Molecular Neurobiology

ISSN0893-7648/05/32(1): 19–26/$30.00

Novel Eicosanoid Pathways

The Discovery of Prostacyclin/6-keto Prostaglandin $F_{1\alpha}$ and the Hepoxilins

Cecil R. Pace-Asciak

Research Institute, The Hospital for Sick Children, Programme in Integrative Biology, Toronto, Ontario, Canada M5G1X8 and Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5A1A8

Abstract

This article reviews a lecture I was honored to present at the Leon Wolfe Symposium in Montreal on March 25, 2004. The lecture described my research career, which started with my interaction with Wolfe at the Montreal Neurological Institute as a postdoctoral fellow and research associate and was followed by additional research discoveries after I left Montreal for my first academic position at the Research Institute, The Hospital for Sick Children and University of Toronto. The article consists of two parts. The first part involves the discovery (in Wolfe's laboratory) of a new pathway of arachidonic acid, in which a bicyclic prostanoid structure (later called prostacyclin by John Vane and his group) was described, and its further development in Toronto, which led to the discovery of the conversion of the bicyclic prostanoid into 6-keto prostaglandin $F_{1\alpha}$. The second part deals with the hepoxilin pathway, a pathway I discovered during a sabbatical leave in Japan with Professor Shozo Yamamoto, which was followed by a stay of several months in the laboratory of Professor Bengt Samuelsson in Sweden. I deal with the historical aspects of both pathways and end with interesting novel aspects of hepoxilin stable antagonist analogs in the treatment of solid tumors in experimental animals.

Index Entries: Bicyclic prostanoid; prostacyclin; 6-keto prostaglandin $F_{1\alpha}$; hepoxilins; hepoxilin analogs; PBT; leukemia; K562; apoptosis; therapy; cancer.

Introduction

Similar to other contributors to this issue who gave lectures at the Leon Wolfe Symposium in Montreal in 2004, I had my origin in the biological sciences in the laboratory of Leon Wolfe. Unlike others, however, I came to Wolfe's lab at the Montreal Neurological Institute subsequent to being awarded a doctorate in organic chemistry at McGill University in 1966. I worked with Wolfe, first as a postdoctoral fellow and then as a medical research

Received 7/1/04; Accepted 9/14/04.
Author to whom correspondence and reprint requests should be addressed. E-mail: pace@sickkids.ca.

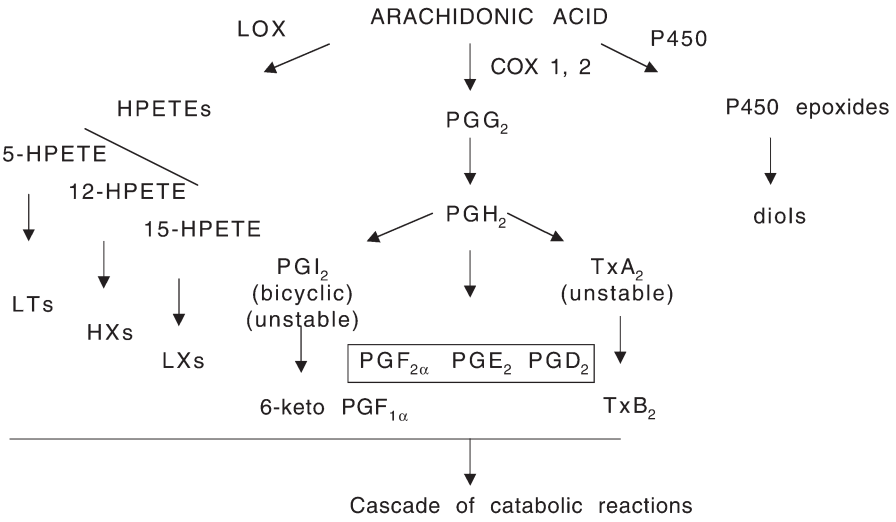

Fig. 1. The arachidonic acid cascade. Boxed compounds are the compounds known at the time of our discovery of the bicyclic compound in 1971. TxA_2 was discovered later but just before 6-keto $PGF_{1\alpha}$. LTs, leukotrienes; HX, hepoxilins; LXs, lipoxins; PG, prostaglandins; TX, thromboxane.

council fellow and research associate from 1966 to 1972, during which time I was exposed to the prostaglandin field, a young field at that time that Wolfe believed was an exceedingly promising area in which I could become involved.

Part 1

Prostacyclin/6-keto Prostaglandin $F_{1\alpha}$

During 1966 to 1972, we made an unexpected discovery with the demonstration of the formation of arachidonic acid of a new compound whose structure was determined to be bicyclic. The formation of this compound, a new member of the prostaglandin family, demonstrated the existence of a novel pathway of arachidonic acid metabolism. Wolfe and I published this seminal paper in 1971 *(1)*. The novelty of this work should be interpreted in light of the established structures and pathways known at the time. Bergstrom, Samuelsson, and their group had discovered the series of prostaglandins known to that point at the Karolinska Institutet in Stockholm. The prostaglandins known at that time were designated as prostaglandins $F_{2\alpha}$, E_2, and D_2, which were derived from arachidonic acid, and the various analogs derived from eicosatrienoic acid and eicosapentaenoic acid *(2–6)*. The unstable intermediate endoperoxides prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2), as precursors to the prostaglandins, had not yet been isolated. Therefore, our report of a novel bicyclic structure at a time when the prostaglandins were all monocyclic, was not well-received. The various compounds derived from the metabolism of arachidonic acid, now known as the "arachidonic acid cascade," involve a cascade of oxygen-incorporating reactions, including prostaglandins and thromboxane formed through cyclooxygenase (COX)-1 and COX-2 as well as products derived through the lipoxygenase and cytochrome P450 pathways (Fig. 1).

In 1972, I moved to the Research Institute of the Hospital for Sick Children in Toronto as an assistant professor and research scientist. Having successfully applied my chemistry background to prostaglandin biology in Wolfe's lab, I continued to investigate the pathway of formation of the bicyclic compound I discovered with Wolfe, and through rather inconvenient cryogenic techniques, I successfully isolated the prostaglandin endoperoxide intermediates that the Samuelsson group in Swe-

den *(7)* and the Nugteren group in Denmark *(8)* had published in 1973 through 1974. To demonstrate that the bicyclic compound was derived through the prostaglandin pathway, I treated the purified prostaglandin endoperoxide PGH_2 with preparations of the rat stomach from which we had originally isolated the bicyclic compound; I discovered that PGH_2 was rapidly and efficiently transformed to the bicyclic prostanoid that Wolfe and I had discovered. In these experiments, I also discovered that this bicyclic compound was further transformed nonenzymatically into a new compound, which I subsequently identified as 6-keto prostaglandin $F_{1\alpha}$ *(9)*. In fact, this compound became the key to the identification (by Vane's group) of the potent, but chemically and biologically unstable, antiplatelet aggregating factor formed from the interaction of PGH_2 with preparations of the rat stomach and aorta. The formation of 6-keto prostaglandin $F_{1\alpha}$ indicated that the biologically active factor was a precursor in this pathway, and chemical synthesis showed it was the bicyclic compound we had identified. Vane subsequently named the factor prostacyclin *(10)*. Vane demonstrated that prostacyclin was a potent inhibitor of platelet aggregation, and this important pharmacological discovery contributed to his sharing of the Nobel Prize with Bergstrom and Samuelsson in 1982 (the announcement was for discoveries concerning "Prostaglandins and Related Biologically Active Substances"). Prostacyclin has become one of the most potent anti-aggregating substances known; its formation by blood vessels controls pro-aggregatory mechanisms in the blood, thereby retaining fluidity of the circulation.

Part 2

The Hepoxilins

The aim of this article is to review some biological features related to the management of cancer by stable analogs of compounds in another pathway: the hepoxilin pathway, which I discovered in the early 1980s during a sabbatical leave in the laboratories of Yamamoto *(11)* and of Samuelsson *(12)*. I later coined the term hepoxilins, a term combining the structural features of hydroxy and epoxide with insulin secretion, their first biological activity, which we discovered *(13)*. The natural hepoxilins are chemically and biologically unstable, so we prepared stable analogs called Pace BioacTive compounds (PBTs) for testing in animals.

Biosynthetic Aspects of the Natural Hepoxilins

Hepoxilins are hydroxyepoxide derivatives of arachidonic acid formed through the 12S-lipoxygenase pathway *(12)*. They are formed through the enzymatic isomerization of the intermediate hydroperoxide 12S-HPETE by an enzyme I termed as hepoxilin synthase *(14)*. Hepoxilin synthase specifically uses 12S-HPETE, but not 12R-HPETE, as substrate *(15)*. Both hepoxilins A_3 and B_3 are formed. Using ^{18}oxygen-labeled 12S-HPETE, I demonstrated that the hydroxyl and epoxide oxygen atoms in both hepoxilins were derived from the hydroperoxy oxygen atoms of 12S-HPETE, indicating an internal isomerization within the enzyme; no dilution with external oxygen from air or water occurred (ref. *14*; Fig. 2). Recently, through immunoprecipitation techniques using antibodies for the leukocyte 12S-lipoxygenase, this synthase was shown to coprecipitate with 12-lipoxygenase and was shown to be an intrinsic catalytic component of the leukocyte- and platelet-type 12S-lipoxygenase *(16)*.

Biological Actions of the Natural Hepoxilins

The most notable biological activity of the hepoxilins probably is their ability to release intracellular calcium from internal calcium stores. This was initially demonstrated in human neutrophils *(17)* but was also observed in neurons *(18)*. This action may be responsible for the effects of hepoxilins in causing the release of insulin from pancreatic islets of Langerhans *(13,19)*. Another important observation was that hepoxilins inhibited the action of inflammatory mediators on intracellular calcium release. This suggests that hepoxilins may antagonize some common pathway

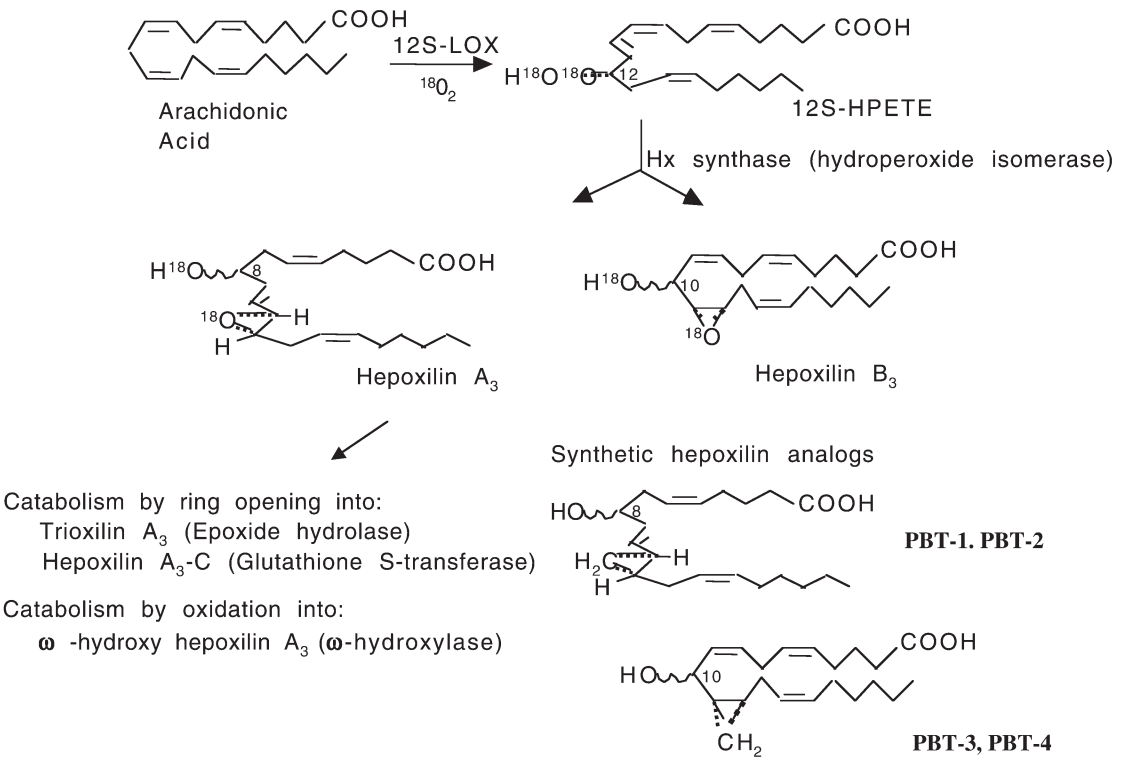

Fig. 2. The biosynthetic pathway of hepoxilin A_3 and hepoxilin B_3 formation, determined through use of ^{18}oxygen-labeling studies. The catabolism of hepoxilin A_3 is shown as are the chemical structures of the cyclopropyl PBT analogs.

related to inflammation, although they release calcium from stores *(20)*. Indeed, hepoxilin A_3 was recently shown to be proinflammatory and was identified as the pathogen-elicited epithelial chemo-attractant, which is formed by epithelial cells and is secreted from their apical surface in response to inflammation. Hepoxilin has been shown to be a key factor promoting the final step in neutrophil recruitment to sites of mucosal inflammation. This is relevant to inflammatory bowel disease *(21)*.

The Need for Stable Analogs (Antagonists) of the Hepoxilins: The PBTs The native hepoxilins showed good biological potential, with the exception that because of their instability in a biological system, their structure had to be modified for the compounds to be expected to have biological actions in vivo. The native hepoxilin structure is subject to rapid catabolism, leading to epoxide ring opening by epoxide hydrolase *(22)* and glutathione *S*-transferase (*23,24*; Fig. 2). Hepoxilin A_3 has been identified as the natural endogenous factor in platelets that regulates cell volume *(25)*. Enhanced hepoxilin synthesis has also been shown in psoriatic lesions, suggesting that they may be formed in response to inflammation *(26)*. Interestingly, hepoxilin B_3 and its product of epoxide hydrolase activity, trioxilins B_3, have been shown to be incorporated into phospholipids *(27)*. Hepoxilins have also been demonstrated to be formed by the Aplysia neurons *(28,29)*.

To stabilize the hepoxilin structure, we chemically synthesized a series of compounds in which the unstable epoxide was replaced by a cyclopropyl group *(30)* (Fig. 2). Preliminary biological testing demonstrated that the cyclopropyl analogs (later abbreviated as PBTs) antagonized the calcium-mobilizing actions of

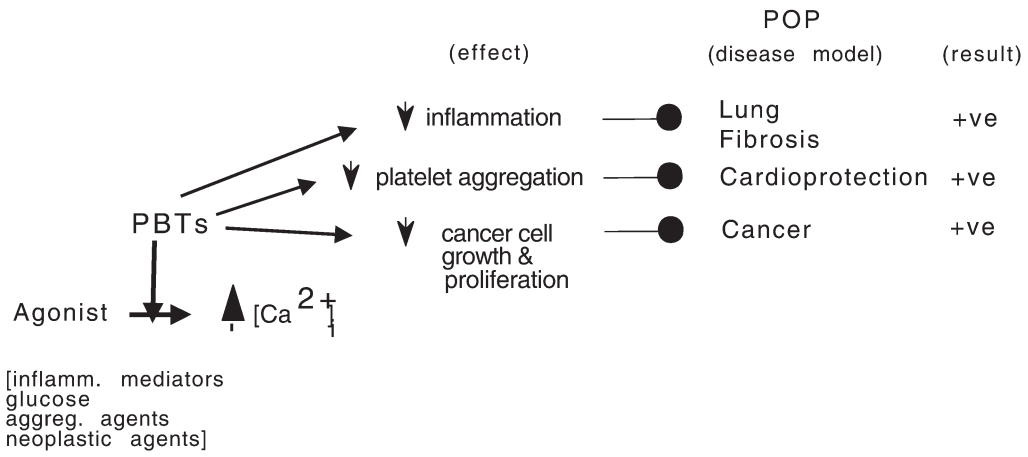

Fig. 3. The biological actions of PBTs are shown in vitro (calcium release) and in vivo (proof-of-principle studies).

the native hepoxilins. Because the PBTs are inactive to degradation chemically (low pH) and to ring opening (catabolism), they should be much more stable for in vivo studies. Indeed, measurable levels of PBTs in blood could be detected by mass spectrometry after intravenous administration in animals.

To date, we have demonstrated in three separate in vivo proof-of-concept studies that the PBTs were active in opposing the bleomycin-induced pulmonary fibrosis *(31)*; in inhibiting platelet aggregation through actions as thromboxane receptor antagonists in vitro *(32,33)*, specifically as TPα receptor antagonists *(34)*, as well as thrombosis in vivo (Dogne et al., private communication, 2004); and in inhibiting the growth of leukemic cells in vitro *(35)* and in vivo *(35a)* (Fig. 3). This article summarizes some of our recent studies implicating the PBTs as potential therapeutics in the management of solid tumors using the K562 leukemia cell line transplanted into immunocompromised mice.

Apoptotic Actions of PBT-3

I refer the reader to two excellent recent review articles on leukemia and the current status of therapy *(36,37)*. Leukemia is a heterogeneous disease characterized by malignant proliferation of cells of the hematopoietic system. The use of chemotherapeutic agents remains the mainstay of antileukemia therapy. However, significant morbidity and mortality still occurs. Chronic myeloid leukemia (CML) is typified by a chromosomal abnormality termed the Philadelphia chromosome (Ph), in which a unique fusion gene called *BCR-ABL* is generated. *BCR-ABL* accounts for the cause of the chronic phase of the disease. The expression of the *BCR-ABL* gene leads to enhanced tyrosine kinase activity, which is required for cell viability. In fact, STI-571 (Gleevec®) has been developed as effective therapy against CML through its inhibition of tyrosine kinase *(38)*. Unfortunately, however, significant resistance to the drug occurs. Hence, novel approaches to the management of the disease are still needed.

Apoptosis (programmed cell death) is defined by a set of distinct morphological (DNA laddering, cell cycle changes, nuclear condensation, and fragmentation) and biochemical (activation of caspases) changes *(39)*. There are several signal transduction pathways regulating cell survival and cell death *(40)*. For example, chemotherapy-induced apoptosis has been shown to involve the mitochondrial pathway; disruption of the mitochondrial membrane results in the release of mitochondrial proteins, including cytochrome c. Cytochrome c interacts with cytoplasmic proteins such as Apaf 1 to cause caspase-9 activation, which in turn results in activation of the downstream

(effector) caspases, such as caspase 3 *(41)*. The Bcl-2 family of apoptosis-regulating proteins regulates mitochondrial membrane permeabilization; this family includes pro-apoptotic proteins such as Bax and Bad and anti-apoptotic proteins such as Bcl-2 and Bcl-xl *(42)*.

CML cells appear to have a defective apoptotic pathway, resulting in their enhanced survival relative to normal cells. We have used the CML leukemic cell line K562, which originated from a patient in blast crisis, because this cell line is accepted as a good model of the disease *(43,44)*. These cells are resistant to apoptosis induction by several chemotherapeutic agents *(45,46)*, and they express the functionally active anti-apoptotic protein BCR-ABL product p210. BCR-ABL protein is associated with CML and interacts with several signal transduction pathways. The K562 cell line has been studied extensively, and there exists much information regarding the apoptosis pathways.

The cyclopropyl hepoxilin analog PBT-3 causes K562 cells to undergo apoptosis in vitro *(35)*. This was demonstrated through a combination of cell changes, including inhibition of ^{3}H-methyl thymidine incorporation into DNA, DNA degradation (laddering) and nuclear fragmentation, increased percentage of cells in the sub-G1 phase, and Annexin V binding as a measure of early stage cell changes; all of these are hallmarks of apoptosis *(35)*. Biochemical signal transduction pathways affected by PBT-3 include increased release of cytochrome c from the mitochondria and associated caspase-3 degradation. These results compare favorably with positive controls involving Gleevec, with the exception that the latter drug was slightly more active in vitro. Interestingly, the effects of PBT-3 were independent of its thromboxane antagonist actions in vitro, because control studies with thromboxane agonists and antagonists were largely ineffective in causing similar apoptotic effects observed with PBT-3 *(35)*.

To define whether PBT-3 was effective in blocking tumor growth in vivo, we adopted a nude mouse model (NU/NU) in which K562 cells were subcutaneously transplanted, resulting in the appearance of solid tumors. These tumors could be observed and measured during the course of the study *(47)*. The animals showed the appearance of solid tumors within a few days following K562 cell transplantation, and the tumors continued to grow during the next 2 to 4 wk. When the tumors reached a certain size (80–100 mm^3), the animals were separated into groups and the groups were treated with the vehicle PBT-3 and Gleevec (as positive control). Preliminary results indicated that PBT-3 was as effective as Gleevec in controlling the growth of the tumors during an 8-d treatment regimen. The size of the tumors from the PBT-3-treated animals did not vary much from the first day of treatment (i.e., 100–200 mm^3) compared to the control animals (vehicle-treated and drug free), which showed a more than 10-fold increase (>2500 mm^3) in tumor volume. Tumors excised at the end of the study from the PBT-3- and Gleevec-treated animals showed DNA laddering and apoptosis, whereas the vehicle control animals did not *(35a)*.

These studies demonstrate a positive antitumor effect of PBT-3 independent of its thromboxane receptor antagonist action, placing it on favorable ground with Gleevec as a potential therapeutic in cancer. Additionally, because PBT-3 is a potent thromboxane receptor antagonist, its dual actions of pro-apoptosis and antithrombosis effects may place PBT-3 in a more favorable position as an anticancer drug because enhanced thrombosis is a feature of many cancers. My colleagues and I believe that the PBT structure has good potential for the development of novel therapeutics in the management and treatment of cancer.

Acknowledgments

Supported by grants from the CIHR and OCRN.

References

1. Pace-Asciak C. and Wolfe L. S. (1971). A novel prostaglandin derivative formed from arachidonic acid by rat stomach homogenates. *Biochemistry* **10,** 3657–3664.

2. Anggard E. and Samuelsson B. (1964). Biosynthesis of prostaglandins from arachidonic acid in guinea pig lung. *J. Biol. Chem*. **240,** 3518–3521.
3. Bergstrom S., Danielsson H., Klenberg D., and Samuelsson B. (1964). The enzymatic conversion of essential fatty acids into prostaglandins. *J. Biol. Chem*. **239,** PC4006–PC4008.
4. Green K. and Samuelsson B. (1971). Quantitative studies on the synthesis in vivo of prostaglandins in the rat. *Eur. J. Biochem*. **22,** 391–395.
5. Samuelsson B. (1964). Identification of a smooth muscle-stimulating factor in bovine brain. *Biochim. Biophys. Acta*. **84,** 218–219.
6. Samuelsson B. (1965). On the incorporation of oxygen in the conversion of 8,11,14-eicosatrienoic acid to prostaglandin E1. *J. Am. Chem. Soc*. **87,** 3011–3012.
7. Hamberg M., Svensson J., Wakabayashi T., and Samuelsson B. (1974). Isolation and structure of 2 prostaglandin endoperoxides that cause platelet aggregation. *Proc. Natl. Acad. Sci. USA* **71,** 345–349.
8. Nugteren D. -H. and Hazelhof E. (1973). Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochim. Biophys. Acta*. **326,** 448–461.
9. Pace-Asciak C. (1976). Isolation, structure and biosynthesis of 6-keto prostaglandin $F_{1\alpha}$ in the rat stomach. *J. Am. Chem. Soc*. **98,** 2348–2349.
10. Gryglewski R. J., Bunting S., Moncada S., Flower R. J., and Vane J. R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins* **12,** 685–713.
11. Pace-Asciak C. R., Mizuno K., and Yamamoto S. (1982). The enzymatic conversion of arachidonic acid into 8,11,12-trihydroxyeicosatrienoic acid. *Biochim. Biophys. Acta*. **712,** 142–145.
12. Pace-Asciak C. R., Granström E., and Samuelsson B. (1983). Arachidonic acid epoxides: isolation and structure of 2 hydroxy epoxide intermediates in the formation of 8,11,12-trihydroxy eicosatrienoic acid and 10,11,12-trihydroxy eicosatrienoic acid. *J. Biol. Chem*. **258,** 6835–6840.
13. Pace-Asciak C. R. and Martin J. M. (1984). Hepoxilin, a new family of insulin secretagogues formed by intact rat pancreatic islets. *Prostaglandins Leukot. Med*. **16,** 173–180.
14. Pace-Asciak C. R. (1984). Arachidonic acid epoxides. Demonstration through oxygen-18 labeled oxygen gas studies of an intramolecular transfer of the terminal hydroxyl group of 12S-hydroperoxy-eicosa-5,8,10,14-tetraenoic acid to form hydroxy epoxides. *J. Biol. Chem*. **259,** 8332–8337.
15. Reynaud D., Demin P., and Pace-Asciak C. R. (1994). Hepoxilin A_3 formation in the rat pineal gland selectively utilizes 12(S)-HPETE but not 12(R)-HPETE. *J. Biol. Chem*. **269,** 23,976–23,980.
16. Nigam S., Shankarnarayanan P., Ciccoli R., et al. (2004). The rat leukocyte-type 12-lipoxygenase exhibits an intrinsic hepoxilin A_3 synthase activity. *J. Biol. Chem*. **279,** 29,023–29,030.
17. Dho S., Grinstein S., Corey E. J., Su W. G., and Pace-Asciak C. R. (1990). Hepoxilin A_3 induces changes in cytosolic calcium, intracellular pH and membrane potential in human neutrophils. *Biochem. J*. **266,** 63–68.
18. Amer R. K., Pace-Asciak C. R., and Mills L. R. (2003). A lipoxygenase product, hepoxilin A_3, enhances nerve growth factor-dependent neurite regeneration post-axotomy in rat superior cervical ganglion neurons in vitro. *Neuroscience* **116,** 935–946.
19. Pace-Asciak C. R., Martin J. M., Corey E. J., and Su W. -G. (1985). Endogenous release of hepoxilin A_3 from isolated perifused pancreatic islets of Langerhans. *Biochem. Biophys. Res. Commun*. **128,** 942–946.
20. Laneuville O., Reynaud D., Grinstein S., Nigam S., and Pace-Asciak C. R. (1993). Hepoxilin A_3 inhibits the rise in free intracellular calcium evoked by formyl-methionyl-leucyl-phenylalanine, platelet-activating factor and leukotriene B_4. *Biochem. J*. **295,** 393–397.
21. Mrsny R. J., Gewirtz A. T., Siccardi D., et al. (2004). Identification of hepoxilin A_3 in inflammatory events: a required role in neutrophil migration across intestinal epithelia. *Proc. Natl. Acad. Sci. USA* **101,** 7421–7426.
22. Pace-Asciak C. R., Klein J., and Spielberg S. P. (1986). Epoxide hydratase assay in human platelets using hepoxilin A_3 as a lipid substrate. *Biochim. Biophys. Acta*. **875,** 406–409.
23. Laneuville O., Chang M., Channa R. C., Corey E. J., and Pace-Asciak C. R. (1990). Isozyme specificity in the conversion of hepoxilin A_3 (HxA_3) into a glutathionyl hepoxilin (HxA_3-C) by the Y_{b2} subunit of rat liver glutathione *S*-transferase. *J. Biol. Chem*. **265,** 21,415–21,418.
24. Laneuville O., Corey E. J., Couture R., and Pace-Asciak C. R. (1991). Hepoxilin A_3 (HxA_3) is formed by the rat aorta and is metabolized into HxA_3-C, a glutathione conjugate. *Biochim. Biophys. Acta*. **1084,** 60–68.

25. Margalit A., Sofer Y., Grossman S., Reynaud D., Pace-Asciak C. R., and Livne A. (1993). Hepoxilin A_3 is the endogenous lipid mediator opposing hypotonic swelling of intact human platelets. *Proc. Natl. Acad. Sci. USA* **90,** 2589–2592.
26. Antón R., Puig L., Esgleyes T., De Moragas J. M., and Vila L. (1998). Occurrence of hepoxilins and trioxilins in psoriatic lesions. *J. Invest. Dermatol*. **110,** 303–310.
27. Antón R., Camacho M., Puig L., and Vila L. (2002). Hepoxilin B_3 and its enzymatically formed derivative trioxilin B_3 are incorporated into phospholipids in psoriatic lesions. *J. Invest. Dermatol*. **118,** 139–146.
28. Piomelli D., Shapiro E., Zipkin R., Schwartz J. H., and Feinmark S. J. (1989). Formation and action of 8-hydroxy-11,12-epoxy-5,9,14-eicosatrienoic acid in aplysia: a possible second messenger in neurons. *Proc. Natl. Acad. Sci. USA* **86,** 1721–1725.
29. Piomelli D. and Greengard P. (1990). Lipoxygenase metabolites of arachidonic acid in neuronal transmembrane signalling. *Trends Pharmacol. Sci.* **11,** 367–373.
30. Demin P. M. and Pace-Asciak C. R. (1993). Synthesis of racemic 11,12-cyclopropyl analogs of hepoxilins A_3 and B_3. *Tetrahedron Lett*. **34,** 4305–4308.
31. Jankov R. P., Luo X., Demin P., et al. (2002). Hepoxilin analogs inhibit bleomycin-induced pulmonary fibrosis in the mouse. *J. Pharmacol. Exp. Ther*. **301,** 435–440.
32. Pace-Asciak C. R., Reynaud D., Demin P., Aslam R., and Sun A. (2002). A new family of thromboxane receptor antagonists with secondary thromboxane synthase inhibition. *J. Pharmacol. Exp. Ther*. **301,** 618–624.
33. Reynaud D., Clark D., Qiao N., Rand M. L., and Pace-Asciak C. R. (2003). The hepoxilin stable analogue, PBT-3, inhibits primary, platelet-related hemostasis in whole blood measured in vitro with the PFA-100. *Thromb. Res*. **112,** 245–248.
34. Qiao N., Reynaud D., Demin P., Halushka P. V., and Pace-Asciak C. R. (2003). The thromboxane receptor antagonist PBT-3, a hepoxilin stable analog, selectively antagonizes the TPα isoform in transfected COS-7 cells. *J. Pharmacol. Exper. Therap*. **307,** 1142–1147.
35. Qiao N., Lam J., Reynaud D., Abdelhaleem M., and Pace-Asciak C. R. (2003). The hepoxilin analog PBT-3 induces apoptosis in *BCR-ABL*-positive K562 leukemia cells. *Anticancer Res*. **23,** 3617–3622.
35a. Li X., Qiao N., Reynaud D., Adelhaleem M., and Pace-Asciak C. R. (2005). The hepoxilin analog, PBT-3, inhibits growth of K-562 CML solid tumours in vivo in nude mice. *In Vivo* **19,** 185–189.
36. Goldbloom J. and Melo J. V. (2003). Chronic myeloid leukemia—advances in biology and new approaches to treatment. *N. Engl. J. Med*. **349,** 1451–1464.
37. Clarkson B., Strife A., Wisniewski D., Lambek C., and Liu C. (2003). Chronic myelogenous leukemia as a paradigm of early cancer and possible curative strategies. *Leukemia* **17,** 1211–1262.
38. Capdeville R., Buchdunger E., Zimmermann J., and Matter A. (2002). Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat. Rev. Drug Discov*. **1,** 493–502.
39. Green D. R. (2000). Apoptotic pathways: paper wraps stone blunts scissors. *Cell* **102,** 1–4.
40. Dragovich T., Rudin C. M., and Thompson C. B. (1998). Signal transduction pathways that regulate cell survival and cell death. *Oncogene* **17,** 3207–3213.
41. Costantini P., Jacotot E., Decaudin D., and Kroemer G. (2000). Mitochondrion as a novel target of anticancer chemotherapy. *J. Natl. Cancer. Inst*. **92,** 1042–1053.
42. Baliga B. C. and Kumar S. (2002). Role of Bcl-2 family of proteins in malignancy. *Hematol. Oncol*. **20,** 63–74.
43. Lozzio C. B. and Lozzio B. B. (1975). Human chorionic myelogenous leukemia cell line with positive philadelphia chromosome. *Blood* **45,** 321–334.
44. Lozzio B. B. and Lozzio C. B. (1977). Properties of the K562 cell line derived from a patient with chronic myeloid leukemia. *Int. J. Cancer* **19,** 136.
45. Martin S. J., Lennon S. V., Bonham A. M., and Cotter T. G. (1990). Induction of apoptosis (programmed cell death) in human leukemic HL-60 cells by inhibition of RNA or protein synthesis. *J. Immunol*. **145,** 1859–1867.
46. Martin S. J. and Cotter T. G. (1991). Ultraviolet B irradiation of human leukemia HL-60 cells in vitro induces apoptosis. *Int. J. Radiat. Biol*. **59,** 1001–1006.
47. Le Coutre P., Mologni L., Cleris L., et al. (1999). In vivo eradication of human *BCR/ABL*-positive leukemia cells with an ABL kinase inhibitor. *J. Natl. Cancer Inst*. **91,** 163–168.