Lymphology 19 (1986) 153-156

# ENHANCED PROSTAGLANDIN $I_2$-FORMATION OF HUMAN LYMPHATICS DURING PULSATILE PERFUSION

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## ABSTRACT

*Previous studies demonstrate that prostacyclin (prostaglandin $I_2$, $PGI_2$) is the main arachidonic acid product in human lymph vessels. Pulsatile perfusion increases and prolongs $PGI_2$-formation as do leukotrienes (LT) such as $LTC_4$. Thus, physical activity besides local mechanical and biochemical influences on lymph pressure and flow also stimulates local lymphatic $PGI_2$ synthesis, a prime counterbalancing factor in lymphatic constriction induced by other eicosanoids.*

Human lymphatics like arteries (1) and veins (2) produce notable amounts of prostaglandin ($PGI_2$) (3). The *in vitro* $PGI_2$-synthesis (4), however, as well as the conversion rate (5) in the presence of exogenously added $^{14}C$-arachidonic acid (AA) does not reflect the actual amount of biologically active $PGI_2$ at the site of its action. Thus, leukotrienes (LT) $C_4$ and $D_4$ are both capable of enhancing the $PGI_2$-generation on a dose-dependent basis (6). This phenomenon, for example, may play an important role during inflammation where white blood cells provide LT's (7) in excess at the inflamed site. Enhanced eicosanoid formation induced by cellular damage (8), hypoxia (9) and mechanical irritation (10) are other factors promoting release of contractile stimulants, such as thromboxane $A_2$ (11), and LT's (12) eicosanoids that offset the vasorelaxant effect of $PGI_2$ (13). Increased intraluminal pressure as with exercise has also been suggested to stimulate $PGI_2$-formation in blood vessels (14).

Because the intraluminal pressure of *in vivo* lymphatics varies widely during physical activity we examined whether graded increments in lymphatic pressure altered formation of prostaglandin $I_2$ *in vitro*.

## MATERIALS AND METHODS

We examined four human peripheral lymphatics from three females and one male (age 15-42 years). Lymphatics were stored in liquid nitrogen (-70°C) until testing. A control segment of the lymph vessel was incubated in the perfusion system (*Fig. 1*) but without perfusion. The incubation buffer (tris-HCl, pH 7.4) was removed every ten minutes for 120 minutes and determined promptly for the presence of prostaglandin $I_2$ using the platelet aggregation bioassay (16). Briefly, 100 $\mu$l of the supernatant was added to an aggregometer one minute prior to induction of aggregation by 1 $\mu$M (100 $\mu$l) ADP. The aggregation inhibition was quantified using a synthetic $PGI_2$-standard. The characteristics of the platelet aggregation inhibitory compound were classified as being $PGI_2$ as described earlier (7). The amount of $PGI_2$ is given in pg $PGI_2$ per $cm^2$/per minute.

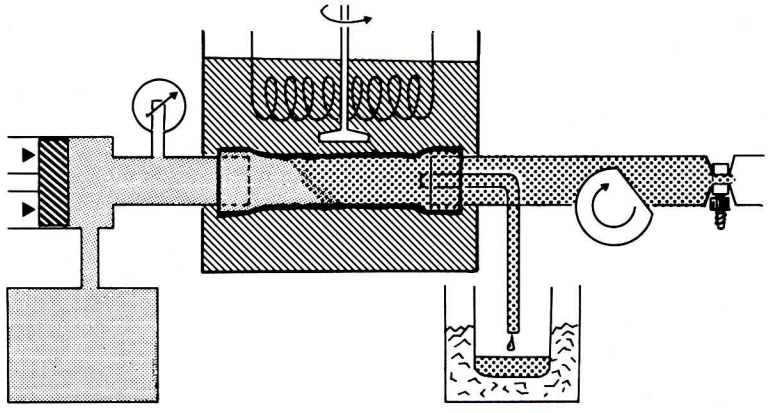

*Fig. 1: The lymph vessel is fixed in a buffer bath and perfused under various pressure conditions provided by the pump to the left and regulator to the right. The effluent is collected on ice for RIA- and bioassay-determination.*

*Perfusion experiments*

Lymph vessels were perfused under pulsatile flow at graded pressures of 30/20, 60/40 and 120/80 mmHg with 60 pulsations per minute. 0.15 ml/min solution was perfused and the effluent was collected in an ice-bath as shown in *Fig. 1.* As with control lymphatics, incubated fluid was collected serially for 120 minutes and the amount of prostacyclin in the effluent tested using the bioassay technique, and the half-life of $PGI_2$-formation was computed.

*Statistics*

The values are given in $\bar{X} \pm SD$; comparison has been done using Student's t-test for assessing statistical significance.

## RESULTS

Perfusion with pulsatile pressure *in vitro* significantly increased and prolonged prostaglandin $I_2$-formation. The increase in prostacyclin formation was dose-dependent *(Table 1);* however, the half-life of $PGI_2$-generation was unchanged (22 ± 4, 24 ± 3 and 21 ± 4 min., respectively). The addition of leukotriene $C_4$ (50 ng/ml) induced a further increase in $PGI_2$-formation, but again the half-life of $PGI_2$ was unchanged (25 ± 4 min.).

## DISCUSSION

Intralymphatic pressure varies considerably depending on physical activity, (e.g., walking, running, sitting, recumbency). Our findings demonstrate, that in an isolated human lymphatic, $PGI_2$-formation is definitely pressure dependent. Nonetheless, it should be noted, that this lymph vessel is removed from its normal physiological environment including neurohumoral regulating controls. Because prostaglandin $I_2$ relaxes precontracted lymphatics and antagonizes vasoconstriction (13), the amount of biologically active $PGI_2$ available at a local site may be important for overall modulation of lymphatic tone. Unfortunately, the active local concentrations of the contractile agents (thromboxane $A_2$, prostaglandin $G_2$, $H_2$, leukotrienes) are difficult if not impossible to assess *in vivo*. Thus, it is still speculative whether this pressure-dependent response of prostaglandin $I_2$-generation operates *in vivo*. It is also noteworthy that clinical conditions with increased lymph flow are associated with increased formation of either thromboxane or leukotrienes (e.g. endotoxemia (20) or local inflammation (6)). Thus, lymph flow with physical movement (21) is not only propelled by mechanical forces, but also by the prostaglandin system. Other environmental

**Table 1.**
**Prostaglandin $I_2$-formation ($cm_2$/min.)**
**With Increasing Pulsatile Pressure ($\bar{x} \pm SD$)**

| Min. | *Static* | *30/20 mmHg* | *60/40 mmHg* | *120/80 mmHg* | *60/40 mmHg +50ng $LTC_4$* |
|---|---|---|---|---|---|
| 10 | 3.62 ± 1.67 | 6.12 ± 1.56 | 11.26 ± 2.17 | 28.41 ± 5.21 | 27.24 ± 7.63 |
| 20 | 1.86 ± 0.61 | 3.12 ± 1.23 | 8.24 ± 1.63 | 21.54 ± 4.17 | 16.32 ± 6.56 |
| 30 | 1.08 ± 0.37 | 2.27 ± 0.64 | 5.26 ± 1.47 | 17.33 ± 3.16 | 10.54 ± 5.21 |
| 40 | 0.26 ± 0.11 | 1.13 ± 0.71 | 4.55 ± 1.16 | 11.62 ± 2.84 | 8.75 ± 3.63 |
| 50 | 0.09 ± 0.05 | 1.47 ± 0.41 | 2.75 ± 0.56 | 8.55 ± 3.06 | 8.54 ± 2.86 |
| 60 | — | 1.33 ± 0.36 | 1.82 ± 0.63 | 6.36 ± 1.86 | 8.64 ± 2.73 |
| 70 | — | 1.55 ± 0.30 | 1.85 ± 0.41 | 3.71 ± 1.24 | 4.13 ± 1.56 |
| 80 | — | 0.83 ± 0.41 | 1.77 ± 0.21 | 4.22 ± 0.86 | 5.34 ± 1.85 |
| 90 | — | 0.74 ± 0.27 | 1.93 ± 0.56 | 2.13 ± 0.71 | 4.13 ± 1.21 |
| 100 | — | 0.26 ± 0.09 | 0.84 ± 0.21 | 2.34 ± 1.12 | 2.65 ± 0.86 |
| 110 | — | 0.37 ± 0.12 | 0.66 ± 0.33 | 1.11 ± 0.26 | 2.24 ± 0.57 |
| 120 | — | 0.22 ± 0.08 | 0.21 ± 0.08 | 0.63 ± 0.31 | 1.23 ± 0.48 |

factors that regulate the biological half-life of $PGI_2$, such as protein concentration and pH, also contribute to the complexity of local lymph propulsion. On the other hand, observations that upstream obstruction of lymphatics (22) promotes a decrease in frequency of spontaneous contraction while downstream obstruction increases lymphatic contractility are still not readily explained. Although the *in vitro* findings favor a pressure-dependent increase in prostacyclin generation, the role of pulsatile flow and $PGI_2$-synthesis is still controversial (15,19). Nonetheless, greater lymphatic tone probably increases lymphatic pulsatility and therefore accelerates forward lymph flow and in this setting local $PGI_2$-production may act as a sensitive feedback control mechanism to regulate human lymphatic motility.

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