Novel Docosanoids Inhibit Brain Ischemia-Reperfusion-mediated Leukocyte Infiltration and Pro-inflammatory Gene Expression

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation
Marcheselli, Victor L., Song Hong, Walter J. Lukiw, Xiao Hua Tian, Karsten Gronert, Alberto Musto, Mattie Hardy, et al. 2003. “Novel Docosanoids Inhibit Brain Ischemia-Reperfusion-Mediated Leukocyte Infiltration and Pro-Inflammatory Gene Expression.” Journal of Biological Chemistry 278 (44): 43807–17. https://doi.org/10.1074/jbc.m305841200.

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:41483534

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Novel Docosanoids Inhibit Brain Ischemia-Reperfusion-mediated Leukocyte Infiltration and Pro-inflammatory Gene Expression

Victor L. Marcheselli§, Song Hong§, Walter J. Lukiv†, Xiao Hua Tian†, Karsten Gronert‡, Alberto Musto†, Mattie Hardy‡, Juan M. Gimenez‡, Nan Chiang‡, Charles N. Serhan‡, and Nicolas G. Bazan‡

From the ‡Neuroscience Center of Excellence and Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112 and the §Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02115

Ischemic stroke triggers lipid peroxidation and neuronal injury. Docosahexaenoic acid released from membrane phospholipids during brain ischemia is a major source of lipid peroxides. Leukocyte infiltration and pro-inflammatory gene expression also contribute to stroke damage. In this study using lipidomic analysis, we have identified stereospecific messengers from docosahexaenoate-oxygenation pathways in a mouse stroke model. Aspirin, widely used to prevent cerebrovascular disease, activates an additional pathway, which includes the 17R-resolvins. The newly discovered brain messenger 10,17S-docosatriene potently inhibited leukocyte infiltration, NF-kB, and cyclooxygenase-2 induction in experimental stroke and elicited neuroprotection. In addition, in neural cells in culture, this lipid messenger also inhibited both interleukin 1-β-induced NF-kB activation and cyclooxygenase-2 expression. Thus, the specific novel bioactive docosanoids generated in vivo counteract leukocyte-mediated injury as well as pro-inflammatory gene induction. These results challenge the view that docosahexaenoate only participates in brain damage and demonstrate that this fatty acid is also the endogenous precursor to a neuroprotective signaling response to ischemia-reperfusion.

Brain ischemia-reperfusion triggers lipid peroxidation that participates in neural injury (1, 2). Docosahexaenoic acid (DHA)† (22:6n-3) esterified in membrane phospholipids is released in brain ischemia (3, 4) and is thought to yield lipid peroxides (5). Leukocyte infiltration and pro-inflammatory gene expression are mediators of ischemic stroke damage (6–8); however, there are no known messengers that down-regulate these events. The biosynthesis of oxygenated arachidonic acid messengers (9, 10) triggered by cerebral ischemia-reperfusion is preceded by an early and rapid phospholipase A₂ activation reflected in free arachidonic and docosahexaenoic acid accumulation (3, 4, 11). These fatty acids are released from membrane phospholipids where they are esterified (3). Both fatty acids are derived from dietary essential fatty acids; however, only DHA is concentrated in the central nervous system (12). It is clear that synaptic membrane and retinal photoreceptor biogenesis is dependent on liver processing of the dietary DHA or of its precursor, linolenic acid, followed by blood lipoprotein transport (13). DHA is involved in memory formation (14), excitable membrane function (15), photoreceptor cell biogenesis and function (16), and neuronal signaling (17) and has been implicated in neuroprotection (18–20). Whether DHA itself or a DHA-derived messenger is involved in these events is not known. Moreover, to date, potent bioactive autacoids from DHA acting in nanomolar concentrations have not been identified in the central nervous system. Although certain docosanoids have been identified in the retina (21) and have been proposed to be neuroprotective (12), their physiologic properties have not been explored.

To test the ability of the mouse brain to synthesize bioactive docosanoids, we used tandem LC-PDA-ESI-MS-MS-based lipidomic analysis in combination with ischemia-reperfusion. The rationale for use of this design was based upon the fact that brain ischemia releases unesterified DHA (3, 4, 11) and that during early stages of ischemia-reperfusion, endogenous signals of repair/neuroprotection may be generated.

EXPERIMENTAL PROCEDURES

Reagents—Human recombinant IL-1β (14019) was from Sigma, and 10,17-dihDHA was prepared as described previously (22, 23). Normal human neural (HN) progenitor cells (CC-2599), neural progenitor maintenance medium, human epidermal and human fibroblast growth factors, gentamicin/amphotericin B (GA1000), and neural survival factor-1 were obtained from Clonetics (Walkersville, MD). AP1, HIF-1α, NF-κBp50/p65, and STAT-1α gel-shift consensus and mutant oligonucleotides were synthesized at the Louisiana State University Health Sciences Center core facility or were purchased from Promega Life Science (Madison, WI).

Middle Cerebral Artery Occlusion (MCA-O) and Reperfusion—Experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center, New Orleans, and followed the National Institutes of Health guidelines for experimental animal use. Mice (20–25 g body weight) were induced with 2% isoflurane in a mixture of 70% nitrous oxide and 30% oxygen. Anesthesia was maintained with 1% isoflurane.

This paper is available on line at http://www.jbc.org
Neuroprotective Docosanoids in Experimental Stroke

Temperature was maintained at 36.5–37.5 °C (Harvard homeothermic blank). F-10 polyethylene catheters were placed in the femoral artery and vein, and blood pressure was monitored. Arterial blood was analyzed for Pao2, Paco2, and pH after 1 h of ischemia and 30 min after the onset of reperfusion. The common carotid and external carotid arteries were temporarily ligated with a retraction suture, and the external carotid artery was dissected just proximal to its bifurcation. The occluding filament was introduced from the external carotid artery and advanced to the internal carotid artery. The arterial venous microclip was removed, and the filament was advanced so that the blunted tip lay in the anterior cerebral artery and the side of the filament occluded the origin of the MCA. The stump of the external carotid artery was ligated, and tension on the retraction suture to the common carotid artery was gently released, restoring blood flow to the region of the MCA. The animals were killed by decapitation, and the hippocampi and brain cortices were rapidly dissected and frozen. Samples were kept at −80 °C until analysis. In some experiments, aspirin (7.5 mg/kg) was administered by gavage 15 min before MCA-O.

Assessment of Stroke Volume—Forty-eight hours after MCA-O, the mice were killed, and their brains were dissected and immersed in ice-cold saline. The brains were embedded in agar blocks and sectioned into coronal slices 1 mm thick by a vibratome (Vibratome Co., St. Louis, MO). The sections were incubated at room temperature in a 3% buffered formalin solution containing 2,3,5-triphenyltetrazolium chloride (TTC). Once the color had developed (10–15 min), sections were fixed in 10% buffered formalin, and phosphorimaged using a Typhoon variable mode imager (Amersham Biosciences). Serial sections were made for all animals.

Brain Ischemia-Reperfusion Triggers the Synthesis of Docosahexaenoic Acid-Oxygenation Pathways—We used 1 h of right middle cerebral artery occlusion in mice followed by reperfusion to assess the formation of docosahexaenoic acid-oxygenation derivatives. Under these conditions there is active release of free docosahexaenoic acid from brain membrane phospholipids (2–4). This model of transient focal ischemia greatly affects the hippocampus, a brain region rich in neurons vulnerable in ischemic stroke and in other neurologic diseases (1, 2).

Fig. 1A shows the time course of formation of novel docosanoids in the ipsilateral hippocampus during ischemia-reperfusion. There was generation of 17-hydroperoxy-DHA, the 15-lipoxygenase-like action on DHA (22), and a novel 10,17-docosatriene. After 48 h, the mice were anesthetized with intracardiac perfusion of ice-cold saline followed by 10% buffered formalin. The brain tissues were allowed to equilibrate overnight in 4% buffered formalin followed by 30% sucrose in 0.1 M PBS. Frozen sections were made at a thickness of 10 μm and mounted on glass slides. The sections were permeabilized with 0.6% Triton X-100 for 10 min, washed in PBS, and blocked in 2% goat serum in PBS for 30 min. Incubation with myeloperoxidase/fluorescein isothiocyanate-conjugated antibody (Dako A/S) was performed at 1:200 dilution for 2 h. The sections were washed in Tween 20 in PBS and then mounted in Vectashield (Vector, CA). Images were recorded by deconvolution microscopy (Intelligent Imaging Innovations, Denver, CO).

RESULTS

Brain Ischemia-Reperfusion Triggers the Synthesis of Docosahexaenoic Acid-Oxygenation Pathways—We used 1 h of right middle cerebral artery occlusion in mice followed by reperfusion to assess the formation of docosahexaenoic acid-oxygenation derivatives. Under these conditions there is active release of free docosahexaenoic acid from brain membrane phospholipids (2–4). This model of transient focal ischemia greatly affects the hippocampus, a brain region rich in neurons vulnerable in ischemic stroke and in other neurologic diseases (1, 2).
therapeutically to manage cerebrovascular diseases, we next asked whether brain biosynthesis of DHA messengers is modified in the presence of aspirin in vivo. Moreover, in non-neural tissues, aspirin triggers the biosynthesis of anti-inflammatory lipid mediators (23, 28). We examined the formation of DHA-derived docosanoids in the presence of aspirin during reperfusion after an ischemic stroke (Fig. 1). Fig. 1E shows the time course of docosanoid formation with aspirin treatment. There

**Fig. 1. Synthesis and metabolism of docosanoids in the ipsilateral mouse hippocampus during reperfusion following transient ischemia.** Structural elucidation of DHA and docosanoids was performed by lipidomic analysis by LC-PDA-MS-MS as described previously (22, 23). A, time course of accumulation of 17S-HDHA, 10,17S-docosatriene, and ω-22-hydroxy-4,17S-diHDHA in the ipsilateral hippocampus during reperfusion after 1 h of MCA-O. There was an enhanced accumulation of ω-22-hydroxy-4,17S-diHDHA, a product of an oxidative pathway, and 10,17S-docosatriene. B, selected ion monitoring (SIM) chromatogram (m/z 359) showing 10,17S-docosatriene. C, MS-MS spectrum for 10,17S-docosatriene. D, SIM (m/z 75) chromatogram showing ω-22-hydroxy-4,17S-diHDHA. E, time course for the accumulation of 17R-series resolvins in hippocampi of mice pretreated with aspirin before MCA-O. Aspirin triggered a metabolic shift toward accumulation of 17R-docosatrienes, 7,8,17R-triHDHA, and 7,17R-diHDHA, products of acetylated COX-2 enzyme. F, representative SIM (m/z 375) chromatogram with peak for 7,8,17R-triHDHA. G, representative SIM (m/z 359) chromatogram with peak for 7,17R-diHDHA. H, typical SIM (m/z 327) chromatogram with peak for DHA.
was a shift away from the products generated from endogenous sources of DHA in the absence of aspirin (Fig. 1A) toward products that included the novel 17R-series resolvins, in particular 7,17R-diHDHA and 7,8,17R-triHDHA, which were formed and were present at the earliest time intervals. There was a marked accumulation in 17R-HDHA, shown recently to be a product of aspirin-acetylated cyclooxygenase-2 (28).

Fig. 1, F and G, shows a representative LC-MS-MS of the ipsilateral hippocampus following treatment with aspirin and depicts the chromatographic profile of products identified via MS-MS and lipidomic analyses. Here we also confirmed the release of DHA under these conditions (3, 4, 11) (Fig. 1H). To assess that the aspirin dose administered by gavage did reach the brain, the time courses for prostaglandin E2, leukotriene B4, and lipoxin A4 production were determined in the hippocampi in parallel assays (Fig. 2, PGE2, LTB4, and LXA4). The rapid accumulation of prostaglandin E2 within 8 h of reperfusion was inhibited by aspirin treatment. Maximal leukotriene B4 was generated in aspirin-treated hippocampi by 8 h, as was the case with lipoxin A4, which also peaked within 8 h. Together these results clearly indicate that the dose of aspirin used in vivo did access the central nervous system.

Fig. 3 depicts the proposed pathway to generate the novel 10,17S-diHDHA α-oxidation product measured in the absence of aspirin. In our experiments, 4,17S-diHDHA did show bioactivity in PMN infiltration, but these were less than those evoked by equimolar concentrations of 10,17S-docosatriene in a non-neural experimental model (Fig. 4). Fig. 4, therefore, confirms that 4,17S-diHDHA inhibits PMN leukocyte exudate formation in murine peritonitis models (28). This α-oxidation product is the likely inactivation product for 10,17S-diHDHA. Fig. 3 also depicts the resolvin or 17R series pathways. These are the products formed in the presence of aspirin.

Polymorphonuclear Leukocyte Infiltration Mediated by Focal Ischemic Stroke in Mice Is Inhibited by 10,17S-Docosatriene—We monitored PMN infiltration, a major factor in mediating brain ischemia-reperfusion damage (7, 8, 29, 30). PMN infiltration is a complex multistep process that is modulated by the coordinated expression of adhesion and signaling molecules (26). DHA-derived messengers were very recently reported to inhibit PMN invasion outside of the central nervous system in the air-pouch model (23, 26, 28).
To determine whether 10,17S-docosatriene displays counteractive bioactivity during brain ischemia-reperfusion-induced PMN infiltration, this docosanoid was constantly infused into the third ventricle during 48 h of superfusion in the amounts shown in Fig. 5. By immunostaining of myeloperoxidase as a marker of PMN leukocytes, we demonstrated a marked inhibition by 10,17S-docosatriene on the ipsilateral side of the brain (Fig. 5A). Myeloperoxidase is a marker of PMN infiltration in the brain (7, 8, 29, 30) and is depicted with a characteristic cytoplasmic granular pattern found only in the focal ischemic side (Fig. 5B). In addition, we dissected both the hippocampus and the neocortex from the focal stroke side (ipsilateral) as well as from the contralateral side and quantified myeloperoxidase enzyme activity. Both the free acid and the carboxymethyl ester of 10,17S-docosatriene inhibited the appearance of myeloperoxidase (Fig. 5, C and D). This was the case both in the ipsilateral hippocampus and in the ipsilateral neocortex. However, the contralateral brain regions did not display increased enzyme activity, nor did they show immunostaining for leukocytes. Infusion of DHA also elicited inhibition of PMN infiltr-
Fig. 5. Inhibition of leukocyte infiltration by 10,17S-docosatriene in mouse hippocampus and neocortex after 1 h of MCA-O and 48 h of reperfusion. Data shown represent averages ± S.D. of the indicated number (n) of individual mice. A and B, immunocytochemical visualization of polymorphonuclear leukocytes (green fluorescence) that exhibit myeloperoxidase immunoreactivity. Ipsilateral brain areas (ischemic area) show positive green fluorescence (Vehicle) as compared with contralateral tissue. The areas shown correspond to the dentate gyrus of the hippocampus. In A, 10,17S-docosatriene-infused animals (0.4 µg over 48 h) exhibited a large reduction in green fluorescence. B shows higher magnifications of the ipsilateral stroke area from vehicle-treated animals, with the cytoplasmic granular appearance of immunoreactivity (arrows) that is characteristic of PMN leukocytes. C, cerebroventricular perfusion of 0.4 µg of 10,17S-docosatriene over 48 h resulted in 80% inhibition of ipsilateral myeloperoxidase activity as compared with vehicle-treated animals. D, perfusion as in A, but with 1 µg or 3 µg of 10,17S-docosatriene methyl ester over 48 h. The free acid exerted more potent inhibition (80 and 99% in hippocampus and cortex, respectively) as compared with the methyl ester derivative, which resulted in 49% inhibition in hippocampus and 97% inhibition in neocortex. E, same as in A, but perfusion with 2, 20, or 200 µg of DHA (free acid) over 48 h. In the hippocampus, dose-dependent inhibition of myeloperoxidase activity was observed (60, 72, and 82%, respectively). In the neocortex, no inhibition was found with 2 µg, but there were 40 and 73% inhibition with 20 or 200 µg, respectively. Statistically significant differences determined by the factorial analysis of variance with comparison of treatment means done by protected Student’s t test (37) are represented as follows: a, p < 0.0001; b, p < 0.001; c, p < 0.003; d, p < 0.006; e, p < 0.01; f, p < 0.03; g, no significant difference.
tion mainly in the hippocampus (Fig. 5E). One interpretation of this effect is that exogenously infused DHA is being utilized for the synthesis of the bioactive docosanoid, 10,17S-docosatriene. This hypothesis is supported by our finding that after 1 h of MCA-O followed by 6 h of reperfusion with DHA, 10,17S-docosatriene was unequivocally generated in the hippocampus at higher levels than in vehicle-perfused mice (Fig. 6).

10,17S-Docosatriene Elicits Neuroprotection in Mouse Focal Ischemic Stroke—To test for in vivo bioactivity of 10,17S-docosatriene in brain tissue during focal ischemic stroke, we devised a combined approach. We implanted Alzet mini-pumps into the third ventricle to deliver the lipid messenger over the 48-h reperfusion following 1 h of MCA-O. Then serial coronal sections of the brain were obtained and incubated with TTC (Figs. 7 and 8). TTC is actively taken up by cells into mitochondria resulting in a reddish color. The focal infarcted right side of the brain, the result of ischemic injury, displays colorless areas indicating severe mitochondrial and cell damage (Fig. 8A). The focal infarcted volume in mice infused with vehicle was about 30% of the total volume, whereas, when 10,17S-docosatriene was infused, there was a reduction to less than 15% of the stroke volume (Fig. 8B).

The Ischemia-induced Up-regulation of COX-2 Expression and NFκB Activation in Brain Tissue Is Inhibited by 10,17S-Docosatriene—Pro-inflammatory gene expression is an important promoter of ischemic brain injury. NFκB is activated in MCA-O (31, 32) as is COX-2 expression, which in turn generates prostaglandin H₂, the substrate for prostaglandin synthetases and a contributor to oxidative stress (33). To investigate whether pro-inflammatory gene expression is also a target of the novel docosanoid messenger 10,17S-docosatriene, we measured DNA binding activity of NFκB and other transcription factors as well as COX-1 and -2 expression in the hippocampus after MCA-O. NFκB was increased over 2-fold in the ipsilateral hippocampus after MCA-O, and the infusion of DHA or 10,17S-docosatriene inhibited ischemia-reperfusion-induced NFκB activation by 28 and 48%, respectively (Fig. 9A). This effect was selective, because DNA bindings for AP1, HIF1α, and STAT-1, which were slightly enhanced by MCA-O, were unaffected by lipid messengers, except for a small effect of 10,17S-docosatriene on STAT-1-DNA binding (Fig. 9B). To determine COX-1 and COX-2 expression we measured mRNA abundance in the ipsilateral hippocampus and found a 3-fold increase of COX-2 (Fig. 9C). The infusion of the precursor DHA

![Fig. 6. LC-MS-MS quantitative analysis of 10,17S-docosatriene and DHA levels in mouse hippocampus after 1 h of MCA-O and 6 h of reperfusion. Shown are representative chromatograms of 10,17S-docosatriene selected reaction monitoring (SRM) for the parent ion 359 m/z on quadrupole 1 (Q1) and the daughter ion 297 m/z on Q3. Also shown are the DHA SRM for parent ion 327 m/z and daughter ion 325 m/z on Q1 and Q3, respectively. A, animals treated by cerebroventricular perfusion of cerebrospinal fluid vehicle for 6 h produced consistent endogenous amounts of 10,17S-docosatriene and DHA. B, animals that were perfused with DHA (20 μg) exhibited greater formation of 10,17S-docosatriene and at least a 3-fold increase in DHA levels.](http://www.jbc.org/content/early/2019/09/25/jbc.RA119.014537/F6.large.jpg)
FIG. 7. Serial sections of the mouse brain exposed to TTC. Sections for TTC staining were made with a vibratome and handled as described under “Experimental Procedures.” The identification numbers of individual mice as well as letters that designate each serial section are shown. Two of the mice depicted were injected with vehicle and two with 10,17S-docosatriene as described under “Experimental Procedures.” Colorless areas indicate mitochondrial damage reflecting the inability to metabolize TTC.

FIG. 8. Neuroprotection by 10,17S-docosatriene in transient focal stroke in mice. A, selected sections from different mice (each mouse is identified by a number and each brain section by a letter) in three groups: sham-operated, vehicle-infused, and 10,17S-docosatriene-infused. Sectioning and TTC staining were performed as described under “Experimental Procedures.” Infusion was performed through an Alzet mini-pump implanted into the third ventricle (see “Experimental Procedures”). B, the graph depicts the percentage of the TTC-stained area with respect to total brain coronal area. Bars are averages ± S.D. from 6 animals each. Asterisk indicates *p < 0.001629, Student’s t test.
as well as its product, 10,17S-docosatriene, inhibited COX-2 expression by 14 and 52%, respectively. The infusion of 10,17S-docosatriene also decreased COX-1 expression by 19%.

Selective Inhibition by 10,17S-Docosatriene of IL-1β-mediated COX-2 Expression and NFκB Activation in Human Neural Progenitor Cells—We tested whether 10,17S-docosatriene affects pro-inflammatory gene signaling in human neural progenitor cells in culture exposed to IL-1β. These cells display dendrites and express neuronal markers (25). IL-1β prominently activated NFκB, and 10,17S-docosatriene down-regulated NFκB to a level below that of unstimulated cells in a concentration-dependent manner (Fig. 9D). HIF1-α was not affected even at 10-fold higher concentrations of DHA. Although IL-1β slightly increased AP1-DNA binding activity, 10,17S-docosatriene did not affect the level of this transcription factor or that of STAT-1 under these conditions (Fig. 9E). The action of 10,17S-docosatriene on intact cells strongly suggests the presence of a receptor-mediated mechanism, which would be consistent with the observation in other cells of evidence for a receptor-mediated event activated by this docosanoid (22). Moreover, the lack of effect of DHA implies that, unlike in whole brain, where infused DHA shows some inhibition of PMN infiltration induced by ischemia-reperfusion, these neural cells in culture exogenous DHA might not be converted to docosanoids in high enough concentrations. Alternatively, other cells may be required for this conversion to occur (e.g. glial cells).

Fig. 9, F and G, show that IL-1β-induced expression of COX-2 in neural progenitor cells was decreased by 10,17S-docosatriene. It is interesting that IL-1β did not activate COX-1 expression, but the docosanoid seemed to decrease the basal level of COX-1 mRNA. Taken together, these observa-
Neuroprotective Docosanoids in Experimental Stroke

Here we have demonstrated for the first time in brain tissue undergoing ischemia-reperfusion the generation of stereospecific DHA-oxygenation pathways that lead to the formation of novel messengers. We found two DHA-oxygenation pathways: the first pathway is responsible for the formation of the messenger 10,17-docosatriene, and the second pathway, which is active in the presence of aspirin, leads to the formation of the resolvin-type messengers (17R-DHA). The pathways described have the potential for exerting counter-regulatory actions on cellular and molecular signaling that promotes brain injury. In the presence of aspirin and during ischemia-reperfusion, we found the formation of characteristic DHA messengers that in non-neural tissues are potent mediators of inflammation resolution (28). These resolvin-type DHA-derived messengers may elicit additional neuroprotective actions in brain ischemia-reperfusion. Our present results demonstrate that the novel 10,17-docosatriene is a potent inhibitor of ischemia-reperfusion-induced PMN infiltration and pro-inflammatory gene induction. This novel DHA messenger also inhibits cytokine-mediated pro-inflammatory gene activation in neural cells in culture. Overall, 10,17-docosatriene potently elicited neuroprotection in vivo by reducing the stroke infarct volume 48 h after MCA-O.

It is important to note that, in the presence of aspirin, there was enhanced formation in brain tissue of 17R-series resolvins that have recently been found to be cytoprotective and are counter-regulators of inflammation outside the nervous system (23). It could be argued that these compounds enhance the actions of endogenously generated docosanoids, which are the counter-regulatory substances generated from DHA to decrease leukocyte recruitment to brain and to limit leukocyte-mediated inflammation and brain damage. The implied switch, from endogenous to aspirin-triggered DHA-derived lipid mediators that enhance this protective action, is of great interest for future studies, given the wide use of aspirin.

The synthesis of 10,17-docosatriene after 1 h of MCA-O coincides with free DHA availability that results from phospholipase A2 activation (2-4) as well as with reperfusion reoxygenation. The endogenous brain synthesis of 10,17-docosatriene that peaks at 8 h of reperfusion may be a response of such insufficient magnitude as to counteract leukocyte infiltration and pro-inflammatory gene induction under the present experimental conditions. Thus the relatively large ischemic insult produced by 1 h of MCA-O followed by several h of reperfusion (Fig. 8) may overcome the ability of the endogenously generated docosanoids to elicit neuroprotection. Thus exogenous administration of 10,17-docosatriene directly into the cerebroventricular system through continuous infusion during the initial 2 days of reperfusion did indeed exert neuroprotection. During 24–72 h of reperfusion is when most brain leukocyte infiltration occurs (7, 8, 29, 30). Leukocytes accumulate in an area surrounding the brain infarct (Fig. 5, A and B) and are thought to possess a multifactorial ability to promote injury in brain ischemia-reperfusion (34). Moreover, PMN infiltration as well as amoeboid microglia along the edges of the stroke infarct may be responsible for expansion of the penumbra region, which occurs from 16 h onward after MCA-O followed by reperfusion (35). Here we demonstrate that 10,17-docosatriene administered continuously by intracerebroventricular perfusion inhibited such infiltration and subsequently reduced the stroke volume by 50% after 48 h. The amount of docosanoid infused was 1 μg over a 48-h period at a rate of 250 nL/h. This observation implies that 10,17-docosatriene is neuroprotective. Once these leukocytes infiltrate the brain, they release IL-1β, tumor necrosis factor-α, and other cytokines, as well as myeloperoxidase, which in turn catalyzes the formation of additional reactive oxygen species. Myeloperoxidase in intercellular spaces of the brain is potentially a highly effective enzymatic catalyst for the initiation of lipid peroxidation (36), and brain cells are richly endowed with a major target of that process, polyunsaturated fatty acyl phospholipids (2, 12).

The bioactivity of the novel docosanoid 10,17-docosatriene was further studied at the cellular level. We chose IL-1β as the trigger, because this cytokine increases during brain ischemia-reperfusion as a result of PMN infiltration as well as activation of microglia and macrophages (35). Whether 10,17-docosatriene bioactive inhibition of PMN infiltration and its blocking of pro-inflammatory gene expression are independent events or part of the same signaling remains to be defined. What is clear is that the outcome of infusing 10,17-docosatriene is neuroprotection from ischemia-reperfusion damage.

These findings have several implications related to both the understanding of how the brain modulates inflammatory injury responses and designing new experimental therapeutics for neurologic diseases. Therefore we have demonstrated novel DHA-signaling pathways that may lead to answers to clinically important questions regarding undiscovered mechanisms in stroke, traumatic head injury, spinal cord injury, and other diseases that involve a neuroinflammatory component. The potent bioactivity of 10,17-docosatriene suggests the existence of a potentially important target for therapeutic neuroprotective interventions in these diseases.

Acknowledgments—We gratefully acknowledge the technical assistance of Brenda Chiappinelli, Eric Tjonahen, and Katie Gotlinger.

REFERENCES
25. Bazan, N. G., and Lukiw, W. J. (2002) *J. Biol. Chem.* 277, 30359–30367
26. Huang, J., Choudhri, T. F., Winfree, C. J., McTaggart, R. A., Kiss, S., Mocco, J., Kim, L. J., Protopsaltis, T. S., Zhang, Y., Pinsky, D. J., and Cennolly, E. S., Jr. (2000) *Stroke* 31, 3047–3053
27. Gronert, K., Clush, C. B., Romano, M., and Serhan, C. N. (1999) *Methods Mol. Biol.* 120, 119–144
28. Serhan, C. N., Hong, S., Gronert, K., Colgan, S. P., Deverhand, P. R., Mirick, G., and Mousignac, R. L. (2002) *J. Exp. Med.* 196, 1025–1037
29. Chopp, M., Li, Y., Zhang, R. L., and Prostak, J. (1996) *J. Cereb. Blood Flow Metab.* 16, 578–584
30. Chatzipanteli, K., Alonso, O. F., Kraydieh, S., and Dietrich, W. D. (2000) *J. Cereb. Blood Flow Metab.* 20, 531–542
31. Mattson, M. P., Culmsee, C., Yu, Z. F., and Camandola, S. (2000) *J. Neurochem.* 74, 443–456
32. Schneider, A., Martin-Villalba, A., Weih, F., Wirth, T., and Schwaninger, M. (1999) *Nat. Med.* 5, 554–559
33. Lukiw, W. J., and Bazan, N. G. (1998) *J. Neurosci. Res.* 53, 583–592
34. Hallenbeck, J. M., and Kochanek, P. M. (1998) in *Cerebrovascular Disease: Pathophysiology, Diagnosis, and Management* (Ginsberg, M. D., and Bogousslavsky, J., eds) Vol. 1, pp. 489–506, Blackwell Science, Inc., Malden, MA
35. Mabuchi, T., Kitagawa, K., Ohtsuki, T., Kuwabara, T., Yagita, Y., Yanagihara, T., Hori, M., and Matsumoto, M. (2000) *Stroke* 31, 1735–1743
36. Zhang, R., Brennan, M. L., Shen, Z., MacPherson, J. C., Schmitt, D., Molenda, C. E., and Haren, S. L. (2002) *J. Biol. Chem.* 277, 46116–46122
37. Shewin, D. J. (2000) *Handbook of Parametric and Nonparametric Statistical Procedures*, 2nd Ed., Chapman and Hall-CRC, Boca Raton, FL
Novel Docosanoids Inhibit Brain Ischemia-Reperfusion-mediated Leukocyte Infiltration and Pro-inflammatory Gene Expression
Victor L. Marcheselli, Song Hong, Walter J. Lukiw, Xiao Hua Tian, Karsten Gronert, Alberto Musto, Mattie Hardy, Juan M. Gimenez, Nan Chiang, Charles N. Serhan and Nicolas G. Bazan

J. Biol. Chem. 2003, 278:43807-43817.
doi: 10.1074/jbc.M305841200 originally published online August 15, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M305841200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 17 of which can be accessed free at http://www.jbc.org/content/278/44/43807.full.html#ref-list-1
Addition and Correction

Vol. 278 (2003) 43807–43817

Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression.

Victor L. Marcheselli, Song Hong, Walter J. Lukiw, Xiao Hua Tian, Karsten Gronert, Alberto Musto, Mattie Hardy, Juan M. Gimenez, Nan Chiang, Charles N. Serhan, and Nicolas G. Bazan

Page 43811, Fig. 4: Two concentrations of 4,17S-diHDHA are shown. The data were labeled incorrectly. The correct figure is shown below.

![Corrected Figure 4](image-url)

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.