

The Effect of Prostaglandin E₁ (PGE₁) on the Plasma-Lymph Barrier of the Hind Limb of Rabbits and its Antagonization by Aescin and Indomethacin*

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Summary

Infusions into the femoral artery of 12.5 to 50 ng/kg/min PGE₁ for 15 min increased the lymph flow by between 13% and 81% for up to 45 min after the infusion, as measured at the plasma-lymph barrier of the hind limbs of rabbits. The lymph flow normalized after 105 min. The ED₅₀ for PGE₁ 15 min after infusion was 19 ng/kg/min. PGE₁ did not influence the permeation of macromolecules (PVP, proteins) from plasma to lymph. With a dose of 50 ng/kg/min severe side-effects appeared. The dose of 25 ng/kg/min did not produce any haemodynamic effects.

Intravenous injections of 0.25 to 0.8 mg/kg of aescin or 2.5 to 20 mg/kg of indomethacin reduced the increased lymph flow, obtained by injecting 25 ng/kg/min of PGE₁, by 45% to 92% and 13% to 68% respectively for up to 45 min after infusion of PGE₁. The dose of 20 mg/kg i.v. of indomethacin as compared with 10 mg/kg did not show an increased efficacy but resulted in severe side-effects. The ED₅₀ of aescin was 0.29 mg/kg i.v. and that of indomethacin 3.4 mg/kg i.v. 45 min after PGE₁ infusion.

According to these results both aescin and indomethacin had an antagonistic effect on the increased permeability of the plasma-lymph barrier of the hind limbs of rabbits induced by PGE₁.

Introduction

The anti-inflammatory effect of the horse chestnut saponin aescin has been demonstrated by its lymphopaenic effect on bradykinin-induced enhanced lymph flow using the plasma-lymph barrier of the hind limbs of rabbits as a model (1, 2).

E-type prostaglandins (PGs) induce inflammatory reactions by increasing the permeability of vascular walls (3-9).

These experiments were designed to examine whether exogenous PGE₁ increases the rate of lymph flow across the plasma-lymph barrier of the hind limbs of rabbits and whether aescin or indomethacin influence this model of pathologically increased vascular permeability.

Materials and Methods

Male rabbits weighing from 2.7 to 3.1 kg were used and divided into 5 experimental groups: PGE₁, n = 36; aescin + PGE₁, n = 27; indomethacin + PGE₁, n = 30; solvent controls, n = 10; aescin controls, n = 9. Strain: Grausilber Bastarde, source: Buchner (Kienberg), food: Altromin K® (Altromin, Lage/Lippe), maintenance: sniff bedding, dust-free (ssniff Versuchstierdiäten GmbH, Soest).

The trachea, the carotid artery, a side vessel of the femoral artery, which supplies the skin, and one of the lymph vessels close to the femoral artery were cannulated. Both hind legs were mechanically exercised 8 times/min, since lymph flow in immobilized animals is very poor. The operation table was heated. Lymph was collected for 30 min periods (lymph collection period = LCP), starting 90 min before (baseline value = LCP 1) and 15 (LCP 2), 45 (LCP 3), 75 (LCP 4) and 105 min (LCP 5) after PGE₁ infusion.

The animals were fasted for 20 h before commencing the experiments. They were anesthetized with pentobarbitone sodium salt using an induction dose of 40 mg/kg/2 ml into the ear vein. All animals were pretreated with 500 mg/kg/10 ml polyvinylpyrrolidone (PVP, K 15, MW 10000, Roth, Karlsruhe) injected into the

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ear vein. 10 min later this was followed by a continuous infusion of 4 mg/kg/0.5 ml PVP using the same route until the experiment was completed. All animals received an infusion of 0.9% NaCl solution into the side vessel of the femoral artery for 90 min. The infusion volume was 0.1 ml/min and was the same for all subsequent infusions. The solvent control group only received the NaCl infusion. The aescin control group received a single intravenous injection of 0.8 mg/kg aescin (sodium aescinate, dissolved in 0.9% NaCl solution, Dr. Madaus & Co., Cologne) into the ear vein 30 min after the beginning of the NaCl infusion. In the PGE₁ group the infusion of 0.9% NaCl solution was followed by an infusion for 15 min of 12.5, 19, 25, or 50 ng/kg/0.1 ml/min of PGE₁ (dissolved in anhydrous ethanol, diluted with 0.9% NaCl solution, Research Laboratories of the Upjohn Company, Kalamazoo, Michigan, USA), whereas the aescin + PGE₁ group and the indomethacin + PGE₁ group received only the PGE₁ dose of 25 ng/kg/min. In addition the aescin + PGE₁ group received a single injection of 0.25, 0.5 or 0.8 mg/kg/2 ml aescin into the ear vein 1 h prior to PGE₁ infusion and the indomethacin + PGE₁ group a single injection of 2.5, 5, 10 or 20 mg/kg/2 ml indomethacin (dissolved in sodium phosphate buffer, pH 7.2–7.4, + 25% of sodium carbonate solution (2%), Sigma Chemical Company, St. Louis, USA) into the ear vein at the same time as PGE₁. After the infusions of PGE₁ the infusion of 0.9% NaCl solution was continued for a further 105 min.

The lymph was collected in heparinized vessels and the volume determined gravimetrically. Determination of protein concentration was carried out according to the biuret method and that of PVP according to the method of *Levy and Fergus* (10). The mean lymph volumes of LCP 1 were compared to those of the LCPs 2, 3, 4 or 5. The mean values were subjected to the Student's t-test for paired samples at a $P = 0.05$ significance level. The increase in lymph flow after PGE₁, aescin (aescin + PGE₁ group) and indomethacin was compared to the solvent controls (= 100%). The increase in lymph flow (%) in the ex-

perimental animals (aescin, indomethacin) was compared to that in the PGE₁ animals (25 ng/kg/min). The increase in lymph flow in the PGE₁ group was taken as 100. The Student's t-test for dependent values was applied. Significance level was $P = 0.05$. ED₅₀ was determined by probit analysis. The calculation was based on the assumption of a $\geq 50\%$ increase of lymph flow (PGE₁ groups) or $\geq 50\%$ inhibition of lymph flow (aescin and indomethacin + PGE₁) and was valid for the time point 15 min after PGE₁ (PGE₁ groups) and 45 min after PGE₁ (aescin, indomethacin) respectively.

Results

See Figs. 1, 2 and 3. Aescin and solvent controls showed no increase in lymph flow. Fifteen min following PGE₁ (LCP 2) the doses up to 25 ng/kg/min showed dose dependent, significant increases in lymph flow rates. In comparison to the 15 min values a leveling off or a decrease in flow rates was observed 45 min after PGE₁ (LCP 3). Lymph flow rates were no longer significantly increased (19 ng/kg/min) as compared to the baseline values or were considerably decreased as compared to LCP 2 and LCP 3 75 min after PGE₁ (LCP 4). Lymph flow had normalized with doses up to 25 ng/kg/min 105 min after PGE₁ (LCP 5). The dose of 50 ng/kg/min showed a differing time response curve: the highest increase in lymph flow as compared to the other LCPs and all other doses was seen 45 min after PGE₁ (LCP 3). Even 105 min after PGE₁ (LCP 5) lymph flow had not yet normalized but was increased by 39%. On account of side-effects such as diarrhoea, muscle tremor, salivation and increased respiration the dose of 50 ng/kg/min was ignored for the calculation of ED₅₀ values.

The PVP and protein concentration values in plasma and lymph after PGE₁ have not been stated here, since they did not differ significantly from the baseline or control values at any time. With lymph-plasma ratios for PVP of 0.5 before PGE₁ to 0.54 after PGE₁ and for proteins of 0.42 before PGE₁ to 0.43 after PGE₁, no increased permeation of PVP and proteins through the plasma-lymph barrier was found.

Fig. 1 The influence of 25 ng/kg/min PGE₁ (●), 0.8 mg/kg aescin (■) and 0.1 ml/min solvent (○) on the lymph flow in the hind limb of the rabbit. Administration of drugs: PGE₁, intra-arterial infusion for the first 15 min of LCP 2; aescin, a single intra-venous injection 30 min after the beginning of the NaCl infusion. ***P < 0.001; *P < 0.05 in comparison to the baseline value = LCP 1

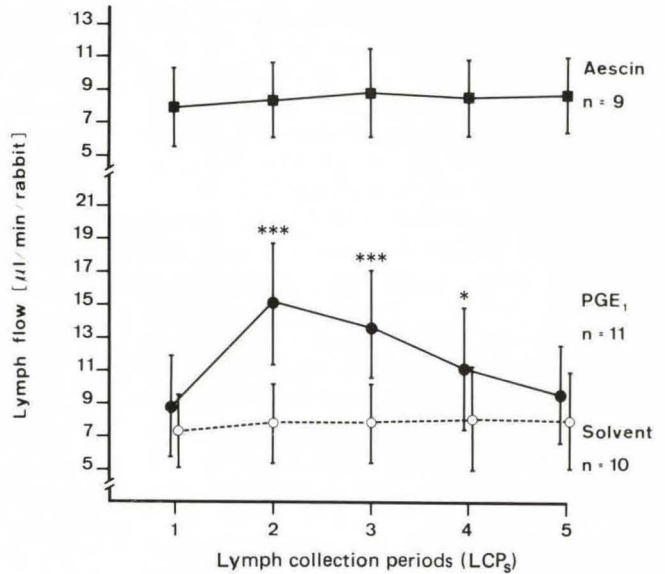
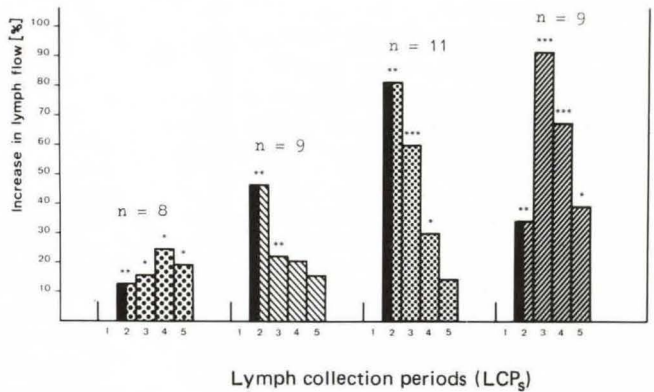


Fig. 2 The influence of various PGE₁ doses on the lymph flow in the hind limb of the rabbit. PGE₁ doses ng/kg/min: □ baseline value, ▨ 12.5, ▩ 19, ▧ 25, ▦ 50. Administration: intra-arterial injection for the first 15 minutes of LCP 2 *P < 0.05; **P < 0.01; ***P < 0.001 in comparison to control animals



Aescin decreased the lymph flow by between 45% and 91%, the dose of 0.8 mg/kg i.v. being already supramaximal (see Figs. 4 and 5). The ED₅₀ of aescin was calculated for 45 min after PGE₁. No analytical determinations of blood and lymph samples in the experimental groups were performed, since the PGE₁ group (25 ng/kg/min) had not shown any significant changes with respect to PVP and protein concentrations in plasma and lymph.

Indomethacin decreased the lymph flow by between 13% and 68%. The dose of 20 mg/kg i.v. did not result in an enhanced effect in

comparison to the other doses. On account of the gastro-intestinal side-effects, which occurred with 20 mg/kg i.v. of indomethacin, this dose was ignored for the calculation of the ED₅₀.

Discussion

Our results show that intra-arterial PGE₁ enhanced the lymph flow in the hind limbs of rabbits dose-dependently. When compared to the results of other authors (11), who used cats and higher doses of PGE₁, this effect must be regarded as very pronounced. Species differences most certainly play a role (3, 12,

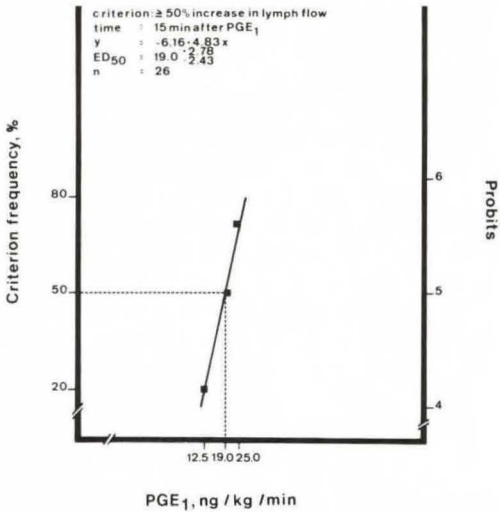


Fig. 3 ED₅₀ of arterially infused PGE₁ on male rabbits

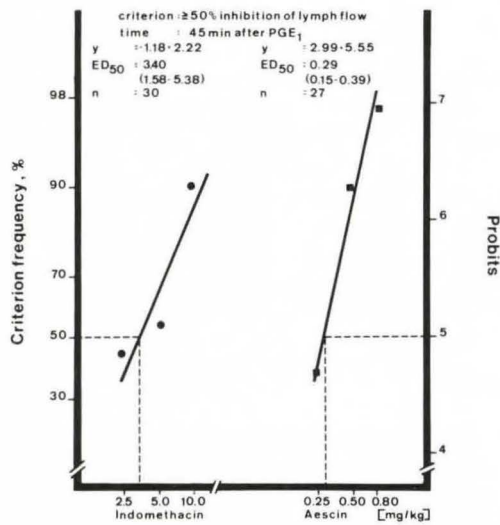


Fig. 4 ED₅₀ of indomethacin (i.v.) and aescin (i.v.) on male rabbits with increased lymph flow induced by arterial infusion (15 min) of 25 ng/kg/min PGE₁

13). The effect of exogenously administered PGE₁ was relatively short lived: 105 min after PGE₁ infusion no further enhanced lymph flow was detectable. Transient effects of E-type PGs after intra-arterial administration were also observed by other authors (9). This

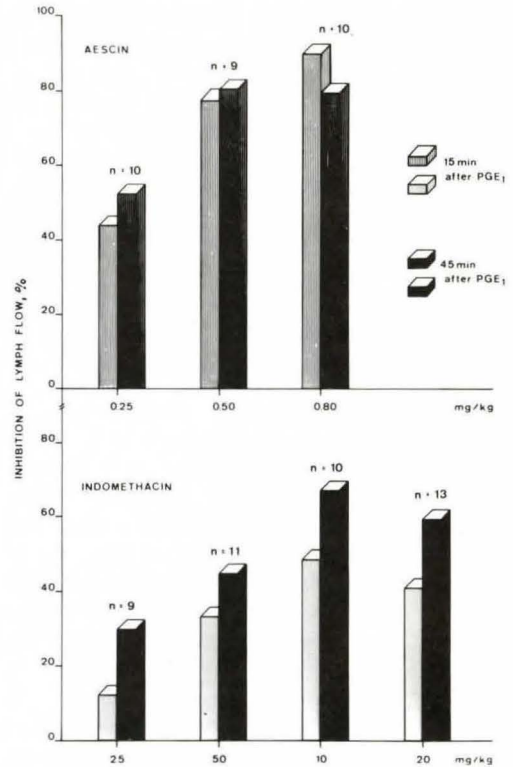


Fig. 5 The antagonistic effect of aescin and indomethacin on the PGE₁-induced (25 ng/kg/min) increase in the lymph flow in comparison to control animals and at different times

effect may be connected with the rapid metabolism of PGE₁ after a single passage through the lungs (12). To obtain a longer lasting effect of exogenous PGE₁, a single injection is not sufficient, but an infusion has to be made. In our experiments an infusion of 12.5 to 25 ng/kg/min for 15 min, which is equivalent to 188 to 375 ng/kg, did not result in any side-effects. At a dose level of 750 ng/kg PGE₁ (50 ng/kg x 15 min) severe side-effects occurred, so that only those doses which were tolerated without symptoms or side-effects have been taken for the determination of the ED₅₀.

In our experiments PGE₁ enhanced lymph flow significantly, whereas the lymph-to-plasma concentrations of PVP and protein did not alter significantly. From this it was concluded that PGE₁ had a pronounced effect on the

permeation of small molecules but not on physiological (proteins) or non-physiological (PVP) macromolecules. These results correspond to those of similar studies by *Joyner* with the lymph flow in dog extremities, in that PGE₁ did not change the selectivity of the capillary membranes but its surface area: he, too, did not find any increased permeation of macromolecules after PGE₁ treatment (16).

Based on molecular weights, exogenous PGE₁ increased the lymph flow approximately as well as bradykinin (2). Similar results were obtained by other authors who had tested the influence of PGE₁ on vessel permeability in rats (4, 5, 9). A connection between bradykinin and PG effects had been demonstrated several times (3, 9, 18, 19). The physiological effect of PGE₁ can be induced by endogenous and exogenous bradykinin (19). In our experiments aescin lowered the increased lymph flow caused by PGE₁ much more than that caused by bradykinin (2): after bradykinin the ED₅₀ for aescin was 0.7 mg/kg i.v., whilst after PGE₁ the ED₅₀ for aescin was 0.29 mg/kg i.v. Presumably aescin does not influence bradykinin itself but the PGE₁ released after exogenous administration of bradykinin.

Since 25 ng/kg x 15 min of PGE₁ had not resulted in substantial haemodynamic changes (20) in pilot studies, it could be assumed that PGE₁ affects the vessels directly. *Simpson* (6) found that after PGE₁ the inter-endothelial space was increased and explained this by contraction of the cells. The antagonizing effect of aescin on the PGE₁ effect may occur at this level. Experiments of *Hammersen* (21) show that aescin reduced to normal the diameter of the endothelial interspaces which were widened during inflammation.

There is a certain degree of correspondence between our results and those of *Longiave* (22), who attributes part of the efficacy of aescin to the local stimulation of PGF_{2α}, a physiological antagonist of PGE₁. Therefore, the findings that aescin supported the production of PGF_{2α} on the one hand and antagonized the PGE₁ effect on the other hand complement each other. Whether aescin influences endogenous PGE₁, however, cannot be answered by our experiments.

Indomethacin showed a clear lymphopaenic effect, which was smaller than that for aescin. The effect of indomethacin (and aescin) in these experiments cannot be regarded as PG synthetase inhibition, since PGE₁ (and not a PG precursor) had been applied exogenously. Apparently, apart from the PG synthetase inhibition, indomethacin may have another mode of action in inflammation, which may be a competition for the same membrane receptors. This may be the case for aescin as well. The experiments presented here show that PGE₁ may increase the small molecule permeability of the plasma-lymph barrier of the hind limbs of rabbits and that anti-exudative or antiphlogistic substances such as aescin and indomethacin are able to antagonize this PGE₁ effect.

Acknowledgements

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