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European Journal of Pharmacology 331 (1997) 259–266

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α-Trinositol prevents increased negativity of interstitial fluid pressure in rat skin and trachea induced by dextran anaphylaxis

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Received 29 April 1997; revised 23 May 1997; accepted 27 May 1997

Abstract

The new anti-inflammatory agent α-trinositol (D-myo-inositol-1,2,6-trisphosphate), is suggested to act on the cellular adhesion receptor towards extracellular matrix components, the β_1-integrins, and may therefore represent a novel principle for therapy of the phenomena associated with acute inflammation. Increased negativity of interstitial fluid pressure (p_{if}) is a major driving force for the rapid edema formation in trachea and skin associated with dextran anaphylaxis in the rat. We therefore used this experimental model to study the effect of α-trinositol in skin and trachea of pentobarbital anesthetized rats. p_{if} was measured with sharpened glass capillaries (3–7 μm) connected to a servocontrolled counterpressure system. α-Trinositol (10 mg) was given before or after dextran. Circulatory arrest was induced 2 min after i.v. dextran to limit the increased capillary fluid filtration associated with the anaphylactic reaction. This increased filtration will otherwise raise interstitial volume and thereby p_{if} and cause an underestimation of a potential increased negativity of p_{if}. In the trachea, p_{if} was 0.0 ± 1.0 mmHg (S.D.) and -1.4 ± 0.5 mmHg in controls given saline vehicle and α-trinositol ($P > 0.05$), respectively, and fell to -8.5 ± 2.7 mmHg after dextran ($P < 0.01$). α-Trinositol given 2 min prior to or after dextran resulted in p_{if} of -1.7 ± 1.2 mmHg ($P > 0.05$ versus control, $P < 0.01$ versus dextran) and -4.7 ± 3.0 mmHg ($P < 0.01$ versus control and dextran), respectively. In skin, i.v. dextran caused p_{if} to fall from -0.6 ± 0.5 to -4.6 ± 1.9 mmHg ($P < 0.001$). When α-trinositol was given prior to dextran the corresponding figures were -0.4 ± 0.8 and -0.9 ± 1.1 mmHg, respectively ($P > 0.05$). Subdermal administration of α-trinositol after i.v. dextran and circulatory arrest normalized p_{if} in concentration of 100, 10 and partly at 1 mg/ml. Thus, α-trinositol prevented the increased negativity of p_{if} induced by dextran anaphylaxis when administered prior to as well as after dextran showing that the α-trinositol also could influence an already started inflammatory reaction. © 1997 Elsevier Science B.V.

Keywords: Edema; α-Trinositol; Capillary permeability; Trachea; Skin (rat); Connective tissue; Inflammation; Anaphylaxis

1. Introduction

The novel anti-inflammatory drug α-trinositol (D-myo-inositol-1,2,6-trisphosphate) is an isomer of the intracellular messenger inositol 1,4,5-trisphosphate and is produced from phytic acid. α-Trinositol has a molecular weight of 529.9 Da and is strongly hydrophilic. Due to its low molecular weight and hydrophilicity it should have free passage across the capillary membrane. α-Trinositol will effectively reduce edema in a number of acute inflammatory reactions. Thus, pretreatment with α-trinositol reduces edema formation following injection of carrageenan in the rat foot pad (Claxson et al., 1990) and lung edema formation after smoke inhalation in the sheep (Nakazawa et al., 1993). In burn injury, capillary leakage and edema formation are reduced also when the drug is administered after the injury has been inflicted (Cassuto et al., 1990; Gunther et al., 1993; Lund and Reed, 1994). A possible insight into the molecular site of action of the drug was obtained in experiments where α-trinositol was shown to attenuate the increased negativity of interstitial fluid pressure (p_{if}) via an action on the β_1-integrins (Rodt et al., 1994). The β_1-integrins mediate cellular attachment towards the structural components of loose connective tissues (Hynes, 1992; Gullberg et al., 1990). The conclusion that α-trinositol acts on the β_1-integrins was based on

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PII S0014-2999(97)01041-8

observations that antibodies towards β_1-integrins induced an increased negativity of p_{if} which was reversed by pretreatment with α-trinositol (Rodt et al., 1994). In vitro experiments in the same study suggested that the drug did not act on the extracellular portion of the receptor, but via the intracellular apparatus eventually connecting this transmembrane receptor to the cytoskeleton (Rodt et al., 1994). Based on the ability of α-trinositol to act via the β_1-integrin system and attenuate an increased negativity of p_{if} in acute inflammation, it would seem permissible to suggest that α-trinositol represents a new principle for drug therapy.

In the present study the effect of α-trinositol has been investigated by measurement of interstitial fluid pressure (p_{if}). The role normally assigned to p_{if} is to maintain normal interstitial volume and transcapillary fluid flux (Aukland and Reed, 1993): Increased capillary filtration will raise interstitial fluid volume and thereby p_{if} which in turn will act across the capillary wall to limit further filtration. During lowered capillary filtration the changes are opposite, but will again act to limit changes in interstitial fluid volume. Contrary to the normal role assigned to p_{if}, recent studies have demonstrated that increased negativity of p_{if} is a major driving force for the rapidly forming edema in acute inflammation (Reed, 1995), including the edema formation in skin (Reed and Rodt, 1991) and trachea (Koller and Reed, 1992; Koller et al., 1993) accompanying dextran anaphylaxis in the rat. Normally 12–24 h is required to filter a volume equal to the interstitial volume across the capillary wall. When visible edema appears in 5–10 min, a volume similar to interstitial volume has been filtered in this same time period. Transcapillary filtration has therefore increased about 100 times and must originate from increased capillary water permeability and/or capillary filtration pressure. The capillary filtration coefficient ('water permeability') is commonly reported to increase 2–3 times above normal even in major insults like burn injury to the skin (Arturson and Mellander, 1964). In burn injuries, Arturson and Mellander (1964) calculated that the filtration pressure required to explain the rapidity of edema formation was in the order of 200–300 mmHg based on measurement of edema formation and the capillary filtration coefficient. More recently it was shown that p_{if} in burn injuries decreases from -1 mmHg in a control to as low as -150 mmHg and this offers an alternative explanation for the rapid edema formation in burn injuries (Lund et al., 1988). Less negative values of p_{if} have been observed in several inflammatory reactions, from a control of -1 to between -5 and -10 mmHg. The normal transcapillary net filtration pressure is 0.5 to 1 mmHg and the increased negativity of p_{if} will raise the capillary fluid filtration by 10 to 20 times above control and will therefore be of major importance to explain the rapid edema formation. Finally, the principle importance of the observations of an increased negativity of p_{if} in acute inflammatory reactions are to change the common notion of the role of loose connective tissues from being passive controllers to 'actively' participate in the inflammatory reactions and play an 'active' role in the transcapillary exchange.

Several conventional anti-inflammatory drugs have been investigated for an effect on the increased negativity of p_{if}, but with little success. These drugs include antihistamines, anti-serotonins, indomethacin, hydrocortisone in acute inflammatory reactions induced by dextran anaphylaxis, carrageenan and xylene (Rodt et al., 1990; Reed and Rodt, 1991; Rodt and Reed, 1993). Only high doses of the protease inhibitor aprotinin was able to partly attenuate the increased negativity of p_{if} accompanying dermal application of xylene (Rodt et al., 1990). The fact that α-trinositol can attenuate the increased negativity of p_{if}, strongly suggests that the drug has a new 'therapeutic target', probably on the β_1-integrin system as described above. Measurement of p_{if} in acute inflammatory reactions seems to represent an easy and reliable measurement of in vivo effects of α-trinositol. In the present study, we investigated the effect of α-trinositol further using an experimental model of anaphylaxis in the rat. In the rat dextran induces an anaphylactic reaction which is characterized by a rapidly occurring edema around the nose and the paws concomitant with increased negativity of p_{if} (Reed and Rodt, 1991; Koller and Reed, 1992). We have used this model in order to study the effect of α-trinositol on increased negativity of p_{if} when given either before or after the dextran response had been initiated.

2. Materials and methods

2.1. Animals and anesthesia

Non-fasted female Wistar-Møller rats weighing 200–250 g were used in the experiments. Anesthesia was induced with intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight). The femoral vein was catheterized with a PE-50 catheter for administration of drugs.

The procedures described in this article have been carried out with the approval of and in accordance with the recommendations laid down by the Norwegian State Commission for Laboratory Animals.

2.2. General procedures

Circulatory arrest was induced during anesthesia with intravenous injection of 0.5 ml saturated potassium chloride 2 min after administration of dextran or 2 min after administration of α-trinositol when the drug was administered after dextran. α-Trinositol (D-myo-inositol-1,2,6-trisphosphate) was obtained from Perstorp Pharma (Lund, Sweden) and used at 40 mg/kg bodyweight given intravenously, i.e., a 250 g rat received 10 mg α-trinositol.

Dextran 70 (60 mg/ml) was obtained from Pharmacia (Uppsala, Sweden).

2.3. Measurements

Measurements of interstitial fluid pressure (p_{if}) were performed with the animal in the supine position. Sharpened glass capillaries (3–7 μm) were connected to a servocontrolled counterpressure unit (Wiig et al., 1981). The pipettes were filled with 0.5 M NaCl colored with Evans blue. Punctures were done under visual guidance using a stereomicroscope (Wild M5, Leitz, Germany) and without applying stretch or compression to the trachea at the site of measurement. The micropuncture technique allows measurement of p_{if} without introducing or removing fluid from the tissue.

Measurements were accepted when the following criteria were met:

(1) Feedback gain could be altered without changing the recorded pressure.

(2) After fulfillment of criterion (1), fluid communication between pipette and the interstitium was verified by applying suction to the servocontrolled pump. Fluid movement into the pipette was visualized as increased electrical resistance in the pipette due to the lower tonicity in the fluid entering the pipette.

(3) Zero recording did not change compared to the prepuncture value after measurement had been performed.

Zero measurement was performed in a plastic cup at the level of the puncture site. Counterpressure created by the servocontrolled pump was recorded with a Hewlett-Packard pressure transducer (1280C) (Hewlett Packard, Palo Alto, CA, USA) connected to a Gould Amplifier (model 13-4615-35 Gould, Ballanviers, France) and recorder (model 8188 220106). The recordings of p_{if} were grouped in 10 min periods for 60 min after circulatory arrest had been induced.

2.4. Experimental protocol

2.4.1. Trachea

All measurements and surgical manipulations were performed postmortem to avoid inflammatory and/or circulatory changes that could create an inflammatory reaction and thereby influence capillary permeability and interstitial fluid volume. The trachea was exposed surgically as soon as possible after circulatory arrest had been induced and immediately covered with mineral oil to prevent evaporation. The surgical preparation of the trachea required 10–15 min and consisted of a skin incision followed by blunt dissection of the sternohyoideus muscle which was then held apart by applying stretch on silk sutures placed in the muscle. The micropipette was advanced from the abluminal side of the trachea and into the intercartilagenous space of the exposed trachea and towards the mucosa. Measurements were performed within 1 mm under the abluminal surface.

Experiments in the trachea were carried out in five subgroups:

(1) Control: The rats ($n = 6$) received 1 ml of isotonic saline i.v. and circulatory arrest was induced with 0.5 ml saturated potassium chloride given i.v. 2 min later as described above. The preparation of the trachea and measurement of p_{if} was performed as described above.

(2) Control with α-trinositol: The rats ($n = 6$) were given 10 mg (0.1 ml) α-trinositol instead of isotonic saline followed by 0.9 ml isotonic saline to flush the catheter, but otherwise treated as group 1.

(3) Dextran: The rats ($n = 7$) received 1 ml dextran i.v.. Circulatory arrest was induced 2 min later and the trachea was prepared free and the punctures were performed as described above.

(4) α-Trinositol followed by dextran: The rats ($n = 6$) received 10 mg α-trinositol and 0.9 ml isotonic saline to flush the catheter. 1 ml dextran was given i.v. 2 min later and 2 min thereafter circulatory arrest was induced with saturated potassium chloride. The trachea was then prepared free and the punctures were performed as described above.

(5) Dextran followed by α-trinositol: 7 rats received 1 ml dextran i.v. and 2 min thereafter 10 mg α-trinositol (0.1 ml) followed by 0.9 ml isotonic saline. Circulatory arrest was induced 2 min thereafter, the trachea prepared free and the punctures were performed as described above.

2.4.2. Skin

Punctures in the skin were made on the dorsal side of the paw. The pipettes were inserted under a stereomicroscope as described above. Punctures were made through intact skin in the dermis and pressures were recorded 0.3–0.7 mm under the skin surface.

Experiments were carried out in three subgroups:

(1) Dextran: After measurement of control p_{if} the rats ($n = 6$) received 1 ml dextran i.v. and circulatory arrest was induced 2 min later with saturated potassium chloride.

(2) α-Trinositol followed by dextran: After measurement of control p_{if}, the rats ($n = 6$) were given 10 mg α-trinositol followed by 0.9 ml to flush the catheter and 10 min thereafter 1 ml dextran was given i.v. Circulatory arrest was induced 2 min later and measurements performed as described above.

(3) Dextran followed by subdermal α-trinositol: After measurement of control p_{if}, dextran was given i.v. and circulatory arrest was induced as described above. The p_{if} was measured again during the first 10 min after circulatory arrest and thereafter 5 μl α-trinositol was injected subdermally in the paw using a 28 Gauge chromatography syringe (Hamilton, Bonaduz, Switzerland). A volume of 10 μl injected in this way will outline a circle 5 mm in diameter (Reed and Rodt, 1991). α-Trinositol was used in

Table 1
Interstitial fluid pressure in skin after intravenous (i.v.) dextran or saline followed by saturated potassium chloride 2 min later to induce circulatory arrest. Ten min thereafter α-trinositol (α-T) or 0.9% saline was injected subdermally in decreasing concentrations. Mean ± S.D

I.v.	Subdermal	Interstitial fluid pressure (mmHg)							
		Control	Dextran	Subdermal injection of α-trinositol or saline					
			0–10 min	0–10 min	11–21 min	21–30 min	31–45 min	46–60 min	61–90 min
Saline	Saline	−0.8 ± 0.3	−0.8 ± 0.5	−0.6 ± 0.6	−0.3 ± 0.4	−0.7 ± 0.3	−0.5 ± 0.6	−0.4 ± 0.2	−0.4 ± 0.2
Dextran	Saline	−0.9 ± 0.2	— [e]	−3.7 ± 0.8 [b,d]	−4.0 ± 1.5 [b,d]	−4.8 ± 1.5 [b,d]	−5.5 ± 0.6 [b,d]	−5.1 ± 1.0 [b,d]	−4.9 ± 1.4 [b,d]
Dextran	α-T, 100 mg/ml		−0.6 ± 0.4	−4.2 ± 0.7 [b,d]	−3.2 ± 0.5 [b,d]	−2.5 ± 1.2 [b,c]	−1.5 ± 0.9 [a]	−0.3 ± 0.7	−0.4 ± 0.9 −0.4 ± 0.2
Dextran [f]	α-T, 10 mg/ml		−1.1 ± 0.3	−3.7 ± 0.6 [a,d]	−3.5 ± 0.7 [a,d]	−2.2 ± 0.2 [a]	−1.7 ± 0.5 [a]	−0.8 ± 0.5	−0.5 ± 0.4 −0.4 ± 0.3
Dextran	α-T, 1 mg/ml		−0.8 ± 0.2	−3.9 ± 0.7 [b,d]	−4.1 ± 0.9 [b,d]	−3.4 ± 0.7 [b,d]	−2.6 ± 1.1 [a]	−2.4 ± 1.3 [c]	−2.3 ± 1.1 [a,d] −1.8 ± 1.2 [a]
Dextran	α-T, 0.1 mg/ml		−0.6 ± 0.3	−4.0 ± 0.8 [b,d]	−4.1 ± 0.7 [b,d]	−3.5 ± 0.8 [b,d]	−3.6 ± 0.7 [b,d]	−3.9 ± 0.8 [b,d]	−3.8 ± 0.8 [b,d] −3.6 ± 0.4 [b,d]

[a] $P < 0.05$, compared to own control.
[b] $P < 0.01$, compared to own control.
[c] $P < 0.05$, at the same time interval compared to i.v. saline follwed by subdermal saline.
[d] $P < 0.01$, at the same time interval compared to i.v. saline follwed by subdermal saline.
[e] Measurements lacking.
[f] $n = 3$ in this group; [a] in this group only means $0.05 < P < 0.10$ (t-test). All experimental p_{if} less than control.

concentrations of 100 mg/ml ($n = 6$), 10 ($n = 3$), 1 ($n = 6$) and 0.1 ($n = 6$) mg/ml. p_{if} was measured up to 90 min after induction of circulatory arrest in the time periods outlined above.

2.5. Statistical methods

The experimental data were averaged for each registration period (see above). Statistical comparisons of the different experimental groups were performed with one-way ANOVA (using repeated measures if possible) and post-hoc Bonferroni and *t*-tests. Data are given as mean ± S.D. unless otherwise specified. $P < 0.05$ was considered statistically significant.

2.6. Drugs

α-Trinositol (Na^+ salt, Na_5H–$InsP_3$) was supplied by Perstorp Pharma (Lund, Sweden) in sealed vials, each containing 1 g of freeze dried powder. α-Trinositol was reconstituted in sterile water to give a stock solution of 100 mg/ml. The reconstituted stock solution was frozen at −20°C in vials containing 0.5 ml. Each vial was used either the day it was opened or the subsequent day after storage at +4°C.

3. Results

3.1. Trachea

Interstitial fluid pressure in the trachea did not change significantly with time throughout the experimental period (Fig. 1). The p_{if} in the five groups are presented in Fig. 2. p_{if} averaged 0.0 ± 1.0 and −1.4 ± 0.5 mmHg in the control groups receiving saline and α-trinositol, respectively. In the animals given dextran p_{if} was −8.5 ± 2.8 mmHg ($P < 0.01$ compared to both control groups). In the animals given α-trinositol prior to dextran, p_{if} averaged −1.7 ± 1.2 mmHg ($P > 0.05$ to control groups). α-Trinositol given after dextran partially attenuated the increased negativity usually observed after dextran with average p_{if} of −4.7 ± 3.0 mmHg ($P < 0.01$ compared to dextran alone and control groups).

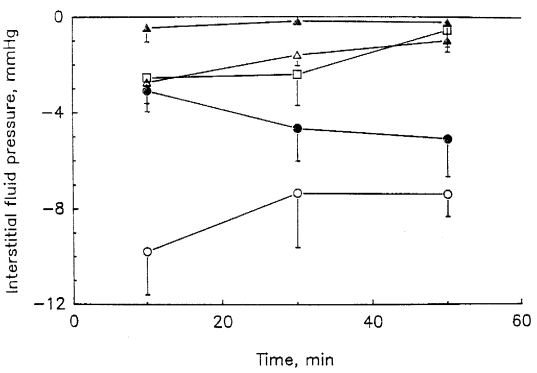

Fig. 1. Interstitial fluid pressure in trachea as a function of time in the five experimental groups. Filled triangle: control with saline; open triangle: control with α-trinositol; open circle: dextran; filled circle: dextran followed by α-trinositol; open square: α-trinositol followed by dextran. Mean ± S.E.

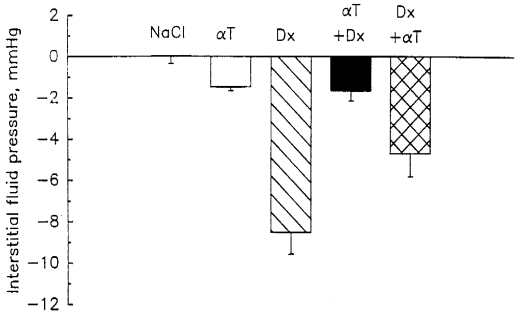

Fig. 2. Average interstitial fluid pressure in trachea as a function of time in the five experimental groups. NaCl: control with saline; αT: control with α-trinositol, Dx: dextran, Dx + αT: dextran follwed by α-trinositol, αT + Dx: α-trinositol followed by dextran. Mean ± S.D.

3.2. Skin

Intravenous dextran caused p_{if} to fall from −0.6 ± 0.5 to −4.6 ± 1.9 mmHg ($P < 0.001$). When α-trinositol was administered prior to dextran the corresponding figures were −0.4 ± 0.8 and −0.9 ± 1.1 mmHg, respectively ($P > 0.05$). When α-trinositol was administered subdermally after dextran and subsequent circulatory arrest, complete reversal of p_{if} was obtained at concentrations of 100 and 10 mg/ml (Table 1). Partial reversal was obtained at 1 mg/ml while α-trinositol at 0.1 mg/ml was without effect.

4. Discussion

The present experiments show that pretreatment with α-trinositol attenuated the increased negativity of interstitial fluid pressure (p_{if}) induced by dextran anaphylaxis in skin and trachea. In trachea, a less marked but still significant effect on p_{if} was observed also when α-trinositol was given after dextran. In skin, a dose-dependent reversal of the increased negativity of p_{if} was demonstrated when α-trinositol was injected subdermally after intravenous injection of dextran. Thus, α-trinositol is also able to reverse the effects of an ongoing inflammatory process.

Acute inflammatory reactions are commonly associated with rapid edema development. The increased capillary fluid filtration needed to generate this edema must result from increased capillary hydraulic conductivity (CFC) and/or increased capillary net filtration pressure (ΔP).

The interstitial fluid volume is controlled by transcapillary fluid flux (J_V) and lymph flow (J_L):

$$J_V = CFC^* \left(P_c - P_{if} - \sigma \left(COP_p - COP_{if} \right) \right) = CFC \Delta P \quad (1)$$

where P and COP are hydrostatic and colloid osmotic pressures, respectively, while subscripts c, if and p denote capillary, interstitial fluid and plasma, respectively. Furthermore, CFC and σ are the capillary filtration coefficient ('water permeability') and capillary reflection coefficient for plasma proteins, respectively. Finally, ΔP is the net capillary filtration pressure, i.e., the pressure imbalance across the capillary wall, which generates the capillary fluid filtration.

The magnitude of the transcapillary net filtration pressure in trachea is not known. In skin and skeletal muscle the net filtration pressure can be estimated at 0.5 to 1 mmHg and will generate a fluid filtration which is equal to interstitial fluid volume in 12–24 h (Reed and Rodt, 1991; Aukland and Reed, 1993). In dextran anaphylaxis the capillary fluid filtration is increased at least 15 times above control in trachea (Koller and Reed, 1992) and an increased negativity of p_{if} from -1 to between -5 to -10 mmHg in skin will raise the net capillary filtration pressure by 5–10 times and thereby become a major contributor to the increased capillary filtration (Koller and Reed, 1992). The capillary filtration coefficient in acute inflammatory reactions in skin will increase only 2–3 times above control values (Arturson and Mellander, 1964) and a doubling has been reported after inhalation injury to the lung (Nakazawa et al., 1993, 1994). In single perfused true and arteriolar capillaries bradykinin induced a fivefold increase in hydraulic conductivity in the first 5 min, but returned to be 3.5 times control (Williams and Huxley, 1993), while there was no change in venous capillaries. However, since the rise in hydraulic conductivity rapidly returns towards control from their peak values, the hydraulic conductivity when averaged over time was of the same magnitude as that observed by Arturson and Mellander (1964). Furthermore, the capillary reflection coefficient (σ) decreased from normal values of 0.9 to 0.6 in skin after burn injury (Pitt et al., 1987). The contribution from lowered capillary reflection coefficient to raise the net filtration pressure may be larger in skin since control σ is higher and therefore the reduction may be larger (Nordin et al., 1978). The lowering of σ will enhance net capillary filtration pressure by a factor equal to the change in the capillary reflection coefficient times the normal transcapillary colloid osmotic pressure gradient, the latter has been estimated at about 10 mmHg (Nordin et al., 1978). The capillary hydrostatic pressures in trachea will be expected to increase during inflammation, but no data seem available on the actual changes in pressure occurring in dextran anaphylaxis. Lymph flow would at least be expected to double due to the increased interstitial volume (Nakazawa et al., 1993). Taken together, the observed changes in the capillary pressures and conductivities seem insufficient to explain the rapid edema development.

The increased negativity of p_{if} observed in cutaneous burns (Lund et al., 1988, 1989) will be the major determinant to explain the rapid edema formation. The quantitative role of p_{if} in the edema formation associated with dextran anaphylaxis will be less, but would nevertheless seem to be of similar importance for the transcapillary fluid flux and edema formation as increased capillary permeability and increased capillary pressure. However, independent of the quantitative importance of p_{if}, the fact that increased negativity of p_{if} contributes to the edema formation, is principally important since it places an 'active' role on the loose connective tissue in the edema formation and transcapillary exchange associated with acute inflammation rather than increased capillary permeability.

The present experiments were performed after the induction of circulatory arrest subsequent to administration of dextran. The anaphylaxis is accompanied by increased capillary fluid flux which will raise interstitial fluid volume and thereby p_{if} towards more positive values which may lead to underestimation of an increased negativity of p_{if}. The edema formation and a potential rise in p_{if} is efficiently prevented by inducing circulatory arrest. We have previously shown that induction of circulatory arrest does not change p_{if} in skin for 90 min (Wiig et al., 1981). In trachea, previous studies have demonstrated that even gentle manipulation of trachea in living animals may induce increased capillary transport of fluid and protein resulting in edema formation (Nordin et al., 1978). The experimental protocol used in this study therefore involves induction of circulatory arrest prior to surgical manipulation of trachea. Like for skin, we have previously shown that p_{if} in trachea is around -1 mmHg up to 1 h after induction of circulatory arrest in control animals (Koller and Reed, 1992).

The present study verified our previous findings of increased negativity of interstitial fluid pressure both in skin and trachea in dextran anaphylaxis (Reed and Rodt, 1991; Koller and Reed, 1992). α-Trinositol abolished the increased negativity of p_{if} both in skin and trachea when given prior to dextran. More importantly, α-trinositol abolished the increased negativity of p_{if} in both tissues also when given after dextran. In skin, a dose-dependent effect could be demonstrated. The highest concentration used was that of the stock solution used for i.v. injection. A concentration down to 10 mg/ml completely reversed the effect of dextran, while a concentration of 0.1 mg/ml was without effect. The rats in this study were given 40 mg/kg α-trinositol which will dilute in an extracellular fluid volume of 24 ml/100 g rat (Reed and Wiig, 1984) and therefore result in a concentration of about 0.5 mg/ml, i.e., similar to the concentration where the effect of α-trinositol disappeared when the drug was given subdermally.

Pharmacological intervention to counteract increased capillary permeability and edema formation in local and

general inflammatory reactions has been attempted with several drugs. In most cases the effect is dependent on administration of the drug prior to the trauma or initiation of the inflammatory reaction. However, α-trinositol when given after experimental cutaneous burns will inhibit the increased capillary leakage of albumin also when given after infliction of the injury (Cassuto et al., 1990; Lund and Reed, 1994). The drug is also unique in several other ways. First, α-trinositol is able to interfere with the function of the β_1-integrins in connective tissue cells, i.e., the receptor by which the connective tissue cells attach to the structural components of the connective tissue (Rodt et al., 1994). Furthermore, α-trinositol is able to abolish or attenuate the increased negativity of p_{if}, occurring in the initial stages of acute inflammatory reactions and which is a major driving force for the rapid edema formation (Lund et al., 1988). In the airways α-trinositol has previously been demonstrated to attenuate an increased negativity of p_{if} occurring in neurogenic inflammation (Woie and Reed, 1994) and in experimental asthma (Woie et al., 1996) Furthermore, α-Trinositol is a vasodilator when given in high doses (Gardiner et al., 1994). It is however, unlikely that the attenuation of p_{if} demonstrated in the present study is related to a vascular effect. The best evidence for this is the effect of α-trinositol when injected subdermally.

Several conventional anti-inflammatory substances have been tested for an effect on attenuation of increased negativity of p_{if} in acute inflammations, but so far only α-trinositol has been able to attenuate the increased negativity of p_{if}. There was no effect of anti-histamine, anti-serotonins or indomethacin on the increased negativity of p_{if} in skin induced by dextran anaphylaxis (Reed and Rodt, 1991). The protease inhibitor aprotinin had some effect on the increased negativity of p_{if} induced by xylene application to the skin (Rodt et al., 1990), but was without effect in carrageenan induced increased negativity of p_{if} (Rodt and Reed, 1993).

Based on the knowledge of α-trinositol and integrin function (Rodt et al., 1994) and the observations in the present study, it is tempting to suggest that the effect of α-trinositol is to strengthen β-integrin function and/or reestablish the adhesion necessary for normal matrix function. An important aspect of the present study is that it points to the possibility of therapeutic interaction with adhesion molecules in acute inflammatory reactions in the airways, thereby pointing to novel therapeutic approaches in acute airway inflammation as well as for inflammatory reactions to the skin.

Acknowledgements

Financial support from The Norwegian Research Council, The Norwegian Heart Association, Laerdal's Foundation for Acute Medicine and Gerd and Lars Volder's Foundation is greatly appreciated.

References

Arturson, G., Mellander, S., 1964. Acute changes in capillary filtration and diffusion in experimental burn injury. Acta Physiol. Scand. 62, 457–463.

Aukland, K., Reed, R.K., 1993. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. Physiol. Rev. 73, 1–78.

Cassuto, J., Jønson, A., Nellgård, P., Hedner, T., 1990. Reduced albumin extravasation in experimental skin burn injury by D-myoinositol-1,2,6-triphosphate infusion. Proceedings American Burn Association. p. 30.

Claxson, A., Morris, C., Blake, D., Siren, M., Halliwell, B., Gustafsson, T., Løfkvist, B., Bergelin, I., 1990. The anti-inflammatory effects of D-myo-inositol-1,2,6 trisphosphate (PP56) on animal models of inflammation. Agents Actions 29, 68–70.

Gardiner, S.M., Kemp, P.A., Fallgren, B., Bennett, T., 1994. Effects of chronic infusions of α-trinositol on regional and cardiac haemodynamics in conscious rats. Br. J. Pharmacol. 113, 129–136.

Gunther, R.A., Gustafsson, T., Brofeldt, B.T., 1993. α-Trinositol (α-T) and decreased fluid balance following thermal injury (abstract). Circ. Shock (Suppl. 1), 51.

Gullberg, D., Tingström, A., Thuresson, A.-C., Olsson, L., Terracio, L., Borg, T.K., Rubin, K., 1990. β_1 Integrin mediated collagen gel contraction is stimulated by PDGF. Exp. Cell Res. 186, 264–272.

Hynes, R.O., 1992. Integrins: Versatility, modulation, and signalling in cell adhesion. Cell 69, 11–25.

Koller, M.-E., Reed, R.K., 1992. Increased negativity of interstitial fluid pressure in rat trachea in dextran anaphylaxis. J. Appl. Physiol. 72, 53–57.

Koller, M.-E., Woie, K., Reed, R.K., 1993. Increased negativity of interstitial fluid pressure in rat trachea after mast cell degranulation. J. Appl. Physiol. 74, 2135–2139.

Lund, T., Reed, R.K., 1994. Alpha-trinositol inhibits edema generation and albumin extravasation in thermally injured skin. J. Trauma 36, 761–765.

Lund, T., Wiig, H., Reed, R.K., 1988. Acute postburn edema: Role of strongly negative interstitial fluid pressure. Am. J. Physiol. 255, H1069–H1074.

Lund, T., Wiig, H., Onarheim, H., Reed, R.K., 1989. Mechanism behind increased dermal imbibition pressure in acute burn edema. Am. J. Physiol. 256, H940–H948.

Nakazawa, H., Gustafsson, T.O., Traber, L.D., Herndon, D.N., Traber, D.L., 1994. Alpha-trinositol decreases lung edema formation after smoke inhalation in an ovine model. J. Appl. Physiol. 76, 278–282.

Nakazawa, H., Traber, L.D., Traber, D.L., 1993. Trinositol blocks the increase in pulmonary microvascular permeability produced by inhalation injury (abstract). Circ. Shock. (Suppl 1), 50.

Nordin, U., Källskog, Ö., Lindholm, C.-E., Wolgast, M., 1978. Transvascular fluid exchange in the tracheal mucosa. Microvasc. Res. 15, 287–298.

Pitt, R.M., Parker, J.C., Jurkovich, G., Taylor, A.E., Currerri, P.W., 1987. Analysis of altered capillary pressure and permeability after thermal injury. J. Surg. Res. 42, 693.

Reed, R.K., 1995. Interstitial fluid pressure. In: Reed, R.K., McHale, N.G., Bert, J.L., Winlove, C.P., Laine, G.A. (Eds.), Interstitium, Connective Tissue and Lymphatics. Portland Press, London, 1995, pp. 85–100.

Reed, R.K., Rodt, S.Å., 1991. Increased negativity of interstitial fluid pressure during the onset stage of inflammatory edema in rat skin. Am. J. Physiol. 260, H1985–1991.

Reed, R.K., Wiig, H., 1984. Compliance of the interstitial space in rats. III: Contribution of skin and skeletal muscle interstitial fluid volume to changes in total extracellular fluid volume. Acta Physiol. Scand. 121, 57–63.

Rodt, S.Å., Reed, R.K., 1993. Interstitial fluid pressure in rat skin becomes more negative in the initial phase of carrageenan-induced edema. Int. J. Microcirc. Clin. Exp. 12, 299–312.

Rodt, S.Å., Reed, R.K., Ljungström, M., Gustafsson, T.O., Rubin, K., 1994. The anti-inflammatory agent α-trinositol exerts its edema preventing effect through modulation of β_1 integrin function. Circ. Res. 75, 942–948.

Rodt, S.Å., Wiig, H., Reed, R.K., 1990. Increased negativity of interstitial fluid pressure contributes to development of oedema in rat skin following application of xylene. Acta Physiol. Scand. 140, 581–586.

Wiig, H., Reed, R.K., Aukland, K., 1981. Micropuncture measurement of interstitial fluid pressure in rat skin and skeletal muscle: Comparison to wick-in-needle technique. Microvasc. Res. 21, 308–319.

Williams, D.A., Huxley, V.H., 1993. Bradykinin-induced elevations of hydraulic conductivity display spatial and temporal variations in frog capillaries. Am. J. Physiol. 264, H1575–1581.

Woie, K., Reed, R.K., 1994. Neurogenic inflammation and lowering of interstitial fluid pressure in rat trachea is inhibited by α-trinositol. Am. J. Respir. Crit. Care Med. 150, 924–928.

Woie, K., Westerberg, E., Reed, R.K., 1996. Lowering of interstitial fluid pressure will enhance edema in trachea of albumin-sensitized rats. Am. J. Respir. Crit. Care Med. 153, 1347–1352.