

PROSTAGLANDIN SYNTHESIS IN HUMAN LYMPHATICS FROM PRECURSOR FATTY ACIDS

A. Oguogho, A. A. Aghajanian, H. Sinzinger

Wilhelm Auerswald Atherosclerosis Research Group (ASF) Vienna and Department of Nuclear Medicine, University of Vienna, Austria

ABSTRACT

(Iso)-eicosanoids appear to play a pivotal role in lymphatic contractility. Because prostaglandin (PG)I₂, an arachidonic acid (20:4) metabolite, is a key substance generated by human lymphatics, both from exogenous and endogenous substrates, it is reasonable to assume that altered nutritional intake of precursor fatty acids (FA) influences formation of respective eicosanoids qualitatively and quantitatively, and thereby modify its biological effects on human lymphatics. We, therefore, examined the effect of 2 other FA-precursors, dihomo- γ -linolenic (20:3) and eicosapentaenoic acid (20:5) on the formation of the respective PG-metabolites in human lymphatics removed from the legs in patients undergoing amputation after traumatic injury. 20:3 and 20:5 were poorer substrates to form PGs. Because these PGs exert different biological actions and their synthesis may be altered by vascular environmental risk factors such as cigarette smoking, diabetes mellitus, hyperlipidemia, and availability of FA precursors and therefore nutrition, PGs may profoundly modulate the lymphatic contractile response under a variety of circumstances. The full effect of all the formed compounds of the 1- and 3-series PGs on lymph vessel contractility, however, still needs to be tested.

Arachidonic acid (20:4; AA) is the main precursor fatty acid (FA) for prostaglandin (PG) synthesis in humans. These precursor

FA are usually converted to the respective PGs by cleaving off 2 double bonds. Changing the dietary intake of FA-composition influences precursor FA-availability. Dihomo- γ -linolenic acid (20:3) and eicosapentaenoic acid (20:5) are known to alter the spectrum of PG-synthesis after a modified nutritional ingestion of plants and fish, respectively (Fig. 1). Prostacyclin (PGI₂) is the main compound formed by human lymphatics from both exogenous (1) and endogenous (2-4) substrates. Alterations in synthetic profile of these PGs have been defined in arteries but there is no data on this subject in human lymphatics. Accordingly, we examined the conversion to PGs from its major precursors in human lymphatics.

MATERIALS AND METHODS

Ten samples of human lymphatics were taken from 4 men and 6 women (age 24-56 years). None had a known risk factor for atherosclerosis such as a history of smoking, diabetes, hyperlipidemia, and they were not taking medication for at least 2 weeks prior to study. Lymphatic segments were taken from the legs after lower limb amputation [below (n=4) and above (n=6) the knee] for treatment of traumatic injury and after visualization of the lymphatics by instillation of patent blue into the amputated stump under 50-fold magnification. The study was performed in accordance with the Declaration of Helsinki related to experiments on human subjects.

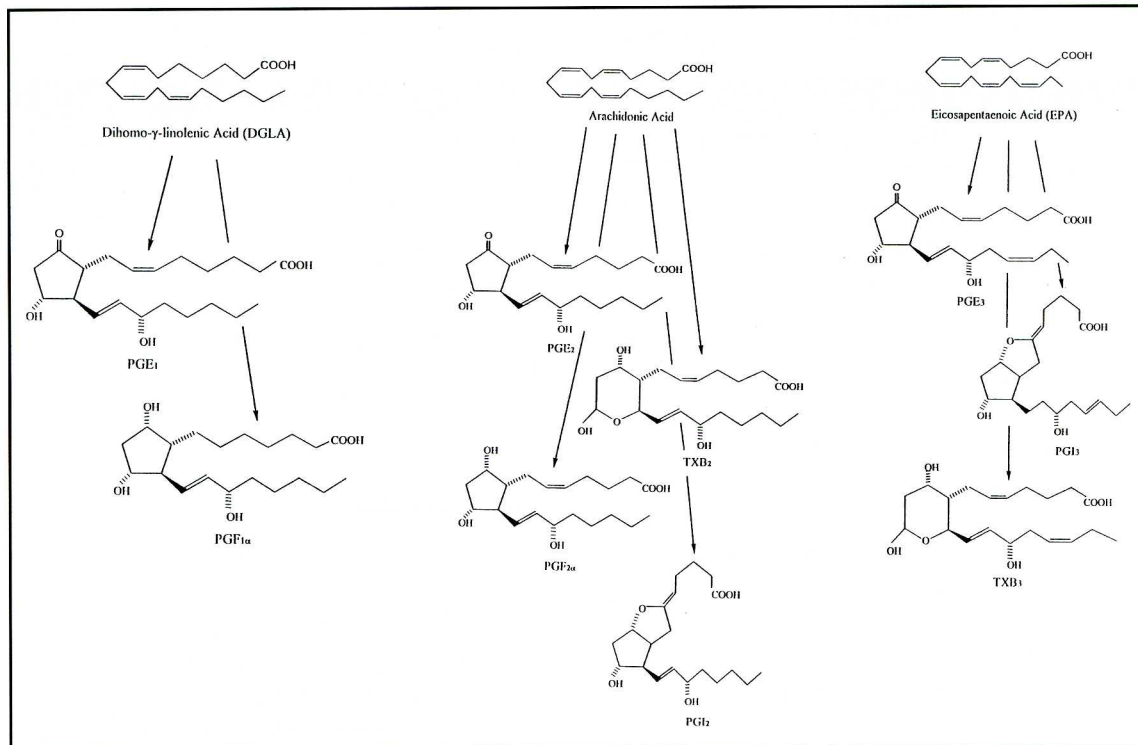


Fig. 1. Pathway showing the conversion products found in human lymphatics after incubation with radiolabeled precursors (^{14}C -DGLA, ^{14}C -AA, and ^{14}C -EPA).

Radiothinlayer-chromatography (RTLC)

Lymphatic vessel samples were freed from surrounding connective tissue, washed in ice-cold tris-HCl-buffer (0.05 M, pH 7.4), and minced and incubated in 1 ml tris-HCl-buffer containing 0.5 μCi ^{14}C -arachidonic acid (AA), ^{14}C -dihomo- γ -linolenic acid (DGLA) or ^{14}C -eicosapentaenoic acid (EPA) (Amersham, Buckinghamshire, UK), in a shaking water bath at 37°C. The reaction was arrested by adding 1M HCl, thereby reaching a pH of 3. After removal of the tissue sample, extraction was done using 2 ml of ethyl acetate. The ethyl acetate fraction was dried under nitrogen, dissolved in 100 μl ethanol (90%) and stored at -20°C. The samples were sputtered to silical-gel plates (Merck, Darmstadt, Germany) and dissolved twice in the following solvent system using the organic fraction: 110 ml ethyl acetate, 50 ml

isooctane, 20 ml acetic acid glacial and 100 ml H_2O . Final detection was performed using a radioactivity scanner (TLC Linear Analyzer B283; Berthold, Wildbad, Germany). Various PGs were identified using the respective synthetic radiolabeled standards (New England Nuclear, Boston, MA, USA).

Statistical Analysis

Values are given as mean \pm SD; calculation for significance was done using Student's t-test and ANOVA. A p value of <0.01 was considered significant.

RESULTS

RTLC using the ^{14}C radiolabeled precursor FA showed a conversion predominantly to PGI_2 or its respective stable

TABLE
Conversion of Exogenous ¹⁴C-Precursor FA to PGs*

Family	DGLA	Compound	AA	Compound	EPA	Compound
I	—	—	3.76±0.73	PGI ₂	2.84±1.27	PGI ₃
E	0.47±0.16	PGE ₁	1.64±0.42	PGE ₂	1.06±0.74	PGE ₃
F	0.86±0.24	PGF _{1α}	0.79±0.26	PGF _{2α}	—	PGF _{3α}
TX	—	TXB ₁	0.23±0.07	TXB ₂	0.17±0.08	TXB ₃
OH-FA	4.07±1.36	OH-FA	3.84±1.46	OH-FA	4.96±2.08	OH-FA
precursor FA	94.60±1.44	DGLA	89.74±2.86	AA	91.07±2.16	EPA

*Values are given in % conversion rate ($\bar{x} \pm SD$); see Fig. 1 for abbreviations

metabolites (Table). The total conversion rate of ¹⁴C-AA (10.26%) was higher than that of ¹⁴C-EPA (8.93%). ¹⁴C-DGLA was the poorest substrate, showing a 5.40% conversion. Hydroxy FA was the lowest formed from ¹⁴C-AA and the highest formed from ¹⁴C-EPA. The amount of thromboxane (TX; derived from AA and EPA) was negligible. From ¹⁴C-DGLA no TX-conversion occurred.

DISCUSSION

Previous reports have emphasized the importance of lymphatic contractility in lymph transport (5-10). The introduction of the role of PGs on lymphatic contractility and lymph propulsion (11-13) has opened a new view of lymph dynamics exploring local mediators and their potential interactions in this phenomenon. The findings in human lymphatics now demonstrate for the first time that the synthetic PG-profile varies according to exogenous FA precursor availability, thereby opening a whole host of possible biological responses in lymphatic function. Under physiologic conditions, PGI₂ is the major PG formed by lymph vessels and the main compound in lymph fluid, being

exceeded only by isoprostanes after peroxidation injury (14,15). Because of a lack in the Δ⁵⁻⁶ double bond, a transformation of PGH₁ into PGI₁ is not possible and thus conversion from DGLA is precluded. Interestingly, no conversion to TXA₁ (TXB₁) occurred. The findings have to be interpreted cautiously, however, because the alteration is usually higher if endogenous substrate is investigated which for human studies is limited by methodological shortcomings. Perhaps, a semiquantitative immunohistochemical approach may help resolve this issue. As the respective compounds of the 1-, 2- and 3-series show with different biological actions, changes in nutritional behavior or in effect availability of fatty acids are likely to alter lymphatic contractile capability and thereby lymph propulsion. To get definite information along these lines, these different prostaglandin/arachidonic acid compounds still need to be examined and tested for their contractile and/or vasomodulating effects on isolated human lymph vessel segments. Even for human blood vessels, the metabolic effects of these various PG-compounds of the different precursors (1-3) on blood vascular modulation are largely unknown. Data for

the 1- and 3-series PGs on human lymphatics are also not known. *In vitro* data suggest that polyunsaturated FAs and, in particular, ω 3-FA is a potential source for "oxidative stress." Turpeinen (16), for example, showed that a diet high in linolenic acid induced oxidative stress (increase in isoprostane 8-epi-PGF_{2 α} and decreased nitric oxide) thereby predisposing toward long-term endothelial dysfunction. Whereas no data on nitric oxide and lymphatics are yet available, 8-epi-PGF_{2 α} as one of the F₂-isoprostanes and in this regard an important marker of *in vivo* lipid peroxidation may have notable biological importance (15). Risk factors such as diabetes mellitus, hyperlipidemia, cigarette smoking may enlarge the spectrum of prostaglandin compounds formed and specifically the ones derived from the isoprostane family.

In isolated rat lymph vessels, endothelial nitric oxide and PGs (PGE₂, TXA₂) are important modulators of lymphatic vasomotion (17). PGs released from various cells may enter lymphatics and exert auto- and paracrine effects (18). Thus, PGs (E₁, E₂, F_{2 α} , AA) when perfused through prenodal lymph vessels in the paw of anesthetized dogs generated marked lymphatic vasoconstriction suggesting that PGs released after local injury may modulate lymph flow (19). Considering the number of local enzymes capable of altering FA-chain length and saturation, there appears to be myriad factors capable of regulating lymphatic eicosanoid synthesis and the lymphatic contractile response profile.

The data agree with earlier findings (20) showing that AA is a considerably better substrate than EPA or DGLA for generating vasomodulating PGs for lymphatics. Because hydroxy FA, I- and E-series PGs seem to be the most potent in this regard, more information is needed on their receptor binding, contractile performance, and other biological properties in human lymphatics.

ACKNOWLEDGMENTS

Dr. Anthony Oguogho received a stipend

from the Department of Physiology, Faculty of Basic Medical Sciences, Edo State University, Ekpoma, Nigeria, as supported by the Austrian Academic Exchange Division (ÖAAD).

The valuable help of Eva Unger in preparing and typing the manuscript is gratefully acknowledged.

REFERENCES

1. Sinzinger, H, J Kaliman, E Mannheimer: Arachidonic acid metabolites of human lymphatics. *Lymphology* 17 (1984), 39-42.
2. Mannheimer, E, H Sinzinger, R Oppolzer, et al: Prostacyclin synthesis in human lymphatics. *Lymphology* 13 (1980), 44-46.
3. Sinzinger, H, J Kaliman, E Mannheimer: Effect of leukotrienes C4 and D4 on prostaglandin I₂-liberation from human lymphatics. *Lymphology* 19 (1986), 79-81.
4. Sinzinger, H, J Kaliman, E Mannheimer: Enhanced prostaglandin I₂-formation of human lymphatics during pulsatile perfusion. *Lymphology* 19 (1986), 153-156.
5. McHale, NG, IC Roddie: The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. *J. Physiol.* 261 (1976), 255-269.
6. Okhashi, T, Y Kawai, T Azma: The response of lymphatic smooth muscles to vasoactive substances. *Eur. J. Pharm.* 375 (1978), 183-188.
7. Fruschelli, C, R Gerli, C Alessandrini, et al: Le controle neuro umerale della contritilita dei vasi linfatici. *Arch. Ital. Anat. Embr.* 88 Suppl. (1983), 49.
8. Grunger, DN, RE Parker, EW Anillu, et al: Lymph flow transients. *Acta Physiol. Scand.* 100 (1978), 61.
9. Hall, JG, B Morris, G Wooley: Intrinsic rhythm pulsation of lymph in the unanaesthetized sheep. *J. Physiol.* 180 (1965), 336-349.
10. Hargens, AR, BW Zweifach: Contractile stimuli in collecting lymph vessels. *Am. J. Physiol.* 233 (1977), 57.
11. Johnston, MG, JB Hay, HZ Movat: Kinetics of prostaglandin production in various inflammatory lesions, measured in draining lymph. *Am. J. Pathol.* (1979), 225-238.
12. Johnston, MG, JL Gordon: Regulation of lymphatic contractility by arachidonic acid metabolites. *Nature* 293 (1981), 294-297.
13. Sinzinger, H, J Kaliman, E Mannheimer: Regulation of human lymph contractility by

- prostaglandins and thromboxane. *Lymphology* 77 (1984), 43-45.
14. Oguogho, A, J Kaliman, H Sinzinger: Levels of eicosanoids (6-oxo-PGF_{1α} and 8-epi-PGF_{2α}) in human and porcine lymphatics and lymph. *Lymphology* (in press).
 15. Sinzinger, H, A Oguogho, J Kaliman: Isoprostane 8-epi-prostaglandin F_{2α} is a potent contractor of human peripheral lymphatics. *Lymphology* 30 (1997), 155-159.
 16. Turpeinen, AM, S Basu, M Mutanen: A high linolenic acid diet increases oxidative stress in vivo and affects nitric oxide metabolism in humans. *Prostagl. Leukotr. Essent. Fatty Acids* 59 (1998), 229-233.
 17. Mizuno, R, A Koller, G Kaley: Regulation of the vasomotor activity of lymph microvessels by nitric oxide and prostaglandins. *Am. J. Physiol.* 274 (1998), 790-796.
 18. O'Neill, C: The biochemistry of prostaglandins: A primer. *Aust. and New Zealand J. Obstetrics and Gynaecol.* 34 (1994), 332-337.
 19. Dabney, JM, MJ Buehn, DE Dobbins: Perfused prenatal lymphatics are constricted by prostaglandins. *Am. J. Physiol.* 260 (1991), 1-5.
 20. Lands, WEM: The biosynthesis and metabolism of prostaglandins. *Ann. Rev. Physiol.* 41 (1979), 663-640.

Professor Helmut Sinzinger, M.D.
Wilhelm Auerswald Atherosclerosis
Research Group (ASF) Vienna,
Nadlergasse 1, A-1090 Vienna, AUSTRIA
Tel.: +43.1.4082633
Fax: +43.1.4081366
E-mail: Helmut.Sinzinger@akh-wien.ac.at