Oxford Biomedical Research
SUPERIOR SCIENCE. RELIABLE RESULTS.™

Cyclooxygenase-catalyzed bioactive prostaglandin $F_{2\alpha}$: An unique biomarker of luteolysis and inflammation

Samar Basu, Ph.D.
Clinical Nutrition and Metabolism,
Department of Public Health and Caring Sciences
Faculty of Medicine
Uppsala University
Uppsala, Sweden

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

P.O. Box 522 • Oxford, MI 48371 • tel: 800.692.4633 • fax: 248.852.4466 • hppt://www.oxfordbiomed.com

Introduction

Prostaglandin F_2 ($PGF_{2\alpha}$), a major stable unique cyclooxygenase-catalysed primary prostaglandin that regulates several major physiological functions such as ovarian function and endometrial cyclic changes, embryo development, tubal function, maintenance of pregnancy and parturition (1-4) and also involved in regulation of complex pathophysiological processes, such as acute and chronic inflammation (5-9). Since the discovery of a second isoform of prostaglandin endoperoxide H synthase (PGHS) or cyclooxygenases, it evidenced that primary $PGF_{2\alpha}$ can be formed from arachidonic acid through both isoforms of cyclooxygenases, namely cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The purpose of this short illustration is to elaborate the unique role of bioactive and stable $PGF_{2\alpha}$ in inflammation with its earlier well-defined characteristics as a luteolysin that control major reproductive functions in mammalians, and further establish 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of $PGF_{2\alpha}$ as a novel biomarker of acute and chronic low-grade inflammation.

Biosynthesis of $PGF_{2\alpha}$

Although 15-keto-dihydro-$PGF_{2\alpha}$ is further efficiently degraded by one or two steps of β-oxidation of the carboxyl side chain to yield dinor (C_{18}) or tetranor (C_{16}) metabolites, we have recently shown that a large amount of 15-keto-dihydro-$PGF_{2\alpha}$ is found in both normal basal condition (12-13), during a certain physiological state (1,2,14,15), pathophysiological situations (5-7,16) and also following a dietary or drug supplementation (13,17-20). This allowed us to study the role of $PGF_{2\alpha}$ in clinical situation by collecting a single urine sample instead of collecting frequent plasma samples during a certain period of time that is often being difficult in large clinical or experimental studies due to ethical and practical reasons.

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

Prostaglandin $F_{2\alpha}$ is found nearly all tissues including lung, liver, kidney, heart, vascular tissues, muscle, fat, brain, stomach, reproductive organs and eye tissues. When reached to the systemic circulation PGF2a bioconverts to the major metabolite 15-keto-dihydro-$PGF_{2\alpha}$. This metabolite has been found in measurable quantities in most of the biological fluid analysed, including plasma, urine, synovial fluid, bronchoalveolar fluid, bile, lymph, amniotic, pericardial and seminal fluid and microdialysis fluid from various organs. However, the basal level of this metabolite in plasma and urine varies widely between species, and also between individuals, indicating an ongoing enzymatic lipid peroxidation of varying extent in normal conditions that is essential to keep homeostasis of the body (21).

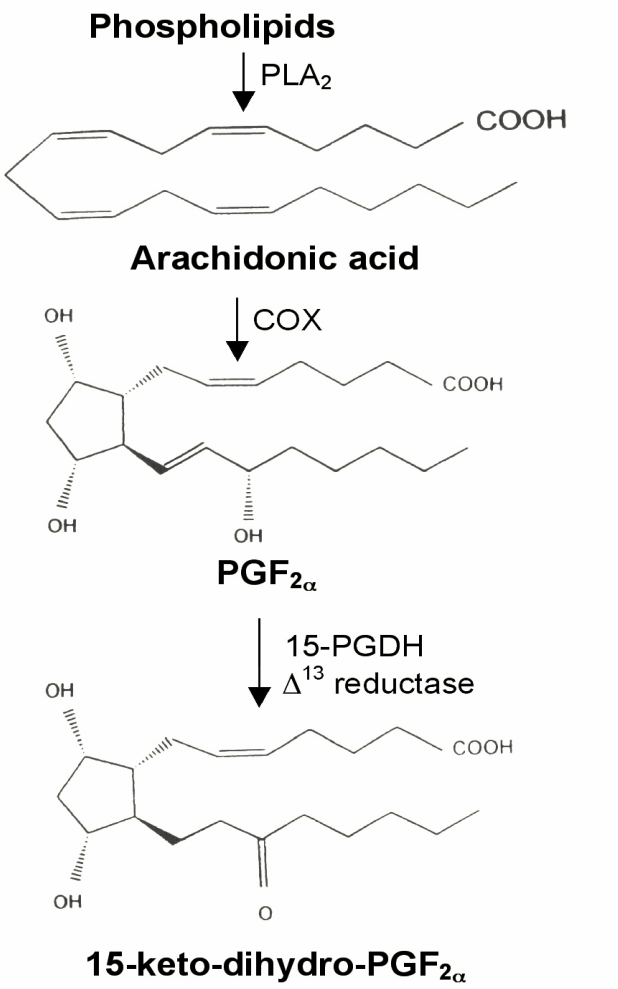

Fig. 1. Cyclooxygenase-catalysed oxidation of arachidonic acid to PGF_{2a} and its further degradation to the major metabolite 15-keto-dihydro-PGF_{2a}. PLA_2 = phospholipase A_2, COX = cyclooxygenases, 15-PGDH = 15-hydroxy prostaglandin dehydrogenase.

Quantification of $PGF_{2\alpha}$

Since $PGF_{2\alpha}$ is found in extremely low amounts in biological fluids it is necessary to apply very sensitive and accurate assay methods. However, identifying 15-keto-dihydro-$PGF_{2\alpha}$ as a major metabolite which is found about 20-30 times higher concentrations in plasma than its parent primary $PGF_{2\alpha}$ and does not form as an artifact through platelets this parameter has

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

long been served as a golden biomarker of $PGF_{2\alpha}$ formation and release into the circulation specially in reproductive endocrinology. Immunological methods such as radioimmunoassay and enzyme immunoassay have shown to be powerful methods to quantify $PGF_{2\alpha}$ formation for decades (1,22,23). Measurement of 15-keto-dihydro-$PGF_{2\alpha}$ in plasma or urine reflects the endogenous formation of $PGF_{2\alpha}$ during basal condition or a certain physiological process that induces extensive $PGF_{2\alpha}$ biosynthesis and release into the circulation (such as luteolysis, pregnancy, parturition or induced abortion; 1,2,14), during acute or chronic inflammation (5-9,16), after drug or dietary supplementation and intravenous infusion of primary $PGF_{2\alpha}$ or their analogues (13,17-20,24).

In experimental studies, frequent plasma and/or continuous urine samples are preferable for observing rapid changes of $PGF_{2\alpha}$ by measuring its metabolite over a certain period of time in the same individual, which are very bioinformative both when studying physiological process (such as luteolysis, pregnancy and parturition) or pathophysiological changes (acute inflammation or oxidative injury), and also when evaluating effect of a certain drug or test compound of interest. In cross-sectional or longitudinal studies on chronic inflammation urinary samples collected during 24-hours or in the mornings are more suitable, to get an integrated picture of the $PGF_{2\alpha}$ formation over a certain period of time. Blood samples should be collected with anti-coagulants such as heparin, citrate or EDTA to prevent any eventual artifactual formation of $PGF_{2\alpha}$ by the platelets. Urine samples should be collected without any addition of other exogenous compounds.

Pathophysiological Roles of $PGF_{2\alpha}$

$PGF_{2\alpha}$ is intimately involved in many aspects of human or animal reproduction, such as ovulation, oocyte maturation, implantation, development of embryo, maintenance of pregnancy, cervix dilatation and labor. $PGF_{2\alpha}$ is also associated with various pathophysiological reproductive conditions i.e. dysmennorhoea, mennorhoea and

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

endometriosis etc. A luteolytic substance or luteolysin is defined as a compound acting upon the ovary and induces regression of the corpus luteum that controls endometrial cyclicity; luteolysis or menstruation (3,25). In the domestic animals, $PGF_{2\alpha}$ is the only unique luteolysin that is known (3,4,25). No such clear-cut utero-ovarian relationship seems to exist in the primates including human although menstrual fluid contains more $PGF_{2\alpha}$ than PGE_2 specially in the secretory phase of menstrual cycle. In early pregnant primates or human, involvement of $PGF_{2\alpha}$ in corpus luteum function is not still fully uncovered.

Prostaglandins are well known mediator of cardinal signs of inflammation; namely erythema, fever, pain, and swelling (26,27). Administration of $PGF_{2\alpha}$ leads to acute inflammation, and nonsteroidal anti-inflammatory drugs such as aspirin, mefenamic acid, indomethacin etc. and COX-2 inhibitor (Vioxx^R) inhibit $PGF_{2\alpha}$ biosynthesis both *in vitro* (28,29) and *in vivo* confirms the role of $PGF_{2\alpha}$ as a potent inflammatory compound (19,27-31). In addition, there are many early reports evidenced that $PGF_{2\alpha}$ is increased in various inflammatory status. We have studied the role of $PGF_{2\alpha}$ in inflammation by several experimental models of inflammation and clinical conditions such as rheumatic diseases and several risk factors of cardiovascular diseases etc (21).

In a series of studies from our laboratory showed that both plasma and urinary metabolites of $PGF_{2\alpha}$ is increased dramatically in a well-established porcine model of septic shock following intravenous administration of *LPS/E. Coli,* and there was an increase of arterial $PaCO_2$ (5,6,9). Further, in an experimental porcine model of cardiopulmonary resuscitation, inflammatory response was assessed by the measurement of 15-keto-dihydro-$PGF_{2\alpha}$ in plasma samples collected frequently from the systemic circulation and jugular bulb, the organ that mainly drains the brain (7). 15-Keto-dihydro-$PGF_{2\alpha}$ is increased rapidly in systemic circulation and locally in the brain as measured in jugular bulb plasma after cardiac arrest and resuscitation (7,16,32,33). However, the local cerebral production was much higher than the systemically measured levels of 15-Keto-dihydro-$PGF_{2\alpha}$, which suggest that COX-mediated product $PGF_{2\alpha}$ is crucially involved in stroke and brain damage.

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

High levels of vasoconstrictive $PGF_{2\alpha}$ metabolite had seen locally in the knee joints and systemically among the patients suffering from rheumatoid arthritis, psoritic arthritis, reactive arthritis and osteoarthritis (8). Urinary $PGF_{2\alpha}$ metabolite and plasma interleukin-6 (IL-6) were increased in subjects with metabolically well-controlled type 1 diabetes patients compared to a matched control population. Further, urinary levels of $PGF_{2\alpha}$ correlated with the degree of glycemic control, HbA_{1c} (Hemoglobin A_{1c}, 34). A large cohort study with elderly type 2 diabetes male patients showed a significantly elevated levels of urinary $PGF_{2\alpha}$ metabolite compared to the control subjects irrespective of disease duration (35). In addition, in adolescents (13-17 years) $PGF_{2\alpha}$ metabolite levels were significantly correlated with BMI, waist circumference and fasting insulin (36). Among the boys, $PGF_{2\alpha}$ metabolite levels were correlated with waist and insulin levels. Children in the highest quartile of BMI and waist circumference had the highest levels $PGF_{2\alpha}$ metabolite. In consistent, $PGF_{2\alpha}$ metabolite levels were significantly elevated in current smokers than non-smokers. Former smokers had also increased levels of $PGF_{2\alpha}$, IL-6 than did non-smokers (37). It was also shown that both cyclooxygenase-mediated $PGF_{2\alpha}$ and C-reactive protein (CRP) independently associated with common carotid artery intima-media thickness, suggesting an involvement of $PGF_{2\alpha}$ in atherogenesis (38).

Together, it could be assumed that vasoactive $PGF_{2\alpha}$ is involved in acute and chronic inflammation and equally related to low-grade inflammation and several traditional risk factors for atherosclerosis.

Summary

measurement of 15-keto-dihydro-$PGF_{2\alpha}$ metabolite by validated immunoassays, as a parameter of biosynthesis and release of $PGF_{2\alpha}$ *in vivo* is a novel approach not only to evaluate the physiological changes in reproduction, such as luteolysis, pregnancy, labor and parturition but also it could be used as an unique biomarker of acute and chronic

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

inflammation. It is a valuable tool to explore the role of inflammatory response in many human diseases, surgical situations and drug evaluation studies with cyclooxygenase inhibitors and other compounds of interest.

References

1. Basu, S., H. Kindahl, D. Harvey and K.J. Betteridge. Metabolites of PGF_{2a} in blood plasma and urine as parameters of PGF2a release in cattle. Acta vet. scand., 1987, 28, 409-420.
2. Basu, S., A. Albihn and H. Kindahl. Studies on endogenous inhibitors of prostaglandin biosynthesis in the bovine endometrial tissue collected by biopsy. J. Vet. Med. A., 1988, 35, 141-151.
3. Poyser, N.L. The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prost. Leuk. Ess. Fatty Acids, 1995, 53, 147-195.
4. McCracken, J.A., Custer E.E. and Lamsa J. Luteolysis: A neuroendokrine-mediated event. Physiol. Rev., 1999, 79, 263-323.
5. Basu, S. and M. Eriksson. Oxidative injury and survival during endotoxemia. FEBS Letters, 1998, 438, 159-160.
6. Basu, S. and M. Eriksson. Lipid peroxidation induced by an early inflammatory response in endotoxemia. Acta Anaesth. Scand., 2000, 44, 17-23.
7. Basu, S., A. Nozari, X.L. Liu, S. Rubertsson, and L. Wiklund. Development of a novel biomarker of free radical damage in reperfusion injury after cardiac arrest. FEBS Letters, 2000a, 470,1-6.
8. Basu, S., M. Whiteman, D. Mattey and B. Halliwell. Elevated levels of F_2-isoprostanes and prostaglandin F2a in different rheumatic diseases. Annals Rheumatic Dis., 2001, 60, 627-631.
9. Mutschler, D., M. Eriksson, G. Wikström, L. Lind, A. Larsson, A. Lagrange, R. Kiiski, A. Norgren and S. Basu. Early myocardial COX-mediated inflammation measured by microdialysis as a cause of circulatory depression. Crit. Care. Med., 2003, 31, 1780-1785.

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

10. Narumiya, S., Sugimoto, Y. and Ushikubi, F. Prostanoid receptors: structures, properties and functions. Physiological Rev., 1999, 79, 1193-1226.

11. Hansen, H.S. 15-Hydroxy prostaglandin dehydrogenase. Prostaglandins 1976, 12, 647-679.

12. Helmersson, J. and S. Basu. F_2-Isoprostane and prostaglandin PGF2a metabolite excretion rate and day to day variation in healthy humans. Prost. Leuk. & Ess. Fatty Acids, 2001, 65, 99-102.

13. Basu, S., A. Smedman and B. Vessby. Conjugated linoleic acid induces lipid peroxidation in humans. FEBS Letters, 2000b, 469, 33-36.

14. Basu, S. and H. Kindahl. Development of a continuous blood collection technique and a detailed study of prostaglandin F2a release during luteolysis and early pregnancy in the heifer. J. Vet. Med. A., 34, 1987, 487-500.

15. Ishihara, O., M. Hayashi, H. Osawa, K. Kobayashi, S. Takeda, B. Vessby and S. Basu. Isoprostanes, prostaglandins and antioxidants in preclampsia, normal pregnancy and non-pregnancy. Free Radical Research, 2004, 38, 913-918.

16. Basu, S., Lui. X.L., A. Nozari, S. Rubertsson, A. Miclescu and L. Wiklund. Evidence for time-dependent maximum increase of eicosanoid formation in the brain as related to duration of cardiac arrest and CPR. Free Radical Research, 2003, 37, 251-256.

17. Risérus, U., S. Basu, S. Jovinge, J. Ärnlöv and B. Vessby. Dietary supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein-A potential link to fatty acids-induced insulin resistance. Circulation 2002, 106,1925-1929.

18. Risérus, U., B. Vessby., J. Ärnlöv and S. Basu. Effects of *cis* 9*trans*11 conjugated linoleic acid supplementation on insulin sensitivity, lipid peroxidation and proinflammatory markers in obese men. American J. Clin. Nutr. 2004, 80, 279-283.

19. Smedman, A., B. Vessby and S. Basu. Regulation of conjugated linoleic acid induced eicosanoid biosynthesis and release in humans by vitamin E and COX-2 inhibitors. Clin. Sci. (Lond.), 2004, 106, 67-73.

20. Nälsen, C., B. Vessby, L. Berglund, M. Uusitupa, K. Hermansen, G. Riccardi, A. Rivellese, L. Storlien, A. Erkkilä, S. Y.-Herttuala, L. Tapsell and S. Basu. (N-3) fatty acids reduce plasma F2-isoprostanes but not prostaglandin F2a formation in healthy humans. J. Nutr. (In press).

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

21. Basu, S. Novel cyclooxygenase-catalysed bioactive and luteolytic prostaglandin F2a with a new dogma in inflammation. 2006 (submitted).

22. Granström, E., Kumlin, M. and Kindahl, M. Radioimmunoassay of eicosanoids. In Prostaglandins and related substances. A practical approach. Eds. Benedetto, McDonald-Gibson, Nigam and Slater, IRL press, UK, 1987, 167-195.

23. Basu, S. Radioimmunoassay of 15-keto-13,14-dihydro-prostaglandin F2a. An index for inflammation via cyclooxygenase catalysed lipid peroxidation. Prost. Leuk. Ess. Fatty Acids, 1998, 58,347-352.

24. Goff, A.K., S. Basu and H. Kindahl. Measurement of 11-ketotetranor PGF metabolites: An approach for monitoring prostaglandin F2a release in the mare. Theriogenology, 1984, 21, 887-896.

25. Basu, S. Maternal Recognition of Pregnancy. Nord. Vet.-Med. 37, 57-79, 1985.

26. Vane JR. Prostaglandins as mediators of inflammation. Adv Prostaglandin Thromboxane Res 1976, 2, 791-801.

27. Takayama, K. et al., Thromboxane A_2 and prostaglandin F2a mediate inflammatory tachcardia. Nature Medicine, 2005, 11, 562-566.

28. Basu, S. Endogenous inhibition of arachidonic acid metabolism in the endometrium of the sheep. Pros. Leuk. & Ess. Fatty Acids, 1989, 35, 147-152.

29. Odensvik, K., N. Cort, S. Basu and H. Kindahl. Effect of flunixin meglumine on prostaglandin F2a synthesis and metabolism in the pig. J. Vet. Pharmacol. Therap., 1989, 12, 307-311.

30. Vane, J.R. Inhibition of prostaglandins synthesis as a mechanism of action for aspirin-like drugs. Nature 1971, 231, 232-235.

31. Helmersson, J., B. Vessby, A. Larsson and S. Basu. Cyclooxygenase-mediated prostaglandin F2a is decreased in an elderly population treated with low-dose aspirin. Prost. Leuk. Ess. Fatty Acids, 2005a, 72, 227-233.

32. Liu, X.L., L. Wiklund, A. Nozari and S. Basu. Differences in cerebral reperfusion and oxidative injury after cardiac arrest in pigs. Acta Anaesthesiol. Scand., 2003, 47, 958-967.

33. Wiklund L., H.S. Sharma and S. Basu. Circulatory arrest as a model for studies of global ischemic injury and reperfusion. Ann. NY Acad. Sci., 2005, 1053, 205-219.

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.

34. Basu, S., A. Larsson, J. Vessby, B. Vessby and C. Berne. Type 1 diabetes is associated with increased cyclooxygenase- and cytokine-mediated inflammation. Diabetes Care 2005a, 28, 1371-1375.

35. Helmersson, J., B.Vessby, A. Larsson and S. Basu. Association of type 2 diabetes with cyclooxygenase-mediated inflammation and oxidative stress in an elderly population. Circulation, 2004, 109, 1729-1734.

36. Basu, S., Steffen, L.M., Vessby, B., Steinberger, J., Moran A., Jacobs, D.R., Hong, C.-R. and Sinaiko, A. Obesity in 15-year-old adolescent is relates to oxidative stress and inflammation. Diabetologia, 2005b, Supplement 48, Abs. 366.

37. Helmersson, J., A. Larsson, B. Vessby, S. Basu. Active smoking and a history of smoking are associated with enhanced prostaglandin F2a, interleukin-6 and F2-isoprostane in elderly men. Atheroscelerosis 2005b, 181, 201-207.

38. Wohlin M., J. Helmersson, J. Sundström, J. Ärnlöv, B. Vessby, A. Larsson, B. Andrén, L. Lind and S. Basu. Both cyclooxygenase- and cytokine-mediated inflammation are associated with carotid intima-media thickness. Cytokine. 2007, 38:130-6.

© 2007 Oxford Biomedical Research, Inc. All Rights Reserved.