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LEVELS OF EICOSANOIDS (6-OXO-PGF$_{1\alpha}$ AND 8-EPI-PGF$_{2\alpha}$) IN HUMAN AND PORCINE LYMPHATICS AND LYMPH

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ABSTRACT

Prostaglandin (PG)I_2 is the primary eicosanoid synthesized by human lymphatics and 8-epi-PGF$_{2\alpha}$, an isoprostane formed during free radical catalyzed peroxidation, is the most potent stimulator of lymphatic contraction tested thus far. We now examine the respective concentrations in the lymphatic wall of both human and porcine lymphatics and lymph fluid using specific immunoassays. Although both compounds are detectable in the lymphatic wall and lymph fluid, PGI$_2$- (via its main metabolite 6-oxo-PGF$_{1\alpha}$) is greater in the lymphatic wall whereas 8-epi-PGF$_{2\alpha}$ dominates in lymph fluid. Because inflammation is associated with oxidative injury, which in turn stimulates release of isoprostane, eicosanoid derivatives may modulate lymphatic tone during acute tissue reaction.

Prostaglandins (PG) are generated by lymphatics of various species. In human, PGI$_2$, a potent antiaggregatory and vasodilatory eicosanoid (1), is a key metabolite found in the wall of lymphatics (2). Several eicosanoids are involved in modulating lymphatic contractility (3-5). The isoprostane 8-epi-PGF$_{2\alpha}$, another eicosanoid stimulates smooth muscle contraction in various tissues (6,7) including lymphatics (8). Because isoprostanes (9) are the products of free radical catalyzed peroxidation of arachidonic acid, they accumulate during oxidative injury (10,11). Accordingly, the potential role of eicosanoids as modulators of lymphatic motility especially during inflammation is raised. As preliminary to future studies, we examined prostaglandin I$_2$-formation (via the stable metabolite 6-oxo-PGF$_{1\alpha}$) and the content of 8-epi-PGF$_{2\alpha}$ in the wall of human and porcine lymphatics and their respective lymph fluid.

MATERIALS AND METHODS

Lymphatic Vessels

Lymphatic segments were obtained from 7 patients undergoing conventional lymphography (5 men, 2 women; age range 15-47 years). They were non-smokers and were not taking drugs known to affect either oxidation or the PG system in the previous

week. The protocol was carried out in accordance with the Declaration of Helsinki. Written informed consent of each patient was obtained. Lymph vessels (n=12) were also obtained from the thigh of minipigs undergoing unrelated experimentation. Tissue samples were rinsed in ice-cold buffer (pH 7.4), weighed and homogenized by Ultraturex and extracted and purified by chromatography. To measure PGI_2-production, the lymphatics were incubated after a single wash in ice-cold (0˚C; pH 7.4) Tris-HCl-buffer for 3 minutes and in 300 µl Tris-HC1-buffer at 37˚C. After extraction and weighing (mg wet weight after drying by filter paper) of the lymphatics, the incubation buffer was frozen and stored at -70˚C until assay during the next 2 weeks.

Lymph Fluid

Lymph was obtained using microhematocrit tubes by capillary action in the presence of 1% EDTA and 10 mg acetylsalicylic acid/ml. After centrifugation (4˚C, 10 minutes, 1500xg) lymph samples were stored at -70˚C until determination.

Assays

Fig. 1 shows the chemical structure of the two eicosanoids assayed. 8-epi-$PGF_{2\alpha}$ was determined using a modified specific enzyme immunoassay (Cayman Chemicals, Ann Arbor, MI, USA) and radioimmunoassay (6-oxo-$PGF_{1\alpha}$). PGI_2-synthesis was measured as pg 6-oxo-$PGF_{1\alpha}$/mg tissue wet weight/minute and the 8-epi-$PGF_{2\alpha}$ content as pg/mg. Interassay and intraassay variation was 8.9 ± 2.3% (8-epi-$PGF_{2\alpha}$; n=11), 5.4 ± 1.6% (6-oxo-$PGF_{1\alpha}$; n=24), 5.8 ± 2.2% (8-epi-$PGF_{2\alpha}$; n=11), and 3.2 ± 1.1% (6-oxo-$PGF_{1\alpha}$; n=24), respectively. The lowest concentration for detection was 2.4 pg (8-epi-$PGF_{2\alpha}$) and 1 pg (6-oxo-$PGF_{1\alpha}$), respectively.

RESULTS

Isoprostane 8-epi-$PGF_{2\alpha}$ was detected in both human and porcine lymphatic walls and the concentrations were similar (*Table 1*). Lymphatic PGI_2-synthesis determined via its stable derivative 6-oxo-$PGF_{1\alpha}$ was also similar in human compared with the minipig (*Table 2*). Lymphatic PGI_2 synthesis via 6-oxo-$PGF_{1\alpha}$ correlated with the content of 8-epi-$PGF_{2\alpha}$ ($r=0.78$) ($p<0.01$).

Lymph fluid also contained both 6-oxo-$PGF_{1\alpha}$ and 8-epi-$PGF_{2\alpha}$ with the latter eicosanoid in higher concentration in both species (*Table 2*).

DISCUSSION

Whereas PGI_2 has an extremely short half-life (1), 8-epi-$PGF_{2\alpha}$, a major isoprostane isomer of enzymatically formed PGs, is a relatively stable compound (9), and has been used as an indicator of *in vivo* oxidative injury (12). 8-epi-$PGF_{2\alpha}$ is also a smooth muscle stimulant (7), including having a potent effect on human lymphatic contractility (6). Because 8-epi-$PGF_{2\alpha}$/6-oxo-$PGF_{1\alpha}$ ratio was high in both human and minipig lymph fluid, it is reasonable to speculate that lipid peroxidation is ongoing in lymphatics as it has been demonstrated previously for arteries. A variety of other mediators, cytokines, and growth factors are key modulators of eicosanoid production and are involved in oxidative injury. Thus, preliminary human data have shown 8-epi-$PGF_{2\alpha}$ in atherosclerotic arteries (obtained during carotid endarterectomy) to be notably higher than in normal arteries (14). Immunohistochemistry has revealed macrophage-rich subendothelial infiltration as the major site of 8-epi-$PGF_{2\alpha}$ where in normal arterial segments, this eicosanoid was

undetectable. During low-density lipoprotein oxidation, abundant amounts of isoprostanes form which in turn are influenced by PG production. Some of these agents have also been shown to alter lymphatic contractility (13) and, accordingly, lipoproteins may influence lymphatic vessel tone perhaps in inflammation indirectly via isoprostane formation.

Because cigarette smoking (1 pack per day) (11,16) and inflammation increase isoprostane production, patients who smoke or have active inflammatory processes were excluded in this study. Of late, isothromboxane (8-epi-TXB_2) has been demonstrated in blood vessels (17) and is conceivable that this compound may be present in lymphatics and lymph and may prove even more potent as a stimulator of lymphatic contraction than 8-epi-$PGF_{2\alpha}$. In fact, many drugs influence isoprostane formation (15,18), including calcium antagonists, angiotensin converting enzyme inhibitors, and some β-blockers.

The 6-oxo-$PGF_{1\alpha}$ levels found after static incubation of lymphatics correlated with concentrations on bioassay of the parent compound PGI_2 (2). Perhaps greater lymphatic wall tension and intraluminal pressure induced by 8-epi-$PGF_{2\alpha}$ enhances the formation of antagonizing PGI_2 (19). This equilibrium may be regulated by the stability of 8-epi-$PGF_{2\alpha}$ compared with the relatively unstable PGI_2 compound with its half-life determined to a major extent by local tissue pH, protein content, and other chemical factors. Thus far, no information is available on the stability of PGI_2 in lymph fluid or concentrations of these compounds under pathologic conditions. Overall, however, the data suggest that under physiologic conditions sufficient concentrations of 8-epi-$PGF_{2\alpha}$ are found in both lymphatics and lymph to influence lymph propulsion.

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