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ARACHIDONIC ACID METABOLITES OF HUMAN LYMPHATICS

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ABSTRACT:

Human lymphatics convert exogenously added ^{14}C-arachidonic acid into PGE_2, PGF_2alpha and the main metabolite 6-keto-PGF_1alpha. Thromboxane formation is undetectable either by radiothinlayer-chromatography or by radioimmunology. These data confirm and extend our earlier findings indicating an important PGI_2-synthetic mechanism in human lymphatics. Assuming that lymphatic contractility is regulated at least in part by thromboxane A_2, we propose that this derivative of arachidonic acid derives from extralymphatic sources.

Moncada and co-workers (1) originally reported that prostacyclin and thromboxane A_2 (TXA_2) are important regulators of platelet function and vascular tone. On the other hand, information concerning the role of prostaglandin activity in lymph vessels and especially human ones is limited. Using the platelet aggregation bioassay technique (2) we first detected formation of prostacyclin (PGI_2) in human lymphatics (3,4). Stimulated by the report of Johnston and Gordon (5) that TXA_2 is mainly responsible for spontaneous contraction in sheep and bovine lymph vessels, we reexamined human lymphatic prostaglandin metabolism using newer, improved methodology. Because human lymphatics, at least in certain areas and under specific conditions, are theoretically capable of forming TXA_2 (6,7), we also examined this comparatively neglected subject (8).

MATERIALS AND METHODS

We investigated 10 samples of human lymphatics from 3 males and 7 females aged 15 to 56 years. The material was stored in liquid nitrogen (−70°C) until final determination. The following techniques were used:

1. Radiothinlayer-chromatography: Tissue samples were washed in ice cold tris-buffer (0.05 M, pH 7.4) and incubated in 1 ml tris-buffer containing 0.5 μCi ^{14}C-arachidonic acid (AA) (Fa. Amersham) in a shaking water bath at 37°C. The reaction was stopped by adding 1N HC1, thus reaching a pH of 3. After removal of the lymphatic vessel, extraction was done using 2 ml of ethylacetate. The ethylacetate fraction was dried under nitrogen, dissolved in 100 μl ethanol (96%) and stored at −20°C. The samples were sputtered to silica-gel plates (Fa. Merck) and dissolved twice in the following solvent system using the organic fraction: 110 ml ethylacetate, 50 ml isooctan, 20 ml acetic acid glacial and 100 ml H_2O. Final detection was performed using a radioactivity scanner (Fa Berthold) TLC Linear Analyzer (B282). Various prostaglandins were identified using synthetic radiolabelled standards (Fa. NEN).

2. Radioimmunoassay for 6-keto-PGF_1 alpha and thromboxane B_2: Lymphatic tissue samples were incubated at 22°C for 3 minutes in 1000 μl tris-HCL buffer. Thereafter, the vessels were removed and

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the incubation fluid stored at −20°C until radioimmunological detection. Non-extracted incubation medium was mixed with ^{3}H-labelled 6-keto-PGF_1 alpha or thromboxane B_2 respectively, the specific antibody (6) was added, and the mixture incubated at 4°C for 24 hours. 500 μl charcoal was added for separation of free and bound antigen followed by centrifugation at 1500g for 20 minutes. The supernatant was added to a scintillation fluid (Pico-Fluor TM 30) and counted. Preparation of specific antibodies was performed using the carbodiimid-technique (9).

3. Bioassay: The tissue samples were incubated at 22°C for 3 minutes in tris-HC1 buffer. At the end of the incubation period 100 μl were removed and immediately added to platelet rich plasma 1 minute prior to the ADP-induced aggregation (3). Inhibition of the aggregation response was quantified by means of a synthetic standard (kindly supplied by Dr. John E. Pike, The Upjohn Company, Kalamazoo, Michigan, USA). The identity of the substance was characterized by means of physiochemical properties (10) and inhibition by specific antiserum.

Statistics: All values are shown as $\bar{x} \pm SD$; significance was tested using Student's t-test.

RESULTS

Radiothinlayer-chromatography demonstrates conversion of exogenously added ^{14}C-arachidonic acid (Table 1) to metabolites, namely 6 -keto-PGF_1alpha, PGE_2 and PGF_2alpha (Fig. 1). In all samples studied, conversion to the stable derivative PGI_2 predominated. This finding was confirmed by the bioassay results (Table 2) showing significant amounts of PGI_2 generated from the incubation buffer. Radioimmunoassay of this incubation medium revealed significant amounts of 6-keto-PGF_1alpha (Table 3), whereas in lymphatics examined for thromboxane assayed via the stable degradation product of TXA_2 or thromboxane B_2 none was detected. Methodologic testing revealed in each case the substance measured was actually PGI_2 or 6-keto-PGF_1alpha. As TXB_2-radio-immunoassay has a detection limit of 1 pg/ml, trace synthesis was not excluded.

DISCUSSION

Our findings confirm earlier preliminary data (3) that human lymph vessels generate considerable PGI_2. To our knowledge it is now demonstrated for the first time that other prostaglandin metabolites are also formed by human lymphatics. In fact, the qualitative prostaglandin synthesis profile resembles that of various human arteries (Sinzinger, H and Reiter, S. unpublished observations). The role of PGI_2 in the function of lymph vessels, however, remains unclear with further clarification limited by current methodology. A promising approach is immunohistochemical identification and quantification of a prostaglandin synthesis profile (11) in a lymphatic at rest and after changes in position and activity (12). In addition to external forces and surrounding muscle contraction (13) human lymph vessels undergo intrinsic, rhythmic contraction both in vivo and in vitro (14-17). In a previous study Johnston and Gordon demonstrated a regulatory role for thromboxane A_2 and prostaglandin endoperoxides in contraction of sheep and bovine lymph vessels (5). Our findings, however, of negative radiothinlayer-chromatography and undetectable radioimmunoassay for thromboxane more or less excludes its derivation from the lymphatic wall and suggests an origin from surrounding tissue. Moreover, a contractile role for PGI_2, observed in animal arteries from various locations (18) and human veins (19) likely holds true for human lymphatics as well. Better insight into prostaglandin activity and that of its metabolites in diseased lymphatics is needed.

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Table 1

^{14}C-Arachidonic Acid (AA) Conversion By Human Lymph Vessels*

SUBSTANCE	6-KETO-PGF$_{1\alpha}$	PGE_2	$PGF_{2\alpha}$	AA
Conversion rate (%)	3.2 ± 0.8**	1.4 ± 0.5	0.7 ± 0.4	94.8 ± 3.5

*N = 10
**$\bar{x}$ ± SD

Table 2 ($\bar{x}$ ± SD)

PGI_2-Formation by Human Lymph Vessels

	N	PGI_2 (pg/mg/min)
♂	3	4.86 ± 2.71
♀	7	4.61 ± 2.65

Table 3

TXA_2 and PGI_2 Formation by Human Lymph Vessels*

	N	6-KETO-PGF$_{1\alpha}$	TXB_2
♂	3	9.61 ± 3.74	not detectable**
♀	7	8.97 ± 3.61	not detectable

*$\bar{x}$ ± SD (pg/ml/min)
** < 0.05 pg/ml/min

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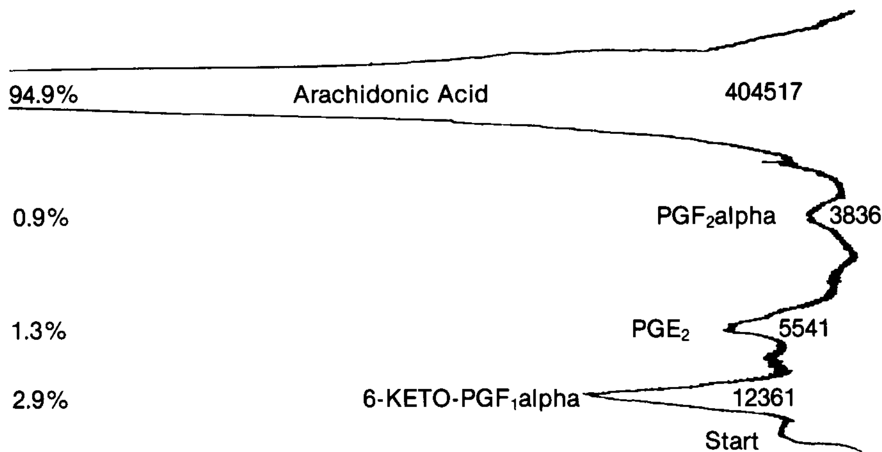

Figure 1:Radiochromatogram of human lymph vessel demonstrating the conversion (%) of arachidonic acid to various prostaglandins (prostaglandins and counts are shown).

REFERENCES

1. Moncada, S, RJ Gryglewski, S Bunting, et al: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263 (1976), 663-665.
2. Moncada, S, EA Higgs, JR Vane: Human arterial and venous tissues generate prostacyclin (prostaglandin x), a potent inhibitor of platelet aggregation. Lancet 1 (1977), 18-20.
3. Mannheimer, E, H Sinzinger, R Oppolzer, et al: Prostacyclin synthesis in human lymphatics. Lymphology 13 (1980), 44-46.
4. Mannheimer, E, H Sinzinger, R Oppolzer, et al: Prostacyclinsynthese in menschlichen Lymphgefaben. Angioarchiv 1 (1981), 19.
5. Johnston, MG, JL Gordon: Regulation of lymphatic contractility by arachidonic acid metabolites. Nature 293 (1981), 294-297.
6. Sinzinger, H, W Firbas: Gefabwandzellen synthetisieren Thromboxan. Wr. klin. Wschr. 95 (1983), 758-760.
7. Silver, MJ, AN Sedar, M Nissenbaum, et al: The influence of endothelial cells, subendothelium, and platelets in atherosclerosis. Atherogenesis 3 (1980), 76.
8. Gryglewski, RJ, S Bunting, S Moncada, et al: Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin) which they make from prostaglandin endoperoxides. Prostaglandins 12 (1976), 685-693.
9. Anhut, H, W Bernauer, BA Peskar: Radioimmunological determination of thromboxane release in cardiac anaphylaxis. Europ. J. Pharmacol. 44 (1977), 85-88.
10. Silberbauer, K, H Sinzinger, M Winter: Prostacyclin release in rat kidney is stimulated by angiotensin II. Brit. J. Exp. Pathol. 81 (1979), 41-49.
11. Ulrich, W, M Matejka, H Porteder, et al: Immunohistochemischer Nachweis von Prostaglandinen. Wr. klin. Wschr. 95 (1983), 809-811.
12. Olszewski, WL, A Engeset: Twenty-four-hour variation of leg lymph protein of different molecular weight in normal man. Acta Physiol. Scand. 100 (1978), 98-101.
13. Rusznjak, I, M Foldi, G Szabo: Lymphatics and lymph circulation. Pergamon, Oxford, 1967.
14. Hall, JG, B Morris, G Woolley: Intrinsic rhythmic pulsation of lymph in the unanaesthetized sheep. J. Physiol. 180 (1965), 336-349.
15. McHole, NG, IC Roddie: The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. J. Physiol. 261 (1976), 255-269.
16. Hargens, AR, BW Zweifach: Contractile stimuli in collecting lymph vessels. Am. J. Physiol. 233 (1977), 57-65.
17. Okhashi, T, Y Kawai, T Azmna: The response of lymphatic smooth muscles to vasoactive substances. Europ. J. Pharm. 375 (1978), 183-188.
18. Levy, SV: Contractile response to prostacyclin (PGI_2) of isolated human saphenous and rat venous tissue. Prostaglandins 16 (1978), 93-97.
19. Sinzinger, H, P Fitscha: Prostacyclin contracts normal and varicose human saphenous veins. VASA (1984) in press.

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