, and cerebellum of rats at day 0, 1, 3, 7, 14, 21 and 28 after acute intoxication with DFP at levels that produced robust seizure activity. The spatiotemporal profiles of prostaglandins and epoxy fatty acids suggest potential targets for developing effective therapeutic approaches to treat neuroinflammation induced by acute OP intoxication.

2. Methods

2.1. Animals

Animals were maintained in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and all studies were performed with regard for alleviation of pain and suffering under protocols approved by the UC Davis Institutional Animal Care and Use Committee. Adult male Sprague Dawley rats (8 weeks old; 250–280 g; Charles River Laboratories, Hollister, CA) were housed individually in standard plastic cages under controlled environmental conditions (22 ± 2 °C, 40–50% humidity) with a normal 12 h light/dark cycle. Food and water were provided *ad libitum*. All animals were allowed to acclimate for 7 d prior to initiation of experiments.

2.2. Dosing

Prior to use, DFP (Sigma-Aldrich, Saint Louis, MO) was determined to be > 97% pure using previously published ^{1}H-, ^{13}C, ^{19}F and ^{31}P NMR methods (Gao et al., 2016). DFP was aliquoted and stored at −80 °C, conditions under which DFP is stable for at least 400 days (Heiss et al., 2016). At the beginning of each exposure day (~8:00 AM), rats were randomly divided into either DFP or vehicle (VEH) groups using a random number generator. All animals were pretreated with 0.1 mg/kg pyridostigmine bromide (TCI America, Portland, OR, USA) in sterile isotonic saline (im) 30 min prior to injection of either DFP or VEH. DFP was prepared in sterile, ice-cold phosphate buffered saline (PBS, 3.6 mM Na_2HPO_4, 1.4 mM NaH_2PO_4, 150 mM NaCl, pH 7.2) within 5 min of administration at 4 mg/kg, sc. VEH animals received an equivalent volume (300 μL) of sterile PBS. Within 1 min, both DFP and VEH animals were administered atropine sulfate (2 mg/kg, Sigma-Aldrich) and 2-pralidoxime (2-PAM, 25 mg/kg, Sigma-Aldrich) in sterile saline in a combined im injection to prevent lethality in DFP animals from peripheral cholinergic symptoms (Li et al., 2011). Seizure behavior was continuously monitored for 4 h after DFP injection and scored using a modified Racine scale as previously described (Siso et al., 2017). Briefly, seizure behavior was scored at 5 min intervals from 0 to 120 min and every 20 min from 120 to 240 min post-DFP injection using a 5-point scale in which 0 = no behavioral abnormalities; 1 = cholinergic symptoms (salivation, lacrimation, urination, defecation); 2 = tremors and muscle fasiculations; 3 = forelimb clonus; 4 = rearing with forelimb clonus; and 5 = rearing and falling. At the end of the 4 h monitoring period, all DFP animals were administered 10 mL of 5% dextrose in sterile saline (sc, Baxter Healthcare Co., Deerfield, IL, USA), returned to their home cages and given soft chow until they resumed normal consumption of water and solid food (Pessah et al., 2016).

Tissues were collected at 1, 3, 7, 14 and 25–31 days post-exposure with an n = 5–8/group. 2–3 vehicle animals were collected at each timepoint. At euthanasia, rats were deeply anesthetized with 5% isoflurane in oxygen. Blood was collected into EDTA tubes *via* cardiac puncture for plasma for analysis before perfusing the animals with cold PBS. The brains were removed and rapidly dissected on ice into hippocampus, cortex, and cerebellum. These brain tissues were snap frozen in liquid nitrogen prior to storage at −80 °C until further analysis.

2.3. Extraction

The extraction process is similar to the plasma protocol as described in a previously published paper (Yang et al., 2009). 10 μL of deuterated internal standard solution, a mixture of d4 PGF1α, d4 TXB2, d4 PGE2, d4 LTB4, d11 14,15 DiHETrE, d6 20 HETE, d8 9 HODE, d8 5 HETE, d11 11,12 EpETrE (Cayman Chemical, Ann Arbor, MI) was added to approximately 100 mg each of hippocampus, cortex and cerebellum. 400 μL of cold methanol with 0.1% of acetic acid and 0.1% of butylate hydroxytoluene, BHT (Sigma-Aldrich, St. Louis, MO) solution was added to these tissue samples and stored at −80 °C for 30 min. After freezing, samples were homogenized using Retsch MM301 ball mills (Retsch Gmbh, Germany) at 30 Hz for 10 min and then kept at −80 °C overnight. The homogenates were centrifuged at 16,000*g* for 10 min, the supernatants were collected, and remaining pellets were washed with 100 μL of ice-cold methanol with 0.1% of acetic acid and 0.1% of BHT and centrifuged at 16,000*g* for 10 min. The supernatants of each sample were combined and diluted with 2 mL of H2O and loaded onto Waters Oasis HLB 3 cc (Waters, Milford, MA) solid phase extraction (SPE) cartridges. The final concentrations of internal standards are 100 nM, which were obtained by spiking 10 uL of 500 nM solutions before extraction. The recovery rate combined with the matrix effects ranges from 40%–100%.

2.4. LC/MS/MS method

The regulatory lipid mediators were measured on a 1200 SL ultra-high-performance liquid chromatography (UHPLC) (Agilent, Santa Clara, CA) interfaced with a 4000 QTRAP mass spectrometer (Sciex, Redwood City, CA). The separation conditions for LC were optimized to separate the critical pairs of regulatory lipid mediators, which share the same multiple reaction monitor (MRM) transitions. In brief, separation was achieved on an Agilent Eclipse Plus C18 150 × 2.1 mm 1.8 um column with mobile phases of water with 0.1% of acetic acid as mobile phase A and acetonitrile/methanol (84/16) with 0.1% of acetic acid as mobile phase B. The detailed separation gradient is listed in Supplementary Table S1. All the parameters on the mass spectrometer were optimized with pure standards (purchased from Cayman Chemical, Ann Arbor, MI) under negative mode. The schedule MRM scan mode was employed to increase the sensitivity of the measurement. All the conditions of LC and mass spectrometry are shown in Supplementary Table S1.

2.5. Data analysis

The multivariate data analyses such as partial least square

discriminant analysis (PLS-DA) and Heatmap were performed using MetaboAnalyst 3.0 (www.metaboanalyst.ca/) and JMP 13 pro (SAS). Data were centered and auto-scaled before the analyses.

3. Results

Rats were monitored for seizure activity during the first 4 h post DFP injection. Seizure severity scores were quantified as previously described (Siso et al., 2017), and the individual seizure scores for each animal collected at 5 min intervals from 0 to 120 min and every 20 min from 120 to 240 min post-DFP injection are provided in the supplemental materials (Supplementary Table S2). Seizure severity was determined as the average seizure score for each animal over the 4 h monitoring period. Based on our previous studies, seizure behavior scored as ≥3.0 is consistent with electroencephalographic evidence of seizure activity. All DFP-exposed animals included in this study exhibited seizure behavior consistent with *status epilepticus* (*e.g.*, seizure scores ≥3.0 for > 5 min) and among DFP-intoxicated animals, the mean average seizure score and SD was 2.50 ± 0.48. Collectively, we monitored 31 regulatory lipid mediators in all three brain tissues from both the DFP and Vehicle group. As shown in Fig. 1, these regulatory lipid mediators include the metabolites of the COX, LOX and cytochrome P450 enzymes derived from arachidonic acid (ARA), docosahexaenoic acid (DHA) and linoleic acid (LA). These lipid mediators showed very different patterns in different regions of brain in a time-dependent manner after induction of inflammation by DFP. The full dataset is provided in the Supplementary Table S3. Since there are 31 lipid mediators detected, in the following discussion, we describe the overall distribution or pattern first and then discuss the interesting individual lipid mediators after it.

3.1. Region-specific effects of acute DFP intoxication on regulatory lipid mediators in the brain

The composition of regulatory lipid mediators in different regions (*i.e.* cortex, hippocampus, and cerebellum) of the brain on day 1 are represented in Fig. 2A–C.

The most abundant lipid mediators in the cortex are 11 HETE, PGF2a, PGD2, 15 HETE, and 7,8 EpDPE (Fig. 2A). The hippocampus (Fig. 2B) has more prostaglandins as a percentage of the total detected lipid mediators than either the cortex or the cerebellum. Approximately 50% of the metabolites in the hippocampus are derived from the COX pathway compared to 40% in the cortex. The predominant metabolites in the cerebellum include LOX metabolites, especially 12 HETE, 13 HODE, 9 HODE and EKODE and epoxides, such as EpDPEs and EpETrEs.

The relative abundance of these lipid mediators in three different brain regions is shown in Fig. 2D. In hippocampus, 26 out of 31 lipid mediators have higher concentration than their concentrations in cortex. All five lipid mediators with lower concentration are the diols that are the metabolites of sEH. In contrast, in cerebellum, only 7 out of 31 lipid mediators have higher concentration than their concentration in cortex. Five of these seven lipid mediators are diols. In addition, the cerebellum shows a much lower percentage of COX metabolites (6 keto PGF1α, TXB2, PGF2α, PGE2, PGD2) compared to either the cortex or the hippocampus.

3.2. Temporal change of the lipid mediators varies by brain region

The temporal profile of changes in these lipid mediators following acute DFP intoxication also varies in the different regions of the brain. Fig. 3a shows a Hierarchy Cluster Analysis (HCA) plot based on the concentrations of regulatory lipid mediators in the cortex. The same analyses were done for hippocampus and cerebellum shown in Supplementary Fig. S1 A and B. There are small differences among the three tissues, but the whole trends are the same. The lipid mediators clearly clustered into several different groups roughly reflecting the pathway of their metabolism. In general, all the pro-inflammatory lipid mediators including 6 keto PGF1a (stable surrogate of PGI2), 11 HETE, 15 HETE, PGD2, PGF2α, PGE2 clustered together. The epoxides from DHA and ARA, which are considered to be anti-inflammatory lipid mediators, clustered together. The diols, including DHETs and 5 HETE, 12 HETE, 8 HETE, HODEs and EpOMEs, are close to each other. These lipid mediators also show similar temporal change as well. For example, PGE2 and other pro-inflammatory lipid mediators, including PGD2, and PGF2α, are increased on days 1 and 3 after DFP intoxication, and then decrease in concentration on subsequent days post-exposure. In contrast, the epoxides from ARA are initially decreased after DFP intoxication but then recover to higher concentrations at days 7 and 14 post-exposure.

In order to show clear trends in temporal changes, we plotted the concentrations of representative lipid mediators derived from arachidonic acid against the time after DFP challenge in Fig. 3B. Some of these lipid mediators, such as PGE2, PGF2α and some of the LOX (lipoxygenase) metabolites (*e.g.* 15 HETE), respond very quickly with

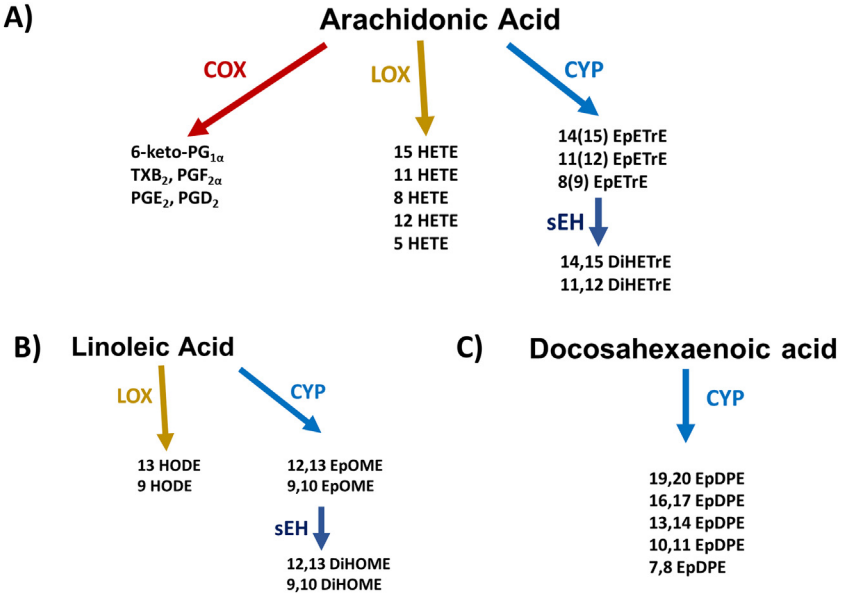


Fig. 1. The regulatory lipid mediators detected in rat brain tissues are derived from arachidonic acid (A), linoleic acid (B) and docosahexaenoic acid.

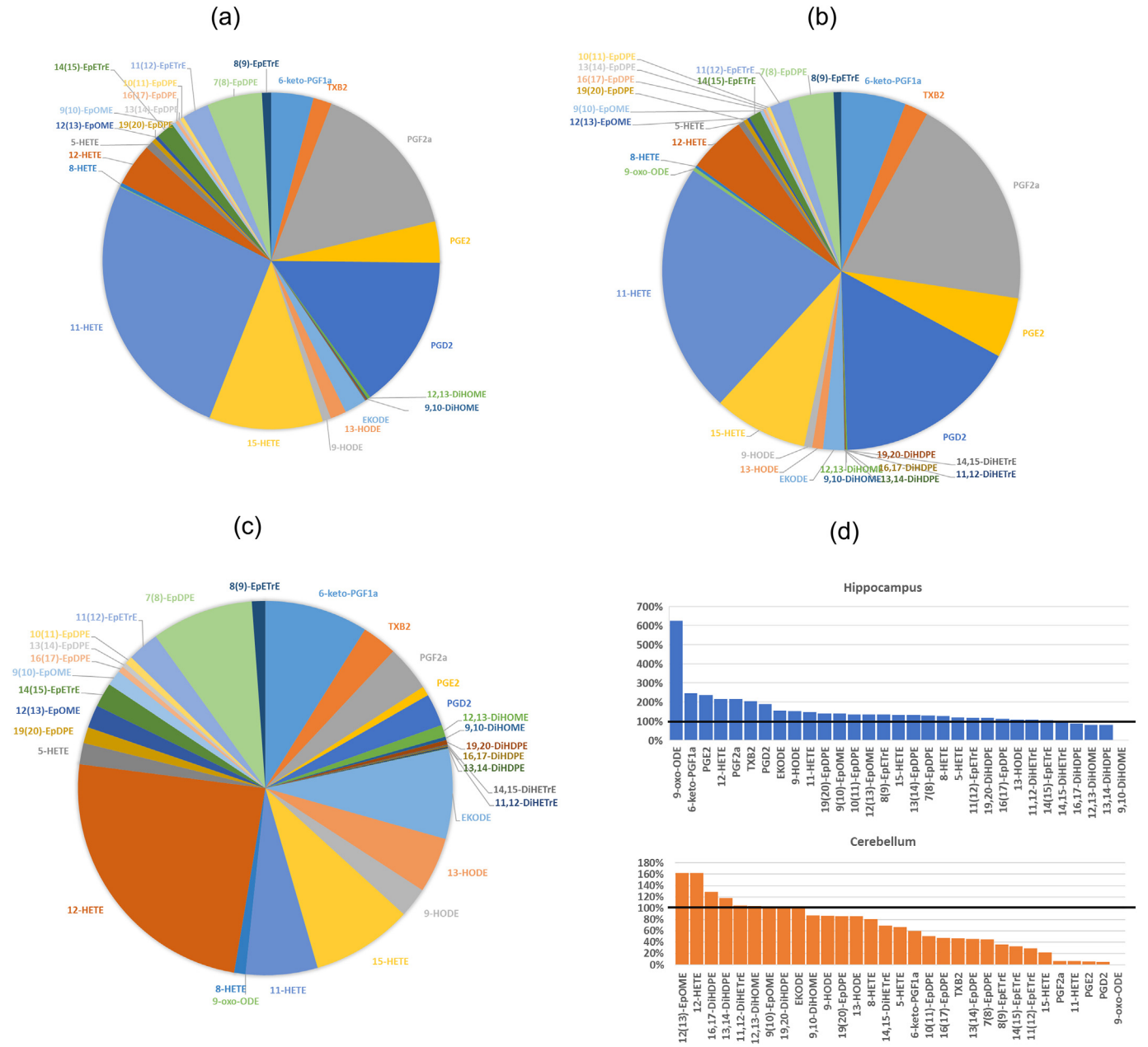


Fig. 2. The relative abundance of regulatory lipid mediators detected in three brain regions: cortex (A), hippocampus (B), cerebellum (C) at one day after acute DFP intoxication shows distinct responses between the different brain regions. The relative ratios (D) of 31 lipid mediators in hippocampus (up) and cerebellum (bottom) to cortex further describes the distinct responses in quantitative manner.

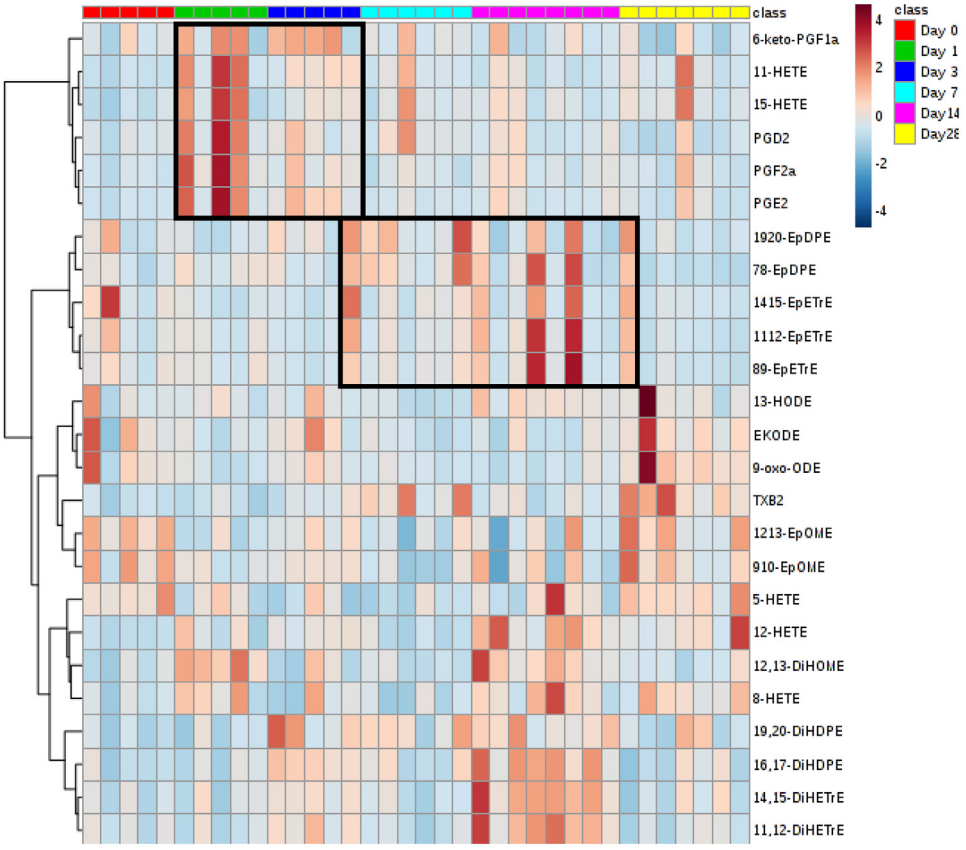
concentrations increasing immediately after DFP intoxication, peaking at day 1 and declining thereafter. There are some lipid mediators that exhibit a slower recovery response, such as TXB2 (stable surrogate *in vivo* for TXA2) and 6 keto-PGF1α (the stable surrogate *in vivo* for PGI2α), which peaked in concentration on day 3 or day 7 post-exposure. Most of these lipid mediators are pro-inflammatory, which respond rapidly to the insult in the first few days post-exposure and then recover by 14 days post-exposure. In contrast, another class of lipid mediator, metabolites of the P450 pathway, show a very different profile. For example, the concentrations of 14,15 EpETrE decreased at day 1 and began to recover at day 7 post-exposure.

3.3. Multivariate data analyses of regulatory lipid mediator profiles

One of the major advantages of lipidomics study is to utilize the multivariate data analysis to provide a more holistic visualization of the changes and interactions of the lipid mediators. In the following sections, we implemented partial least square discriminant analysis (PLS-DA) to identify the potential biomarkers correlated to the different treatment and time points and implemented hierarchy cluster analysis (HCA) to group lipid mediators according to the association of the treatment and time points as well as to visualize the temporal change of the lipid mediators as a whole. The results shown below are based on the cortex data. The same analyses were also implemented for hippocampus and cerebellum data. The results are shown in Supplementary Fig. S2.

Fig. 4 shows the results from PLS-DA based on data from the cortex. PLS-DA is a statistical method that creates a linear regression model to find the relationships between the variables (lipid mediators) and observation's classes (treatment, time points *etc.*) (Ballabio and Consonni, 2013; Worley et al., 2013; Kalivodova et al., 2015; Ankam and Bouguila, 2018). As shown in the score plot (Fig. 4A), there are similar

(a)



(b)

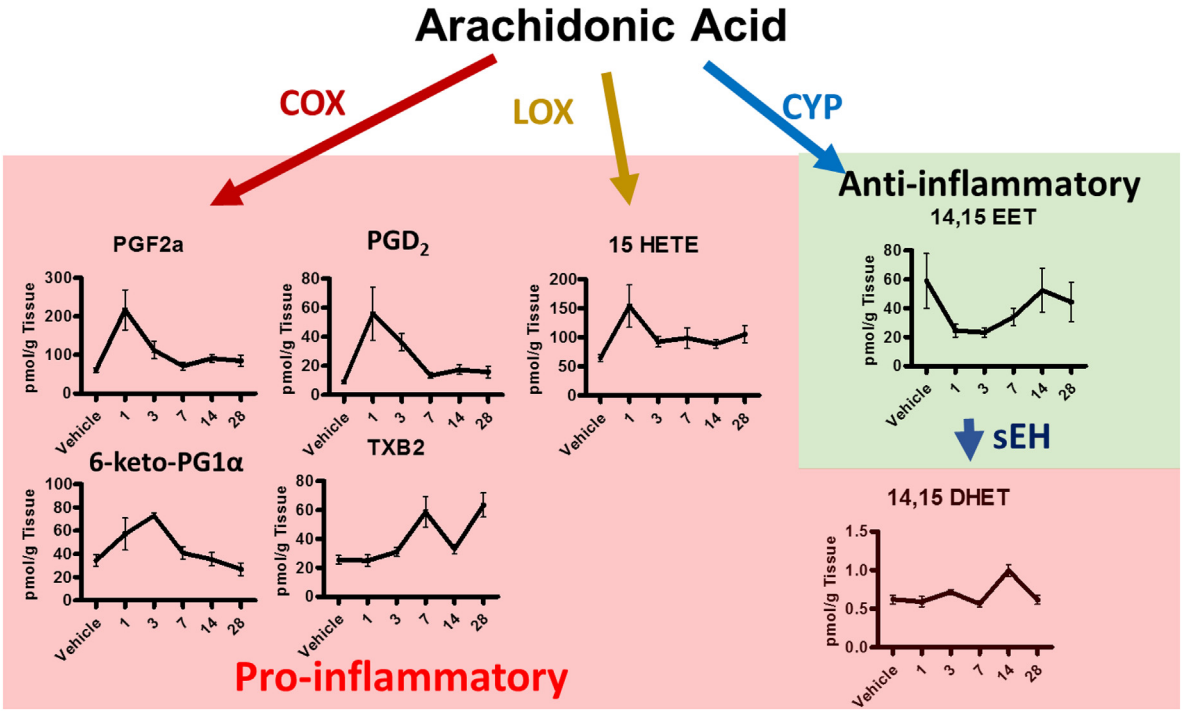


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Fig. 3. Hierarchy Cluster Analysis (HCA) based on concentrations of regulatory lipid mediators in the cortex of vehicle control and DFP animals at days 1, 3, 7, 14 and 28 post-exposure suggests six different groups of lipid mediators with similar temporal change after intoxication of DFP. The heatmap (Fig. 3A) shows that one group of them includes 6 keto PGF1a (surrogate of PGI2), 11 HETE, 15 HETE, PGD2, PGF2α and PGE2. This group of lipid mediators are well known as pro-inflammatory lipid mediators. Another group of lipid mediators includes the epoxides from arachidonic acid and DHA, known as the anti-inflammatory lipid mediators. The temporal profile of selected lipid mediators (Fig. 3B) based on the same data set (n = 4–8) clearly shows that acute DFP intoxication induces the proinflammatory lipid mediators while decreasing the anti-inflammatory lipid mediator, 14,15 EpETrE.

trends that can be found in inflammatory status: the profiles at day 1 post-intoxication are farthest from the vehicle group, and slowly shift back toward the vehicle control over the 28 days following DFP exposure; although profiles do not fully return to that of the vehicle control group. The Variable Importance in Projection (VIP) score (Fig. 4B), which describes a quantitative estimation of the discriminatory power of each individual feature, implies that 11 HETE, PGF2α, PGD2, 15 HETE, PGE2 are the major lipid mediators driving the difference shown in the score plot (Fig. 4A).

Fig. 5 shows a heatmap (HCA) correlation coefficient among the lipid mediators based on the lipidomic profiles of cortex at different days post-exposure. It clearly shows there are several different categories of lipid mediators that follow the same trends after acute DFP intoxication. The two strongest correlation categories are: (1) the epoxides derived from arachidonic acid and docosahexaenoic acid (DHA); and (2) the main COX metabolites (6 keto PGF1a, PGD2, PGF2α, PGE2) and 15 HETE, 11 HETE.

The heatmaps based on representative lipid mediators at different days are built to visualize the temporal change of these lipid mediators. Fig. 6 shows the heatmap on days 1, 3, 7 14 and 21 post-exposure based on data from cortex tissue. Consistent with Fig. 5, which is based on the data at all the time points, the lipid mediators derived from COX are highly correlated with each other and the epoxy fatty acids are correlated with each other. One intriguing finding is the negative correlation between epoxy fatty acids and their corresponding diols that appeared at day 3 post-intoxication and reached the highest negative correlation at day 7 post-exposure. Since several studies implicate the sEH protein as an inflammatory marker (Imig et al., 2002; Ren et al., 2018), these data suggest that the sEH, the enzyme that degrades epoxy fatty acids was induced at day 3 and peaked at day 7.

4. Discussion

Here, we report the spatiotemporal profiles of lipid mediators in the rat brain following acute intoxication with DFP. These lipid mediators are a class of signaling molecules (Yang et al., 2011; Dennis and Norris, 2015; Lawrence et al., 2002; Wymann and Schneiter, 2008; Bazinet and Laye, 2014). Enzymes such as COX that are involved in the metabolism pathways of these lipid mediators have been identified as targets for many diseases (Pannunzio and Coluccia, 2018; Schmelzer et al., 2006; Goswami et al., 2016). Characterizing the spatiotemporal changes in lipid mediator levels induced by acute DFP intoxication complements previous efforts to characterize the neuropathological changes in this model using other methods (Li et al., 2011; Liu et al., 2012; Flannery et al., 2016; Siso et al., 2017). Moreover, the data from this study may suggest potential targets for therapy.

Table 1 lists the changes in representative lipid mediators in the three different regions, the cortex, hippocampus and cerebellum throughout the time course of the experiment. The pro-inflammatory PGE2 shows very similar trends in both the cortex and hippocampus, which has the highest peak on Day 1, indicating that the inflammation was induced immediately following DFP intoxication. The hippocampus has the highest concentration of PGE2, followed by levels found in the cortex. Contrary to this, the concentration of PGE2 in the cerebellum is about 50 times lower with negligible change following DFP exposure. Previously, we demonstrated using classic histopathologic techniques that glial fibrillary acidic acid (GFAP), a biomarker of activated astrocytes, and IBA-1, a biomarker of microglia, increased

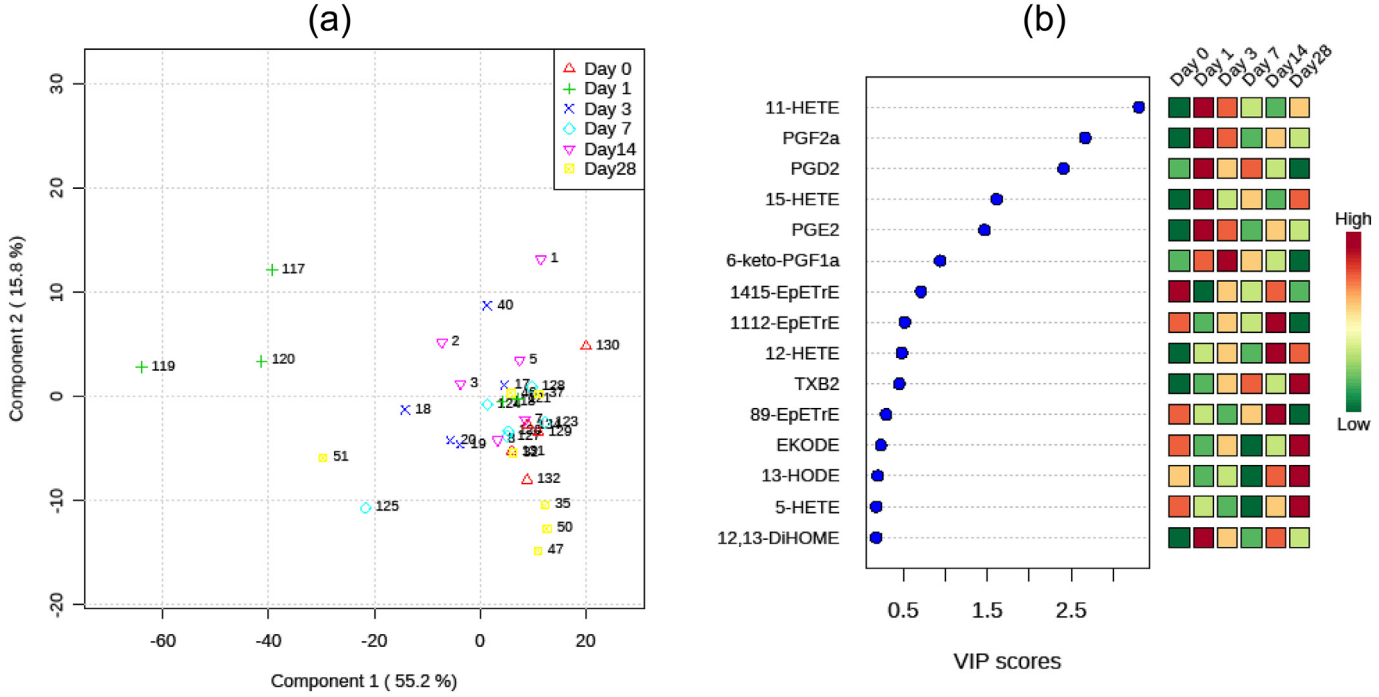


Fig. 4. The score plot (A) of the Partial Least Square Discriminant Analysis (PLS-DA) based on cortex data shows the regulatory lipid mediators profile tracks well with the inflammatory status. The Variable Importance in Projection (VIP) score (B) indicates the relative importance of the regulatory lipid mediators to differentiate the different time points.

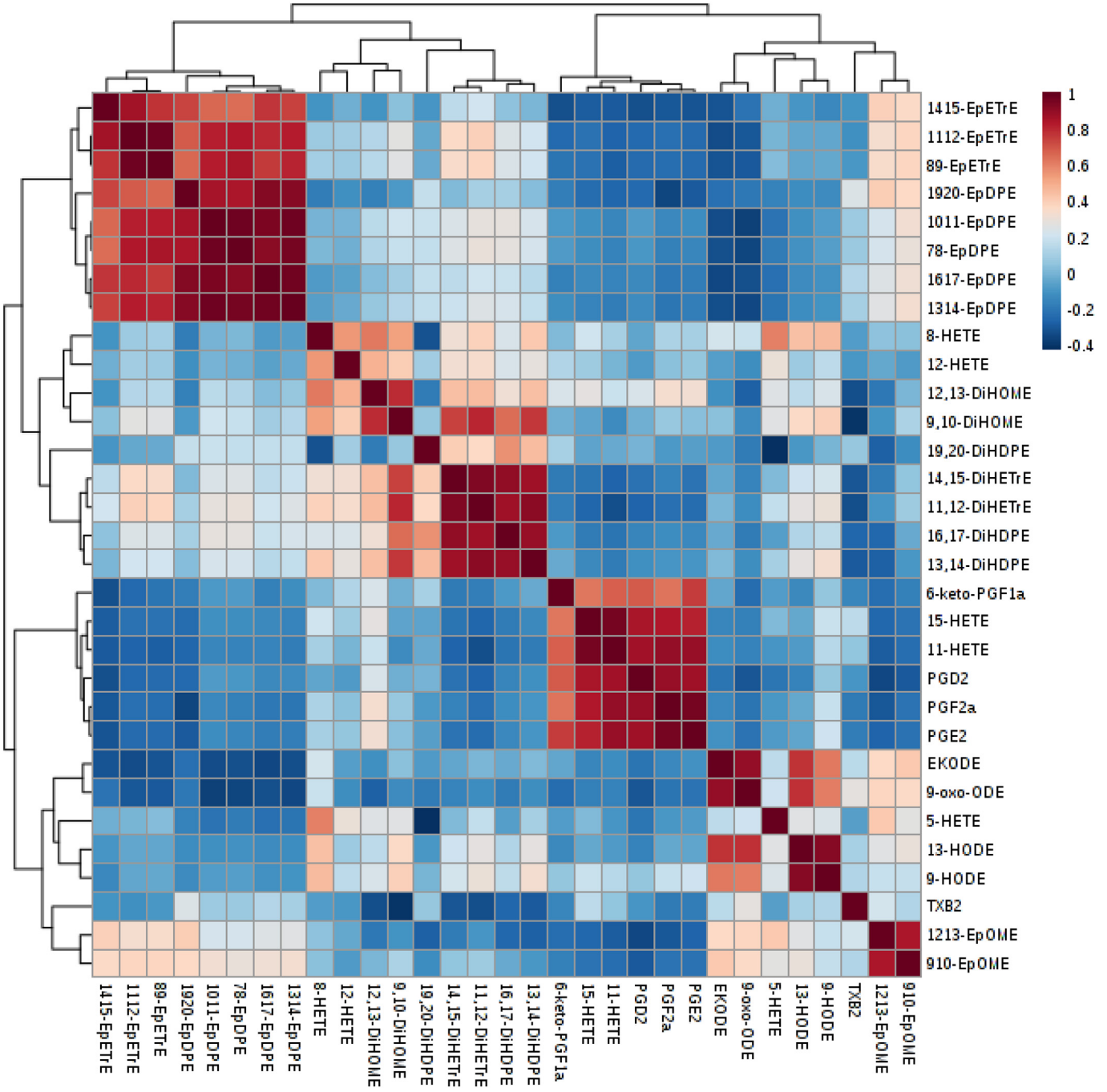


Fig. 5. The heatmap based on the correlation coefficient of regulatory lipid mediators reveals the similarity among them.

significantly in the hippocampus, piriform/entorhinal cortex and dorsolateral thalamus of rats acutely intoxicated with DFP ((Liu et al., 2012; Siso et al., 2017) Additional studies using PET imaging of [11C]-(*R*)-PK11195, a ligand for the 18-kDa mitochondrial membrane translocator protein (TSPO), similarly showed that acute DFP intoxication rapidly triggered neuroinflammation in the hippocampus and cortex that persisted at 1 month post-exposure (Collombet, 2011). The distribution of PGE2 as well as other prostaglandins in this study is consistent with the neuroinflammation induced by DFP. Moreover, a previous report about COX1 and COX2 distribution suggested the induction of COX2 expression was higher in hippocampus and cortex than cerebellum after DFP intoxication (de Araujo Furtado et al., 2012). This might explain why we observed higher levels of prostaglandins in the hippocampus and cortex than in the cerebellum. In contrast, we did not notice such large difference in the distribution among different brain regions for other lipid mediators derived from cytochrome P450 and LOX pathways.

Another interesting finding is the temporal change of 14,15 EpETrE. The concentrations of 14,15 EpETrE decreased at day 1 and remained at a low concentration from day 1 to day 3, then began to recover after day 7. The change of 14,15 EpETrE shows the opposite trends of PGE2. EpETrEs have been shown to be anti-inflammatory or inflammation resolving lipid mediators in several studies (Guignet and Lein, 2018; Yang et al., 2011; Liu et al., 2010; Wang et al., 2017). A recent study shows that EpETrEs have a critical role in monocyte lineage recruitment and resolution during inflammation (Liu et al., 2010).

Both temporal changes of these pro-inflammatory lipid mediators (PGE2, PGD2, *etc.*) and the anti-inflammatory lipid mediators

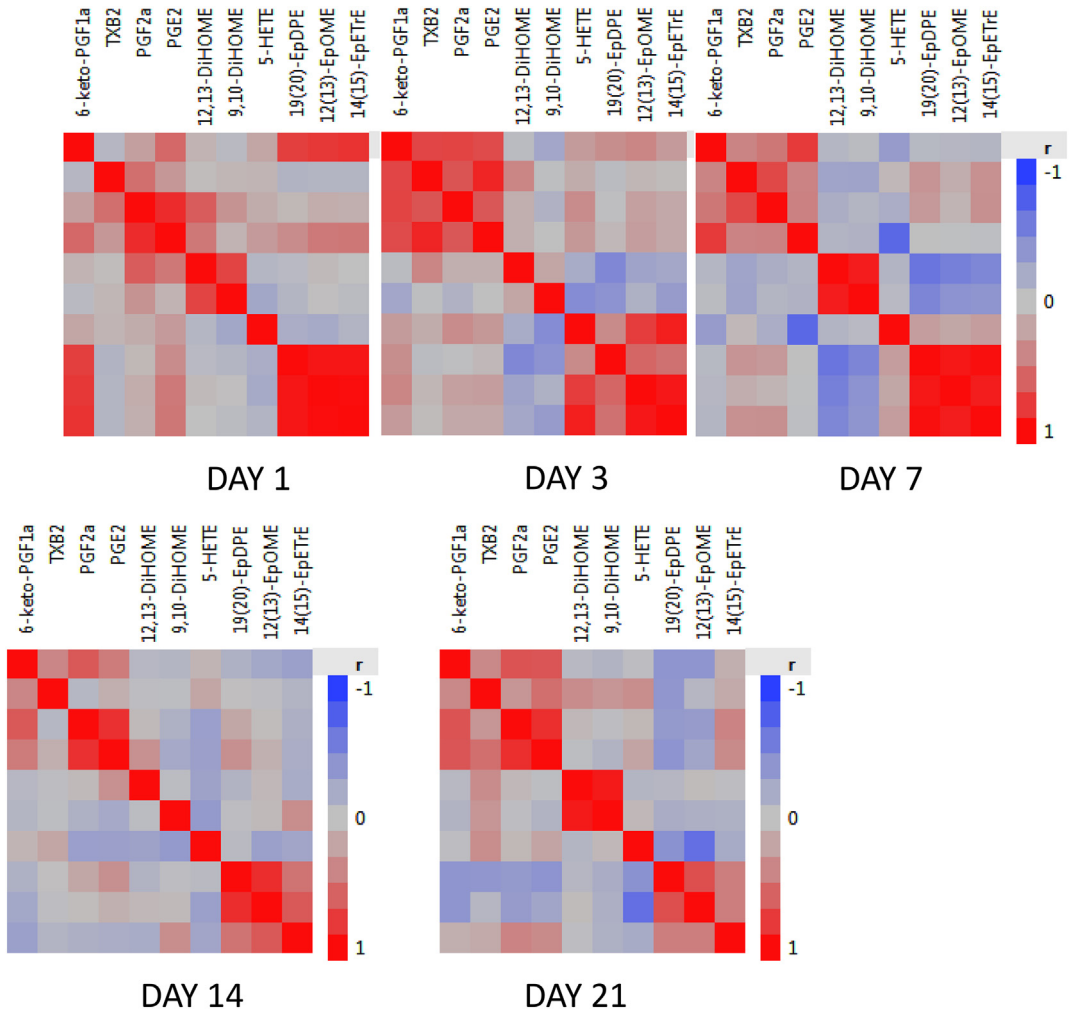


Fig. 6. The heatmap based on correlation coefficient of selected regulatory lipid mediators at different time points illustrated the distinct dynamic pattern change of different lipid mediators.

(EpETrEs) suggests that lipid mediators might contribute coordinately to the neuroinflammation induced by DFP. As a countermeasure, it is attractive to modulate these lipid mediators by inhibition of COX2 and/or inhibition of sEH to treat the neuroinflammation induced by DFP. Inhibitors including NSAID have been widely studied in neuroinflammation research (Yang et al., 2009; Gao et al., 2016; Heiss et al., 2016). The inhibition of sEH has also been shown to be a promising treatment for many diseases with a neuroinflammatory component (Pessah et al., 2016; Ballabio and Consonni, 2013; Smith et al., 2005; Schmelzer et al., 2005; Morisseau and Hammock, 2013). Recent publications on neuroinflammatory mechanisms of brain disease (Kalivodova et al., 2015) support the hypothesis that inhibition of sEH can effectively mitigate neuroinflammation and the progression of neurologic disease by several mechanisms (Dennis and Norris, 2015; Lawrence et al., 2002; Wymann and Schneiter, 2008; Bazinet and Laye, 2014; Pannunzio and Coluccia, 2018). Furthermore, given the temporal change of PGE2 and EpETrEs, the dual inhibition of COX2 and sEH is a very attractive strategy. In support of this approach, inhibition of sEH was shown to synergize with COX inhibition to relieve pain (Schmelzer et al., 2006) and this combinatorial approach alleviated the adverse effects associated with COX inhibitors (Goswami et al., 2016). Dual inhibitors for COX2 and sEH have been developed (Ferrer et al., 2018) and successfully applied in several disease models (Mitchell and Kirkby, 2019; Rojas et al., 2019). By improving their PK-ADME and blood brain barrier penetration, the dual inhibitors could represent a strategy toward developing therapies for inflammation following OP intoxication.

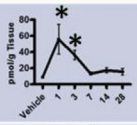
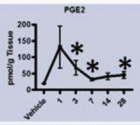
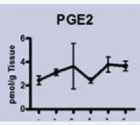
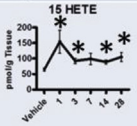
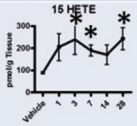
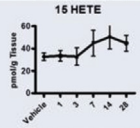
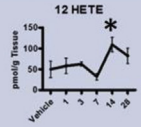
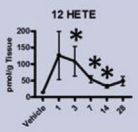
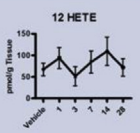
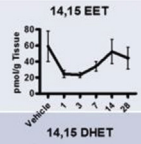
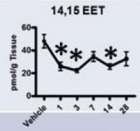
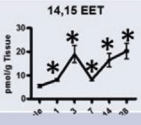
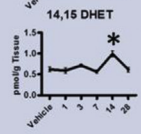
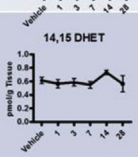
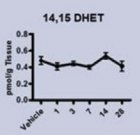
In a recent published review (Rojas et al., 2019), Rojas and colleagues summarized studies relevant to COX-2 signaling cascades in seizure disorders and the potential therapeutic targets (receptors of these prostaglandins). As pointed out in this review, the animal literature is inconsistent regarding the potential benefits of COX-2 selective coxibs in epilepsy models (Rojas et al., 2019). One of the possible reasons is that the modulation of COX-2 might alter the concentrations of other lipid mediators, which also contribute to the biology of COX-2 inhibitors on seizure disorders. Here, we monitored the whole panel of the lipid mediators. According to the spatiotemporal change of these lipid mediators after neuroinflammation induced by DFP, modulation of several enzymes (*e.g.* COX2 and sEH) simultaneous might be a potential way to treat the neuroinflammation.

A key question is whether our data generated in a model of acute DFP intoxication are generalizable to other OPs. While different toxicity profiles have been observed across different OPs (Pope, 1999), the spatiotemporal profile of neuroinflammatory responses observed following DFP-induced *status epilepticus* are very similar to those observed in preclinical models of soman and sarin-induced *status epilepticus* (Siso et al., 2017; Guignet et al., in press.; Hobson et al., 2019; Li et al., 2015). Therefore, we would expect that the brain lipidome following acute seizures would exhibit similar trends across diverse OPs.

5. Conclusion

In the current study, we characterized the spatiotemporal changes

Table 1
The time profiles of selected regulatory lipid mediators in three different brain regions (cortex, hippocampus and cerebellum) show distinct patterns. The data were shown in mean ± SE. * means there is statistical significance between the value and the vehicle ($p < .05$) according to *t*-test result.

Metabolites	Cortex	Hippocampus	Cerebellum
PGE2			
15 HETE			
12 HETE			
14,15 EET			
14,15 DHET			

Note: All the measurements in significant changed groups passed the normality test (Shapiro-wilk test) except 15 HETE 28 days group.

of lipid mediators in the rat brain after acute DFP intoxication. The levels of 31 lipid mediators in three brain regions (cortex, hippocampus and cerebellum) at six time points (vehicle control, 1, 3, 7, 14, 21, and 28) were documented. Among many significant changes, the prostaglandins including PGE2, PGD2 in cortex and hippocampus increased after intoxication of DFP, which correlate with the induction of neuroinflammation reported previously. In contrast, the levels of anti-inflammatory epoxy fatty acid lipid mediators decreased after DFP intoxication and recovered after day 7. While these results are largely phenomenological, and limited in that only male animals were investigated, they suggest that dual inhibition of COX2, the enzyme that produces prostaglandins, and sEH (the enzyme that metabolizes epoxy fatty acids) might be an attractive strategy to treat the neuroinflammation induced by acute OP intoxication. Given the urgent need for improved medical countermeasures to protect the brain from long-term neurologic sequelae, further studies to establish mechanistic relationships and to determine whether these data can be extrapolated to females are warranted.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.taap.2019.114749.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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