Published in final edited form as:

J Mol Neurosci. 2007 September ; 33(1): 80–86.

Electrophilic Cyclopentenone Isoprostanes in Neurodegeneration

Erik S. Musiek,

Division of Clinical Pharmacology, Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, 526 RRB, 23rd and Pierce Aves, Nashville, TN 37232-6602, USA

BethAnn McLaughlin, and

Departments of Neurology and Pharmacology, Vanderbilt University School of Medicine, MRB III Room 8110, 465 21st Avenue South, Nashville, TN 37232-8548, USA

Jason D. Morrow

Division of Clinical Pharmacology, Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, 526 RRB, 23rd and Pierce Aves, Nashville, TN 37232-6602, USA

Jason D. Morrow: jason.morrow@vanderbilt.edu

Abstract

Although oxidative stress has been implicated in the pathogenesis of numerous neurodegenerative conditions, the precise mechanisms by which reactive oxygen species (ROS) induce neuronal death are still being explored. The generation of reactive lipid peroxidation products is thought to contribute to ROS neurotoxicity. Isoprostanes (IsoPs), prostaglandin-like molecules formed *in vivo* via the ROS-mediated oxidation of arachidonic acid, have been previously demonstrated to be formed in increased amounts in the brains of patients with various neurodegenerative diseases. Recently, we have identified a new class of IsoPs, known as A₂- and J₂-IsoPs or cyclopentenone IsoPs, which are highly reactive electrophiles and form adducts with thiol-containing molecules, including cysteine residues in proteins and glutathione. Cyclopentenone IsoPs are favored products of the IsoP pathway in the brain and are formed abundantly after oxidant injury. These compounds also potently induce neuronal apoptosis by a mechanism which involves glutathione depletion, ROS generation, and activation of several redox-sensitive pathways that overlap with those involved in other forms of oxidative neurodegeneration. Cyclopentenone IsoPs also enhance neurodegeneration caused by other insults at biologically relevant concentrations. These data are reviewed, whereas new data demonstrating the neurotoxicity of J-ring IsoPs and a discussion of the possible role of cyclopentenone IsoPs as contributors to neurodegeneration are presented.

Keywords

Isoprostane; Oxidative stress; Lipid peroxidation; Neurodegeneration; Alzheimer's disease; Arachidonic acid; Cyclopentenone; 12-lipoxygenase; Glutamate

Introduction

Oxidative stress has recently emerged as a major contributor to numerous neurodegenerative diseases (Beal 1995; Andersen 2004). Substantial research efforts have been targeted at identifying the mechanisms by which the excessive generation of reactive oxygen species (ROS) causes neuronal death and dysfunction. One leading hypothesis is that lipid peroxidation, the free radical-mediated oxidation of membrane lipids, contributes to the

pathological effects of oxidative stress in the brain. In support of this theory, increased levels of bioactive lipid peroxidation products have been identified in affected brain regions from humans with various neurodegenerative diseases, as well as in corresponding animal models (reviewed in Keller and Mattson 1998; Montine et al. 2002, 2004).

Lipid peroxidation can lead to the formation of highly reactive electrophilic compounds, such as 4-hydroxynonenal, acrolein, and levuglandin-like compounds in the brain (Montine et al. 1997; Sayre et al. 1997; Lovell et al. 2001; Davies et al. 2004; Zagol-Ikapitte et al. 2005). Furthermore, cellular studies have shown that some of these lipid peroxidation products can independently cause neuronal dysfunction and death. Indeed, many of the pathogenic events associated with oxidative stress can be recapitulated by application of lipid peroxidation products to neuronal cultures (Kruman et al. 1997; Mattson et al. 1997; Neely et al. 1999; Picklo et al. 1999; Camandola et al. 2000; Shringarpure et al. 2000). Furthermore, overexpression of glutathione transferase enzymes, which metabolize electrophilic lipids, protects neurons from oxidative insults (Xie et al. 1998, 2001). Thus, lipid peroxidation likely contributes to the observed effects of ROS in neurons (Keller and Mattson 1998; Kruman et al. 1997), although many of the exact oxidized lipid species involved are unknown.

The IsoP Pathway and Neurodegeneration

A well characterized pathway of lipid peroxidation is the formation of isoprostanes (IsoPs), prostaglandin (PG)-like molecules generated nonenzymatically via the ROS-dependent oxidation of arachidonic acid (Morrow et al. 1990; Roberts and Morrow 2002). IsoPs containing various prostane ring structures are formed *in vivo*, including F₂-IsoPs, which are isomeric to PGF_{2α} (Morrow et al. 1990), and D₂/E₂-IsoPs, which are isomers of PGD₂ and PGE₂, respectively (see Fig. 1; Morrow et al. 1994). Because of their stability, the measurement of F₂-IsoPs by mass spectrometry has been extensively employed as a marker of oxidant stress, and is widely considered to be the gold-standard index of lipid peroxidation *in vivo* (Morrow and Roberts 1998; Musiek et al. 2005). Increased cerebral F₂-IsoP formation has been associated with numerous neurodegenerative diseases, including Huntington's Disease (Montine 1999a, b, c), Creutzfeldt–Jakob disease (Minghetti et al. 2000), traumatic brain injury (Bayir et al. 2002), multiple sclerosis (Greco et al. 1999), and Alzheimer's disease (AD; Reich et al. 2001). In the case of AD, exposure of neurons or synaptosomes to amyloidogenic Aβ peptide leads to increased F₂-IsoP formation (Mark et al. 1999; Brunetti et al. 2004). Furthermore, significant increases in F₂-IsoPs, which precede amyloid plaque formation, were observed in a mouse model of AD (Pratico et al. 2001). Several human studies have detected significantly elevated levels of F₂-IsoPs in postmortem brain tissue and cerebrospinal fluid obtained from AD patients (Pratico et al. 1998; Reich et al. 2001), as well as in CSF from living patients with probable AD (Montine 1999a, b, c; Pratico et al. 2000). Increased F₂-IsoP formation occurred specifically in affected brain regions and correlated with the degree of neuronal degeneration (Montine 1999a, b, c). These studies suggest that oxidative stress and IsoP formation are conserved characteristics of the neurodegenerative process.

Different Classes of IsoPs are Formed *In Vivo* in Brain

Whereas F₂-IsoPs are the most thoroughly studied IsoP subtype, IsoPs with alternative ring structures are also formed by the same pathway. Indeed, E₂/D₂-IsoPs, which resemble PGE₂ and D₂, respectively, are formed in competition with F₂-IsoPs from the same endoperoxide precursor (Fig. 1a,b; Morrow et al. 1994). Interestingly, the loss of reducing environment in the brain, manifested by depletion of glutathione (GSH) and vitamin E, shifts the IsoP pathway toward the formation of E/D-ring IsoPs and away from reduced F-ring IsoPs (Montine et al. 2003). In peroxidizing brain synaptosomes, E₂/D₂-IsoP are the favored products of the IsoP pathway, and their levels far exceed those of F-ring IsoPs (Montine et al. 2003). Moreover,

E₂/D₂-IsoP levels are significantly elevated in the brains of human AD patients, and the ratio of E/D-ring to F-ring IsoPs is increased in this disease (Reich et al. 2001). Thus, these findings demonstrate that alternative IsoP subtypes are formed along with F₂-IsoPs under neurodegenerative conditions.

Cyclopentenone IsoPs: A Class of Highly Reactive IsoPs, Which are Formed in Neural Tissue

E₂/D₂-IsoPs are unstable compounds and spontaneously dehydrate in vivo to form A₂/J₂-IsoPs, also known as cyclopentenone IsoPs (Fig. 1; Chen et al. 1999a). Cyclopentenone IsoPs are so named because they contain a highly reactive α,β -unsaturated cyclopentenone ring structure and are thus potent electrophiles (Milne et al. 2005). Cyclopentenone IsoPs rapidly form Michael adducts with cellular thiol groups, such as those found on cysteine residues in proteins or in GSH (Chen et al. 1999a,b). Because other electrophilic lipid peroxidation products can damage neurons, we hypothesized that cyclopentenone IsoPs might be neurotoxic products of the IsoP pathway and might contribute to the pathogenesis of oxidative neurodegeneration. In support of this hypothesis, we have recently demonstrated that cyclopentenone IsoPs are considerably more abundant than F₂-IsoPs in both human and rat brain tissue (Musiek et al. 2006). Oxidative injury caused by exposure to the free radical initiator 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH) caused a 12-fold increase in cyclopentenone IsoPs in brain homogenates, generating cyclopentenone IsoP levels as high as ~550 nM. Thus, cyclopentenone IsoPs are formed in biologically relevant concentration in the brain under conditions of oxidative stress and appear to be favored products of the IsoP pathway in the brain.

Cyclopentenone IsoPs Induce Neuronal Death Via an Apoptotic Pathway

We have performed experiments examining the effects of synthetic 15-A_{2t}-IsoP (Fig. 1b; Zanoni et al. 2002), a major cyclopentenone IsoP isomer formed in vivo (Chen 1999b), in primary cortical neuronal cultures. 15-A_{2t}-IsoP is highly neurotoxic, inducing neuronal death with an LD₅₀ of 950 nM (Musiek et al. 2006). 15-A_{2t}-IsoP-induced cell death is apoptotic, as cells exposed to this IsoP exhibited condensed, fragmented nuclei, as well as increased caspase-3 cleavage, and are completely protected by the pan-caspase inhibitors. 15-J_{2t}-IsoPs, a cyclopentenone IsoPs which is formed similarly to 15-A_{2t}-IsoPs, was recently synthesized (Fig. 1b; Zanoni et al. 2003). This J-ring IsoP induces cell death in HT22 hippocampal cells with slightly greater potency than 15-A_{2t}-IsoP (Fig. 1c), suggesting that many if not all cyclopentenone IsoPs are neurotoxic.

Because of their reactivity, cyclopentenone IsoPs rapidly form adducts with GSH (Chen et al. 1999a, b) and are metabolized in cells via conjugation to GSH by glutathione transferases (GSTs, see Fig. 2; Milne et al. 2004). Exposure of neurons to 15-A_{2t}-IsoP overwhelms the GST system, causing rapid GSH depletion. 15-A_{2t}-IsoP also independently induces mitochondrial free radical production, resulting in membrane lipid peroxidation (Musiek et al. 2006). These two processes set in motion a feed-forward cycle of oxidation in neurons, as cyclopentenone IsoPs are initially generated as products of oxidative stress, then in turn promote further ROS production, thereby generating more of themselves (Fig. 2). Accordingly, 15-A_{2t}-IsoP toxicity can be mitigated by free radical scavenging agents, suggesting that redox alterations caused by 15-A_{2t}-IsoP contribute to its toxicity.

Cyclopentenone IsoPs induce apoptosis in neurons through activation of specific signaling pathways, all of which have been implicated in other forms of oxidative neurodegeneration (Fig. 2). The stress-responsive kinase ERK1/2 is phosphorylated in a redox-dependent manner in response to cerebral ischemia (Alessandrini et al. 1999; Namura et al. 2001; Noshita et al.

2002), and inhibition of ERK attenuates ischemic neurodegeneration, as well as neuronal death in response to other oxidative stimuli (Stanciu et al. 2000; Du et al. 2002; Lee et al. 2003; Chu et al. 2004). Further, dysregulated ERK phosphorylation is observed in neurons of patients with several neurodegenerative diseases, including AD (Perry et al. 1999) and Parkinson's disease (Zhu et al. 2002). Similarly, ERK is transiently phosphorylated in neurons exposed to 15-A_{2t}-IsoP downstream of mitochondrial ROS production, whereas inhibition of ERK phosphorylation partially protects from IsoP neurotoxicity (Musiek et al. 2006).

Human and rodent 12/15-lipoxygenase (12/15-LOX), an enzyme which is expressed in neurons and can oxidize arachidonic acid (Nishiyama et al. 1993), has also been shown to participate in several forms of neurodegeneration associated with oxidative stress (Li et al. 1997; Canals et al. 2003; Zhang et al. 2004). 12/15-LOX has been linked to AD, as inhibition of 12/15-LOX protects neurons from β -amyloid toxicity, and increased 12/15-LOX expression has been described in neurons of patients with AD (Lebeau et al. 2004; Pratico et al. 2004). We observed that 15-A_{2t}-IsoP causes 12/15-LOX activation and that the 12/15-LOX inhibitor baicalein protects neurons from IsoP toxicity (Musiek et al. 2006), providing a potential mechanism linking oxidative stress, 12/15-LOX activation, and cell death.

Finally, 15-A_{2t}-IsoP also induces the phosphorylation of the redox-sensitive adaptor protein p66^{shc} in neurons (Musiek et al. 2006). P66^{shc} phosphorylation contributes to oxidative stress-induced cell death, as mice lacking this protein are resistant to oxidant injury and have increased lifespan (Migliaccio et al. 1999; Trinei et al. 2002). Furthermore, p66^{shc} phosphorylation has recently been shown to play a role in β -amyloid neurotoxicity (Smith et al. 2005), revealing another important neurodegenerative pathway activated by cyclopentenone IsoPs. Thus, cyclopentenone IsoPs induce neuronal death through activation of several signaling proteins that have been previously but disparately implicated in oxidative neurodegeneration.

Cyclopentenone IsoPs Synergize with Other Oxidative Insults

As products of oxidative stress, cyclopentenone IsoPs are generated in response to an initial neurotoxic insult. Thus, determining the effect of these IsoPs in combination with other oxidative insults is of utmost importance in understanding their potential impact in vivo. One such oxidative insult is the application of extracellular glutamate to HT22 hippocampal cells or immature primary neurons. Because neither of these cell types express mature *N*-methyl-D-aspartate receptors, millimolar concentrations of glutamate inhibits cellular uptake of cystine in this system, causing rapid GSH depletion and oxidative cell death (Murphy et al. 1989; Tan et al. 2001). Interestingly, previous studies have shown that loss of GSH in these cells induces death via induction of mitochondrial ROS production (Tan et al. 1998) and activation of 12/15-LOX (Li et al. 1997) and ERK (Stanciu et al. 2000), just as in 15-A_{2t}-IsoP-treated neurons. Accordingly, we found that 15-A_{2t}-IsoP strongly potentiates neurodegeneration caused by sublethal concentrations of glutamate in both HT22 cells and primary neurons at concentrations as low as 100 nM (Musiek et al. 2006). Taken together, the data demonstrate that cyclopentenone IsoPs are neurotoxic products of lipid peroxidation that activate several discrete pathogenic signaling pathways associated with oxidative neurodegeneration, and which enhance neuronal death caused by other oxidative insults.

Conclusions

Although the initial studies are intriguing, much remains unknown about the potential role of cyclopentenone IsoPs in neurodegeneration. It is likely that these compounds modulate numerous other signaling cascades in neurons that have not yet been identified. Furthermore, the exact protein targets and cysteine adducts that are generated have not yet been elucidated. Proteomic approaches are currently being employed to identify these adducts in neurons and

other cell types. The mechanism by which cyclopentenone IsoPs promote mitochondrial ROS production is also an area of continued study. Finally, the quantification of cyclopentenone IsoPs in human postmortem brain samples from patients with various neurodegenerative diseases will provide important information about the involvement of these molecules in neurologic disease in vivo. Novel mass spectrometric approaches have been developed in our lab to reliably and accurately quantify 15-A₂- and 15-J₂-IsoPs in brain tissue (Musiek et al. 2006) and are currently being applied to tissue samples from human patients as well as from animal models of neurodegenerative diseases. The findings that cyclopentenone IsoPs are formed via the same pathway and more abundantly than F₂-IsoPs in brain and that F₂-IsoP levels are increased in the brains of patients with various neurodegenerative conditions (Montine et al. 2004) suggest that cyclopentenone IsoPs are likely also formed abundantly in these diseases. Thus, therapeutic interventions that prevent cyclopentenone IsoP formation, adduction, or downstream signaling might hold promise for the prevention and treatment of neurodegenerative diseases.

Acknowledgments

This work was supported by NIH grants DK48831, GM15431, CA77839, RR00095, ES13125, HD15052, and NS050396. ESM was supported by a grant from the PhRMA Foundation.

Abbreviations

12/15-LOX	12/15-lipoxygenase
AA	arachidonic acid
AAPH	2,2'-azobis(2-amidinopropane)hydrochloride
AD	Alzheimer's disease
ERK	extracellular signal-regulated kinase
GSH	glutathione
GST	glutathione transferase
IsoP	isoprostane
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
nM	nanomolar
PG	prostaglandin
μM	micromolar

References

- Alessandrini A, Namura S, Moskowitz MA, Bonventre JV. MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia. *Proceedings of the National Academy of Sciences of the United States of America* 1999;96:12866–12869. [PubMed: 10536014]
- Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? *Natural Medicines* 2004;10 (Suppl):S18–S25.
- Bayir H, Kagan VE, Tyurina YY, Tyurin V, Ruppel RA, Adelson PD, et al. Assessment of antioxidant reserves and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children. *Pediatric Research* 2002;51:571–578. [PubMed: 11978879]
- Beal MF. Aging, energy, and oxidative stress in neurodegenerative diseases. *Annals of Neurology* 1995;38:357–366. [PubMed: 7668820]

- Brunetti L, Michelotto B, Orlando G, Recinella L, Di Nisio C, Ciabattini G, et al. Aging increases amyloid beta-peptide-induced 8-iso-prostaglandin F2alpha release from rat brain. *Neurobiology Aging* 2004;25:125–129.
- Camandola S, Poli G, Mattson MP. The lipid peroxidation product 4-hydroxy-2,3-nonenal inhibits constitutive and inducible activity of nuclear factor kappa B in neurons. *Brain Research. Molecular Brain Research* 2000;85:53–60. [PubMed: 11146106]
- Canals S, Casarejos MJ, de Bernardo S, Rodriguez-Martin E, Mena MA. Nitric oxide triggers the toxicity due to glutathione depletion in midbrain cultures through 12-lipoxygenase. *Journal of Biological Chemistry* 2003;278:21542–21549. [PubMed: 12679339]
- Chen Y, Morrow JD, Roberts LJ 2nd. Formation of reactive cyclopentenone compounds in vivo as products of the isoprostane pathway. *Journal of Biological Chemistry* 1999a;274:10863–10868. [PubMed: 10196163]
- Chen Y, Zackert WE, Roberts LJ 2nd, Morrow JD. Evidence for the formation of a novel cyclopentenone isoprostane, 15-A2t-isoprostane (8-iso-prostaglandin A2) in vivo. *Biochimica Et Biophysica Acta* 1999b;1436:550–556. [PubMed: 9989284]
- Chu CT, Levinthal DJ, Kulich SM, Chalovich EM, DeFranco DB. Oxidative neuronal injury. The dark side of ERK1/2. *European Journal of Biochemistry* 2004;271:2060–2066. [PubMed: 15153095]
- Davies SS, Amarnath V, Roberts LJ 2nd. Isoketals: highly reactive gamma-ketoaldehydes formed from the H2-isoprostane pathway. *Chemistry and Physics of Lipids* 2004;128:85–99. [PubMed: 15037155]
- Du S, McLaughlin B, Pal S, Aizenman E. In vitro neurotoxicity of methylisothiazolinone, a commonly used industrial and household biocide, proceeds via a zinc and extracellular signal-regulated kinase mitogen-activated protein kinase-dependent pathway. *Journal of Neuroscience* 2002;22:7408–7416. [PubMed: 12196562]
- Greco A, Minghetti L, Sette G, Fieschi C, Levi G. Cerebrospinal fluid isoprostane shows oxidative stress in patients with multiple sclerosis. *Neurology* 1999;53:1876–1879. [PubMed: 10563647]
- Keller JN, Mattson MP. Roles of lipid peroxidation in modulation of cellular signaling pathways, cell dysfunction, and death in the nervous system. *Reviews in the Neurosciences* 1998;9:105–116. [PubMed: 9711902]
- Kruman I, Bruce-Keller AJ, Bredesen D, Waeg G, Mattson MP. Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. *Journal of Neuroscience* 1997;17:5089–5100. [PubMed: 9185546]
- Lebeau A, Terro F, Rostene W, Pelaprat D. Blockade of 12-lipoxygenase expression protects cortical neurons from apoptosis induced by beta-amyloid peptide. *Cell Death and Differentiation* 2004;11:875–884. [PubMed: 15105833]
- Lee YJ, Cho HN, Soh JW, Jhon GJ, Cho CK, Chung HY, et al. Oxidative stress-induced apoptosis is mediated by ERK1/2 phosphorylation. *Experimental Cell Research* 2003;291:251–266. [PubMed: 14597424]
- Li Y, Maher P, Schubert D. A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. *Neuron* 1997;19:453–463. [PubMed: 9292733]
- Lovell MA, Xie C, Markesbery WR. Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures. *Neurobiology Aging* 2001;22:187–194.
- Mark RJ, Fuson KS, May PC. Characterization of 8-epiprostaglandin F2alpha as a marker of amyloid beta-peptide-induced oxidative damage. *Journal of Neurochemistry* 1999;72:1146–1153. [PubMed: 10037487]
- Mattson MP, Fu W, Waeg G, Uchida K. 4-Hydroxynonenal, a product of lipid peroxidation, inhibits dephosphorylation of the microtubule-associated protein tau. *Neuroreport* 1997;8:2275–2281. [PubMed: 9243625]
- Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, et al. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 1999;402:309–313. [PubMed: 10580504]
- Milne GL, Zanoni G, Porta A, Sasi S, Vidari G, Musiek ES, et al. The cyclopentenone product of lipid peroxidation, 15-A2t-isoprostane, is efficiently metabolized by HepG2 cells via conjugation with glutathione. *Chemical Research in Toxicology* 2004;17:17–25. [PubMed: 14727915]

- Milne GL, Musiek ES, Morrow JD. The cyclopentenone (a(2)/j(2)) isoprostanes-unique, highly reactive products of arachidonate peroxidation. *Antioxidants & Redox Signalling* 2005;7:210–220.
- Minghetti L, Greco A, Cardone F, Puopolo M, Ladogana A, Almonti S, et al. Increased brain synthesis of prostaglandin E2 and F2-isoprostane in human and experimental transmissible spongiform encephalopathies. *Journal of Neuropathology and Experimental Neurology* 2000;59:866–871. [PubMed: 11079776]
- Montine KS, Olson SJ, Amarnath V, Whetsell WO Jr, Graham DG, Montine TJ. Immunohistochemical detection of 4-hydroxy-2-nonenal adducts in Alzheimer's disease is associated with inheritance of APOE4. *American Journal of Pathology* 1997;150:437–443. [PubMed: 9033259]
- Montine TJ, Beal MF, Cudkowicz ME, O'Donnell H, Margolin RA, McFarland L, et al. Increased CSF F2-isoprostane concentration in probable AD. *Neurology* 1999a;52:562–565. [PubMed: 10025788]
- Montine TJ, Beal MF, Robertson D, Cudkowicz ME, Biaggioni I, O'Donnell H, et al. Cerebrospinal fluid F2-isoprostanes are elevated in Huntington's disease. *Neurology* 1999b;52:1104–1105. [PubMed: 10102447]
- Montine TJ, Markesbery WR, Zackert W, Sanchez SC, Roberts LJ 2nd, Morrow JD. The magnitude of brain lipid peroxidation correlates with the extent of degeneration but not with density of neuritic plaques or neurofibrillary tangles or with APOE genotype in Alzheimer's disease patients. *American Journal of Pathology* 1999c;155:863–868. [PubMed: 10487843]
- Montine TJ, Neely MD, Quinn JF, Beal MF, Markesbery WR, Roberts LJ, et al. Lipid peroxidation in aging brain and Alzheimer's disease. *Free Radical Biology & Medicine* 2002;33:620–626. [PubMed: 12208348]
- Montine TJ, Montine KS, Reich EE, Terry ES, Porter NA, Morrow JD. Antioxidants significantly affect the formation of different classes of isoprostanes and neuroprostanes in rat cerebral synaptosomes. *Biochemical Pharmacology* 2003;65:611–617. [PubMed: 12566089]
- Montine KS, Quinn JF, Zhang J, Fessel JP, Roberts LJ 2nd, Morrow JD, et al. Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chemistry and Physics of Lipids* 2004;128:117–124. [PubMed: 15037157]
- Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclo-oxygenase, free radical-catalyzed mechanism. *Proceedings of the National Academy of Sciences of the United States of America* 1990;87:9383–9387. [PubMed: 2123555]
- Morrow JD, Minton TA, Mukundan CR, Campbell MD, Zackert WE, Daniel VC, et al. Free radical-induced generation of isoprostanes in vivo. Evidence for the formation of D-ring and E-ring isoprostanes. *Journal of Biological Chemistry* 1994;269:4317–4326. [PubMed: 8307999]
- Morrow JD, Roberts LJ 2nd. Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidant stress. *Methods in Enzymology* 1998;300:3–12. [PubMed: 9919502]
- Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron* 1989;2:1547–1558. [PubMed: 2576375]
- Musiek ES, Breeding RS, Milne GL, Zanoni G, Morrow JD, McLaughlin B. Cyclopentenone isoprostanes are novel bioactive products of lipid oxidation which enhance neurodegeneration. *Journal of Neurochemistry* 2006;97:1301–1313. [PubMed: 16638022]
- Musiek, ES.; Morrow, JD. F2-Isoprostanes as Markers of Oxidant Stress. In: Costa, LG.; Hodgson, E.; Lawrence, D.; Reed, DJ., editors. *An Overview*, in *Current Protocols in Toxicology*. Edison, NJ: Wiley; 2005. p. 17.5–17.6.
- Namura S, Iihara K, Takami S, Nagata I, Kikuchi H, Matsushita K, et al. Intravenous administration of MEK inhibitor U0126 affords brain protection against forebrain ischemia and focal cerebral ischemia. *Proceedings of the National Academy of Sciences of the United States of America* 2001;98:11569–11574. [PubMed: 11504919]
- Neely MD, Sidell KR, Graham DG, Montine TJ. The lipid peroxidation product 4-hydroxynonenal inhibits neurite outgrowth, disrupts neuronal microtubules, and modifies cellular tubulin. *Journal of Neurochemistry* 1999;72:2323–2333. [PubMed: 10349841]

- Nishiyama M, Watanabe T, Ueda N, Tsukamoto H, Watanabe K. Arachidonate 12-lipoxygenase is localized in neurons, glial cells, and endothelial cells of the canine brain. *Journal of Histochemistry and Cytochemistry* 1993;41:111–117. [PubMed: 8417106]
- Noshita N, Sugawara T, Hayashi T, Lewen A, Omar G, Chan PH. Copper/zinc superoxide dismutase attenuates neuronal cell death by preventing extracellular signal-regulated kinase activation after transient focal cerebral ischemia in mice. *Journal of Neuroscience* 2002;22:7923–7930. [PubMed: 12223545]
- Perry G, Roder H, Nunomura A, Takeda A, Friedlich AL, Zhu X, et al. Activation of neuronal extracellular receptor kinase (ERK) in Alzheimer disease links oxidative stress to abnormal phosphorylation. *Neuroreport* 1999;10:2411–2415. [PubMed: 10439473]
- Picklo MJ, Amarnath V, McIntyre JO, Graham DG, Montine TJ. 4-Hydroxy-2(E)-nonenal inhibits CNS mitochondrial respiration at multiple sites. *Journal of Neurochemistry* 1999;72:1617–1624. [PubMed: 10098869]
- Pratico D, Clark CM, Lee VM, Trojanowski JQ, Rokach J, FitzGerald GA. Increased 8,12-iso-iPF₂α-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. *Annals of Neurology* 2000;48:809–812. [PubMed: 11079549]
- Pratico D, Lee VMY, Trojanowski JQ, Rokach J, Fitzgerald GA. Increased F₂-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *FASEB Journal* 1998;12:1777–1783. [PubMed: 9837868]
- Pratico D, Uryu K, Leight S, Trojanowski JQ, Lee VM. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *Journal of Neuroscience* 2001;21:4183–4187. [PubMed: 11404403]
- Pratico D, Zhukareva V, Yao Y, Uryu K, Funk CD, Lawson JA, et al. 12/15-lipoxygenase is increased in Alzheimer's disease: Possible involvement in brain oxidative stress. *American Journal of Pathology* 2004;164:1655–1662. [PubMed: 15111312]
- Reich EE, Markesbery WR, Roberts LJ 2nd, Swift LL, Morrow JD, Montine TJ. Brain regional quantification of F-ring and D-/E-ring isoprostanes and neuroprostanes in Alzheimer's disease. *American Journal of Pathology* 2001;158:293–297. [PubMed: 11141503]
- Roberts LJ 2nd, Morrow JD. Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. *Cellular and Molecular Life Sciences* 2002;59:808–820. [PubMed: 12088281]
- Sayre LM, Zelasko DA, Harris PL, Perry G, Salomon RG, Smith MA. 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. *Journal of Neurochemistry* 1997;68:2092–2097. [PubMed: 9109537]
- Shringarpure R, Grune T, Sitte N, Davies KJ. 4-Hydroxynonenal-modified amyloid-beta peptide inhibits the proteasome: possible importance in Alzheimer's disease. *Cellular and Molecular Life Sciences* 2000;57:1802–1809. [PubMed: 11130184]
- Smith WW, Norton DD, Gorospe M, Jiang H, Nemoto S, Holbrook NJ, et al. Phosphorylation of p66Shc and forkhead proteins mediates Aβ toxicity. *Journal of Cell Biology* 2005;169:331–339. [PubMed: 15837797]
- Stanciu M, Wang Y, Kentor R, Burke N, Watkins S, Kress G, et al. Persistent activation of ERK contributes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. *Journal of Biological Chemistry* 2000;275:12200–12206. [PubMed: 10766856]
- Tan S, Sagara Y, Liu Y, Maher P, Schubert D. The regulation of reactive oxygen species production during programmed cell death. *Journal of Cell Biology* 1998;141:1423–1432. [PubMed: 9628898]
- Tan S, Schubert D, Maher P. Oxytosis: A novel form of programmed cell death. *Current Topics in Medicinal Chemistry* 2001;1:497–506. [PubMed: 11895126]
- Trinei M, Giorgio M, Cicalese A, Barozzi S, Ventura A, Migliaccio E, et al. A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. *Oncogene* 2002;21:3872–3878. [PubMed: 12032825]
- Xie C, Lovell MA, Markesbery WR. Glutathione transferase protects neuronal cultures against four hydroxynonenal toxicity. *Free Radical Biology & Medicine* 1998;25:979–988. [PubMed: 9840744]

- Xie C, Lovell MA, Xiong S, Kindy MS, Guo J, Xie J, et al. Expression of glutathione-S-transferase isozyme in the SY5Y neuroblastoma cell line increases resistance to oxidative stress. *Free Radical Biology & Medicine* 2001;31:73–81. [PubMed: 11425492]
- Zagol-Ikapitte I, Masterson TS, Amarnath V, Montine TJ, Andreasson KI, Boutaud O, et al. Prostaglandin H-derived adducts of proteins correlate with Alzheimer's disease severity. *Journal of Neurochemistry* 2005;94:1140–1145. [PubMed: 15992375]
- Zanoni G, Porta A, Vidari G. First total synthesis of A(2) isoprostane. *Journal of Organic Chemistry* 2002;67:4346–4351. [PubMed: 12054973]
- Zanoni G, Porta A, Castronovo F, Vidari G. First total synthesis of J(2) isoprostane. *Journal of Organic Chemistry* 2003;68:6005–6010. [PubMed: 12868940]
- Zhang Y, Wang H, Li J, Jimenez DA, Levitan ES, Aizenman E, et al. Peroxynitrite-induced neuronal apoptosis is mediated by intracellular zinc release and 12-lipoxygenase activation. *Journal of Neuroscience* 2004;24:10616–10627. [PubMed: 15564577]
- Zhu JH, Kulich SM, Oury TD, Chu CT. Cytoplasmic aggregates of phosphorylated extracellular signal-regulated protein kinases in Lewy body diseases. *American Journal of Pathology* 2002;161:2087–2098. [PubMed: 12466125]

Figure 1.

Formation and neurotoxicity of cyclopentenone IsoPs. **a** Exposure of arachidonic acid (AA) to reactive oxygen species (ROS) leads to formation of an unstable endoperoxide intermediate, which can undergo reduction to form F₂-IsoPs, stable biomarkers of oxidative stress, or can isomerize to yield E₂- and D₂-IsoPs. E₂- and D₂-IsoPs are unstable, and spontaneously dehydrate in vivo to form electrophilic A₂- and J₂-IsoPs, also known as cyclopentenone IsoPs. R and R' represent sidechains, which vary depending on which of the 4 IsoP regioisomers are formed. **b** Diagram of 15-A_{2t}-IsoP and 15-J_{2t}-IsoP, endogenous cyclopentenone IsoPs that were chemically synthesized and employed in cell culture experiments. **c** 15-J_{2t}-IsoP induces cell death in HT22 hippocampal neuronal cells with greater potency than 15-A_{2t}-IsoP. HT22 cells were exposed to IsoPs in minimal essential media containing 0.1% fetal calf serum for 24 h, after which cell viability was assessed by MTT assay as previously described (Musiek et al. 2006). Data represent mean+SEM from three independent experiments

Figure 2.

Schematic of cyclopentenone IsoP formation and action in neurons. Reactive oxygen species (ROS) are generated in neurons by various neurodegenerative stimuli (such as β -amyloid, ischemia, glutamate, and the Parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP]), leading to the formation of F₂-IsoPs and cyclopentenone IsoPs (A/J-IsoPs, 15-A_{2t}-IsoP is depicted). A/J-IsoPs can then either be metabolized via conjugation to GSH by GST (schematized by GS-IsoP), leading to cellular GSH depletion and increased ROS levels, or can form cysteine adducts on proteins. Adduction of unidentified protein targets leads to increased mitochondrial ROS production. These events result in a feed-forward cycle of ROS production and lipid peroxidation that leads to the activation of redox-responsive signaling proteins, including 12-LOX, ERK, and p66^{shc}, culminating in caspase activation (not shown) and neuronal apoptosis. A/J-IsoPs likely activate other cytotoxic signaling pathways, and may induce cell death via direct mechanisms in some cells