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ISOPROSTANE 8-EPI-PROSTAGLANDIN $F_{2\alpha}$ IS A POTENT CONTRACTOR OF HUMAN PERIPHERAL LYMPHATICS

H. Sinzinger, A. Oguogho, J. Kaliman

Department of Nuclear Medicine (HS,AO), University of Vienna, Wilhelm Auerswald-Atherosclerosis Research Group (HS), and Department of Internal Medicine, Hospital Göttlicher Heiland (JK), Vienna, Austria

ABSTRACT

Isoprostanes are products of free radical-catalyzed peroxidation and 8-epi-prostaglandin (PG) $F_{2\alpha}$ is the most important vasomodulator of this group of compounds. In human lower leg lymphatics isolated from 5 different patients without a smoking history or hyperlipidemia, 8-epi-$PGF_{2\alpha}$ stimulated in vitro contraction more strongly than the thromboxane receptor agonist U46619. Other isoprostanes (8-epi-PGE_1, 8-epi-PGE_2) had only limited lymphatic contractile potency. These data suggest a potentially relevant role for 8-epi-$PGF_{2\alpha}$ in facilitating lymph transport especially in conditions of inflammation.

Human lymphatics have been shown to convert exogenously added arachidonic acid (20:4 or 20 C atoms, 4 double bonds) into prostaglandin (PG) E_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (1), the main metabolite of PGI_2 (2). It has further been demonstrated that under certain circumstances thromboxane (TX) B_2 may be formed. Recently, isoprostanes, a group of products formed by free radical catalyzed peroxidation (3) of fatty acids, have been discovered, that can be formed independent of the enzyme cyclooxygenase (4). Isoprostane production therefore is not influenced by enzyme inhibitors, such as acetylsalicylic acid or non-steroidal anti-inflammatory drugs. 8-epi-$PGF_{2\alpha}$, the most important and commonest member of this family of compounds (5) exerts a variety of potent biological actions (4,6,7), including thromboxane-receptor mediated potent vasoconstrictor properties (6,7). We therefore examined the contractile properties of this substance in conjunction with other isoprostanes (8-epi-PGE_1 and 8-epi-PGE_2) on human lymphatics *in vitro* and compared the findings with that of TXA_2.

MATERIALS AND METHODS

We studied 5 human lymphatics derived from the lower legs from four men and one woman aged 15 to 47 years. The patients were non-smokers and normolipemic. The lymphatics were removed during amputation after trauma; specifically, the patients did not have ischemia or venous varicosities. Each patient gave written permission and the studies were carried out according to the guidelines of the Institutional Review Board for experimentation at our institution. The lymphatics were cut into small rings with a circumference of approximately 5 mm. Two wires were fixed via the lumen as described by Johnston and Gordon (8). The lower one was fixed to the bottom of a 5 ml perfusion bath while the upper one was connected with an isometric transducer (Harvard Instruments) to a graphic recorder (Pharmacia). Lymph vessels were perfused with an oxygenated

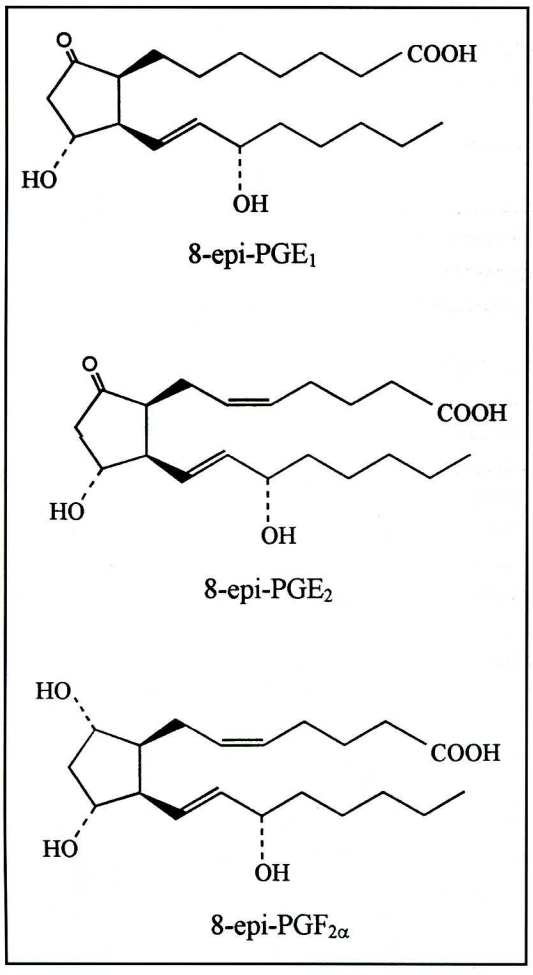

Fig. 1. Chemical formulae of 3 isoprostanes examined.

(95% O_2, 5%, CO_2) Krebs-Ringer solution and kept at a constant temperature of 37°C. The tension of the lymphatics was adjusted to 0.5g. U46619, a selective thromboxane-receptor agonist along with isoprostanes, and eicosanoids were obtained from Cayman Chemical (Ann Arbor, MI, USA). The isoprostanes were dissolved in 70% ethanol (stock solution) and stored at <20°C. The eicosanoids (see *Fig. 1* for formulae) were dissolved in Krebs solution containing the lymphatic vessel. Before starting the experiment, the lymphatic vessels were kept at constant tension for at least 1 hour to allow equilibration. The contraction at each respective dose was determined as % change vs. buffer control.

Statistical Analysis

Values are shown as mean ± standard deviation (SD). Calculation for significance was performed by means of Student's t-test and analysis of variance.

RESULTS

The prostaglandins E_1 and I_2 exerted little or no effect on lymphatic contractility (*Fig. 2*). Even at progressively higher concentrations no dose-dependent contractile response was elicited. The thromboxane receptor agonist U46619 stimulated lymphatic contraction becoming significant at 10 ng and showing thereafter a dose-dependent increase in the contractile response. $PGF_{2\alpha}$ also induced a contractile response; however, its effect was less pronounced than U46619. Among the isoprostanes, 8-epi-$PGF_{2\alpha}$ was the most potent contractile lymphatic stimulator. The other compounds examined, 8-epi-PGE_2 and to a lesser extent 8-epi-PGE_1 also induced a contractile response but significantly only at doses of 50 ng or greater.

DISCUSSION

Johnston and Gordon (9) first reported that TXA_2 and PGH_2 were important contractile stimulants for lymph vessels. Whereas *in vitro* conversion of PGH_2 to TXA_2 has been demonstrated in bovine and sheep (but not in human) lymphatics (1,10,11), the response is nonetheless negligible. During inflammation, however, high amounts of prostaglandins have been documented in draining regional lymph (9) and accordingly under these circumstances these agents become important potential regulators of lymphatic tone (7). To minimize factitious factors, peripheral lymphatics were derived from the same site from patients

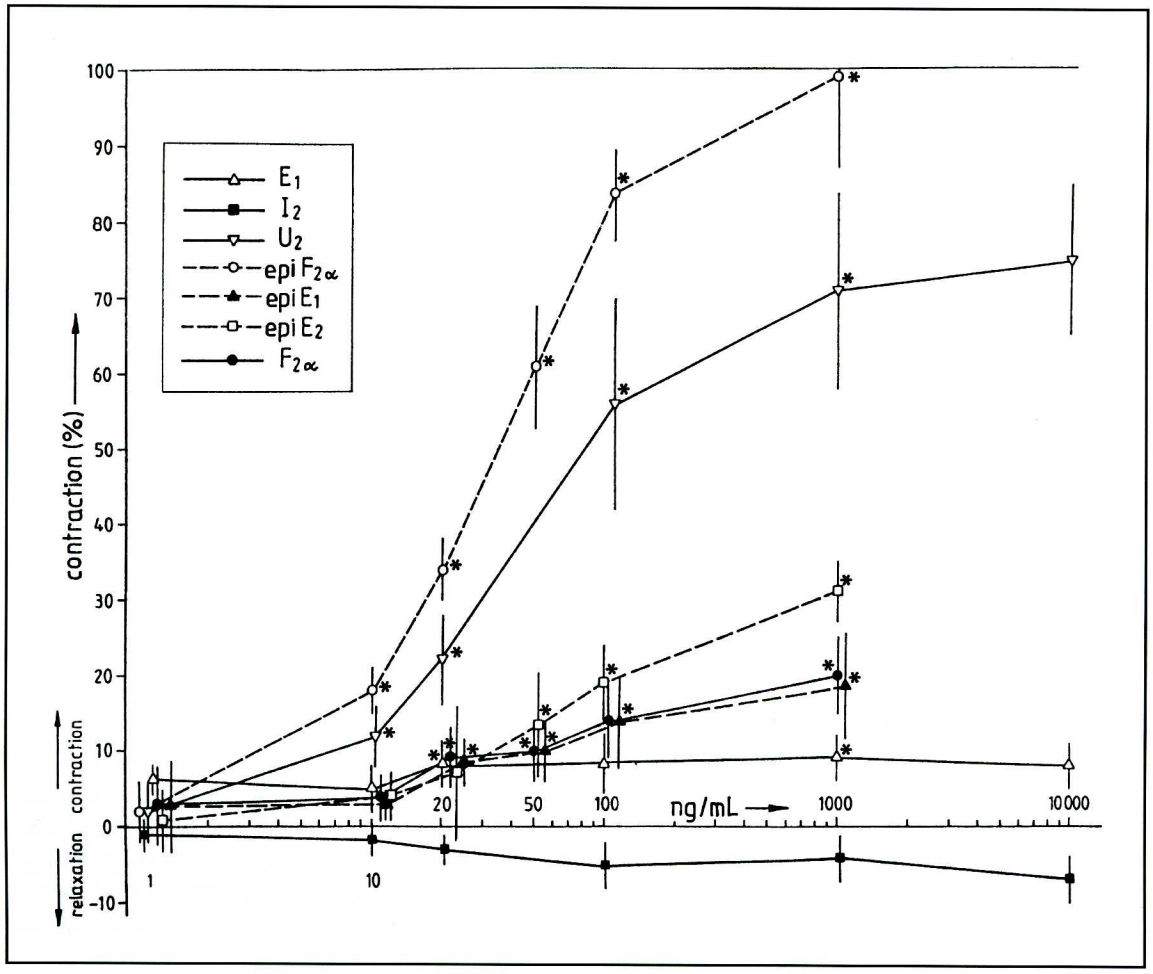

*Fig. 2. Comparison of contractile response of peripheral human lymphatics suspended in vitro to various compounds (isoprostanes, eicosanoids, thromboxane). Data presented as % change vs. buffer control. Note that 8-epi-$PGF_{2\alpha}$ is the most potent contractile agonist, whereas 8-epi-PGE_1 and 8-epi-PGE_2 are less active at each dose level. Abbreviations: E_1=PGE_1; I_2=PGI_2; U=U46619; epi-$F_{2\alpha}$=8-epi-$PGF_{2\alpha}$; epiE_1=8-epi-PGE_1; epiE_2=8-epi-PGE_2; $F_{2\alpha}$=$PGF_{2\alpha}$; *p<0.01.*

without clinical risk factors (e.g., smoking, hyperlipidemia) known to affect eicosanoid synthesis and modulate vascular responsiveness. Recently, isoprostanes a group of compounds formed both *in vitro* and *in vivo* from arachidonic acid by cyclooxygenase-independent free radical catalyzed peroxidation (5,12) have attracted considerable interest as markers of oxidative stress injury and low-density lipoprotein-oxidation (13,14). Specifically, 8-epi-PGE_2 has been unexpectedly found to be a potent renal arterial vasoconstrictor (15), rather than as anticipated a vasodilator. To a lesser extent, this vasoconstrictive response has also been shown for human lymphatics. 8-epi-$PGF_{2\alpha}$ is the most prominent compound of the isoprostane group as demonstrated *in vivo* (16). It is synthesized by human platelets (17) dose-dependently upon activation and its production is not affected by acetylsalicylic acid after various stimuli. Of interest, 8-epi-

$PGF_{2\alpha}$ is much more potent than U46619, a thromboxane receptor agonist, in stimulating renal arterial vasoconstriction (18). 8-epi-$PGF_{2\alpha}$ also exerts a powerful contractile response on human myometrium *in vitro* (19), as well as coronary (20), renal (4,7) and pulmonary (7,21) arteries. Together these data support that 8-epi-$PGF_{2\alpha}$ is a moderate contractile agonist on human lymphatics as shown in these *in vitro* experiments. Its biological action seems to be mediated predominantly via the thromboxane receptor, although more recent findings suggest that 8-epi-$PGF_{2\alpha}$ interacts with a unique receptor distinct from the thomboxane receptor (22,23). It is possible that 8-epi-PGE_2 shares the same receptor (3,24). These data further suggest that under pathological circumstances, such as inflammation and/or oxidative injury in the presence of nitric oxide and superoxide-derived peroxynitrite (9,13), 8-epi-$PGF_{2\alpha}$ may be a biologically important contractor of human lymphatics *in vivo*.

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Helmut Sinzinger, M.D., Professor
Wilhelm Auerswald Atherosclerosis Research Group (ASF) Vienna
Nadlergasse 1
A-1090 Vienna, Austria
Phone: 43-1-4082633
Fax: 43-1-4081366