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Effect of α-trinositol on carrageenan-induced rat paw edema and lowering of interstitial fluid pressure

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Abstract

α-Trinositol attenuates edema formation and capillary albumin extravasation and lowering of interstitial fluid pressure (P_{if}) in several acute inflammatory reactions in rat skin or trachea. The lowering of P_{if} is an important driving force required to explain the rapid edema formation in acute inflammatory reactions. The lowering of P_{if} and edema formation are attenuated by α-trinositol, which is suggested to act on the cellular adhesion receptor for extracellular matrix components. This would represent a novel therapeutic strategy for acute inflammation. To further clarify the mechanisms behind the anti-inflammatory effects of α-trinositol, the effects of pre- and post-treatment with α-trinositol on edema formation and lowering of P_{if} were studied after subdermal injection of carrageenan in the rat. The experiments measuring P_{if} showed that the lowering of P_{if} induced by carrageenan was abolished by 10 mg α-trinositol when administered either prior to or after injection of 5 μl 1% (w/v) lambda carrageenan in the dorsum of the paw. Edema formation after injection of lambda carrageenan (100 μl, 1.5% w/v) into the foot pad was studied in a separate series. In control animals receiving saline vehicle, the volume of the paw injected with carrageenan increased by approximately 30% after 3–4 h. The only significant effect of infusion of 20 mg kg^{-1} h^{-1} α-trinositol was a reduction of edema to approximately 20% when treatment was started 1 h before carrageenan injection. Therefore, the plasma concentration of α-trinositol must already be high when carrageenan is injected in order to prevent edema in the late phase. In conclusion, the present results indicate that the mechanisms involved in the lowering of P_{if} in the early phase of edema development are different from those responsible for the manifest edema measured 3–4 h after carrageenan.

Keywords: Inflammation; α-Trinositol; Interstitial fluid pressure; Edema; Carrageenan; (Rat)

1. Introduction

Intradermal injection of carrageenan is commonly used to induce acute inflammation. Acute carrageenan inflammation induces the production or activation of gelatinases and collagenases (Nakagawa and Sakata, 1986), and also attracts inflammatory cells (Vinegar et al., 1987). It is not associated with histamine, bradykinin, platelets, clotting factors or complement activation (Vinegar et al., 1987), and therefore seems to produce an inflammatory response by a mechanism different from that of agents such as xylene or the anaphylactic reaction towards dextran.

α-Trinositol (D-*myo*-inositol-1,2,6-trisphosphate) is an isomer of the intracellular second messenger inositol-1,4,5-trisphosphate, and is produced from phytic acid. α-Trinositol displays anti-inflammatory potency in several animal models of inflammation, although the mechanism of action is incompletely known. Two commonly studied aspects of inflammation are edema formation and vascular albumin leakage. One or both of these parameters are markedly reduced by α-trinositol treatment in carrageenan-induced foot pad edema (Claxson et al., 1990), adjuvant arthritis (Claxson et al., 1990), neurogenic inflammation of the trachea (Woie and Reed, 1994), thermal injury to skin (Lund and Reed, 1994) and lung injury after smoke inhalation (Nakazawa et al., 1994). α-Trinositol appears to influence several of the processes leading to vascular leakage and edema. One factor contributing to the initial edema in several acute inflammatory reactions is a lowering of the interstitial hydrostatic pressure (P_{if}) from −1 mmHg in control to more subathmospheric pressures. The lowering of P_{if} will contribute to increased fluid flux

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and edema formation, which is part of the inflammatory reaction, since it will raise the net capillary filtration pressure (Reed et al., 1997). This lowering of P_{if} assigns an ''active'' role to the loose connective tissues in generating transcapillary fluid flux. The role normally ascribed to P_{if} is that of a ''passive controller'' of interstitial fluid volume since increased capillary fluid filtration will raise interstitial fluid volume and thereby P_{if}, which in turn will act across the capillary to reduce further fluid filtration (Aukland and Reed, 1993).

α-Trinositol has been suggested to act on the cellular adhesion receptors for extracellular matrix components, the β_1-integrins (Rodt et al., 1994). The integrins are heterodimeric adhesion receptors consisting of an α- and a β-unit where the specificity of the receptor is determined by the α- and β-units from which it is assembled (Clark and Brugge, 1995). The intracellular mechanisms of action of α-trinositol are not fully elucidated, but seems to involve an intracellular Ca^{2+} response (Åhlèn et al., 1998) and intracellular pathways probably involving phosphatidylinositol 3-kinase (Åhlèn et al., 1998), which in turn stimulate the β-integrin system both in vivo and in vitro (Rodt et al., 1994; Åhlèn et al., 1998).

In thermal injury, neurogenic inflammation, and dextran anaphylaxis the lowering of P_{if} is attenuated by α-trinositol given prior to as well as after the injury (Lund and Reed, 1994; Woie and Reed, 1994; Koller et al., 1997). It has not been investigated whether this ability also extends to carrageenan inflammation, which is perhaps the most commonly used experimental model for acute inflammation. It has been demonstrated that continuous infusion of α-trinositol, starting 1 h prior to the subcutaneous carrageenan injection, inhibits edema formation in the rat paw (Claxson et al., 1990). Specifically, the present study investigates whether α-trinositol has anti-inflammatory properties in carrageenan edema, measured as paw volume and P_{if}. Also, the time course of the treatment effects were studied in order to find out whether pre-treatment is necessary in order to achieve edema protection, or whether an already established inflammation may be attenuated by treatment.

2. Materials and methods

2.1. Animals and anesthesia

The two series of experiments were done in two separate laboratories on the strains of rats that each laboratory was normally using. Therefore, female Wistar–Møller rats were used in Series 1 and male Sprague–Dawley rats in Series 2.

2.1.1. Series 1: Interstitial fluid pressure

Female Wistar Møller rats weighing 200–250 g were used in the experiments. The animals were fed ordinary rat pellets and were kept in the animal facilities until the day of the experiment. All surgical procedures and measurements were performed under pentobarbital anesthesia (50 mg kg^{-1}). Animals were kept on a servocontrolled heating pad when anesthetized. The animals were given saturated KCl intravenously to induce circulatory arrest 5 min after i.v. injection of 10 mg α-trinositol. The experimental protocol was approved by the Norwegian State Commission for Laboratory Animals.

2.1.2. Series 2: Edema measurements

Male Sprague–Dawley rats, 200–250 g (Band and Universal), were housed under standard conditions, with free access to food (R3, Laktamin, Vadstena, Sweden) and water. The light schedule was 12-h light:12-h dark and temperature was 20–22°C. The rats were randomly assigned to experimental groups at the start of the experiments. Dose volumes of anesthetics and drugs were calculated from body weight. The animal was given 25 ml kg^{-1} water per os with a feeding tube 1 h before carrageenan injection, and was anesthetized with 50 mg kg^{-1} i.p. thiopental (Pentothal sodium, Abbott). When needed, one additional dose of Pentothal sodium was given. At the end of the experiment, the animal was killed by i.v. injection of 0.5 ml saturated potassium chloride.

2.2. General procedures

2.2.1. Series 1: Interstitial fluid pressure (P_{if})

The jugular vein was cannulated for i.v. administration of α-trinositol. Circulatory arrest was induced 5 min after i.v. injection of α-trinositol or saline vehicle (see below) in order to minimize the vascular phenomena and associated edema formation, which will raise interstitial fluid volume and thereby P_{if}, potentially causing underestimation of a lowering of P_{if}.

2.2.2. Series 2: Edema measurements

The animal was anesthetized throughout the experiment, and body temperature was kept at 37–38°C by external heating. The tail vein was cannulated for drug infusion. The right hind-paw of the rat was washed with 70% ethanol. One hour after water was given per os, 100 μl 1.5% carrageenan was injected subcutaneously into the foot pad of the paw using a glass syringe.

2.3. Measurements

2.3.1. Series 1: Interstitial fluid pressure

Interstitial fluid pressure was measured with glass capillaries (tip diameters of 3–7 μm) connected to a servocontrolled counterpressure system (Wiederhielm et al., 1964) and filled with 0.5 M NaCl colored with Evans Blue. Punctures were performed under visual guidance using a stereo microscope (Wild M5, Heerbrugg, Switzerland) and were accepted when: (1) Feedback gain could be altered

without altering the recorded pressure. (2) After meeting criterion 1, negative pressure was applied to the servocontrolled pump. This should cause increased resistance in the pipette due to the entrance of fluid hypotonic to the 0.5 M NaCl into the pipette. (3) Recording of zero pressure did not change from before the recording. Recording of P_{if} was performed on the dorsal side of the hind paw after the hair had been closely clipped with a pair of scissors. The recordings were grouped in the following periods: Control, 0–20, 21–40, 41–60 and 61–90 min after subdermal injection of 5 μl 1% carrageenan. Data were also averaged for the period of 21–60 min since the decline in P_{if} occurred during the first 20 min and then remained stable. Since not all experiments were continued for 90 min, the period of 61–90 min was not included in this average.

2.3.2. Series 2: Edema measurements

Paw volume was measured in arbitrary units using a plethysmometer (Ugo Basile, Italy). Five measurements were made at each time point. The highest and lowest values were excluded, and the remaining three values were averaged. The paw volume was measured immediately after carrageenan injection and at 0.5, 1, 1.5, 2, 3, and 4 h post-carrageenan.

2.4. Experimental protocol

2.4.1. Series 1: Interstitial fluid pressure (P_{if})

Measurements were performed in six groups. After measurement of control P_{if} with intact circulation, the rats received i.v. either no saline vehicle (Group 1, $n = 7$ and Group 2, $n = 7$), saline vehicle (Group 3, $n = 9$) or 10 mg α-trinositol (Group 4, $n = 9$). Circulatory arrest was induced after completion of control measurements in Groups 1 and 2 and 5 min after i.v. administration of either saline vehicle or α-trinositol in Groups 3 and 4, respectively. After circulatory arrest had been induced, measurement of P_{if} was continued after subdermal injection of 5 μl saline (Group 2) or 5 μl carrageenan (Groups 3 and 4). Group 1 received no subdermal injection. Measurement of P_{if} was continued up to 90 min after induction of circulatory arrest. After measurement of control P_{if} the two last groups received 5 μl carrageenan subdermally while the circulation was intact and 10 min later received either saline vehicle (Group 5, $n = 9$) or 10 mg α-trinositol i.v. (Group 6, $n = 8$). Circulatory arrest was induced 5 min thereafter and measurement of P_{if} was continued up to 60 min after injection of carrageenan.

2.4.2. Series 2: Edema measurements

The animal was randomized to one of the groups below. Infusion of α-trinositol (20 mg kg^{-1} h^{-1}) started at 60 min before carrageenan ($n = 12$), at the time of carrageenan injection ($n = 17$), or 15 ($n = 11$), 30 ($n = 12$) or 60 ($n = 19$) min after subdermal carrageenan injection. A total of 36 control animals were injected with saline at the same time points as for the start of infusion of α-trinositol. No statistically significant differences between the groups of animals receiving saline infusion at different times was found. All control animals were therefore pooled as one group.

2.5. Statistical analysis

Data are reported as means ± S.D. Data for P_{if} were averaged for each registration period and this mean value was analyzed using One-way analysis of variance (ANOVA) and subsequent post-hoc *t*-tests and Bonferroni's tests. Analysis of variance with post-hoc Dunnett's test was used for edema measurements. $P < 0.05$ was considered statistically significant.

2.6. Drugs

Pentobarbital sodium (100 mg ml^{-1}) or thiopental (Penthotal sodium, Abbott) was used as anesthetic. Lambda carrageenan was from Sigma (St. Louis, MO, USA) and was dissolved in 0.9% NaCl to a concentration of 10 or 15 mg ml^{-1}. α-Trinositol (D-*myo*-inositol-1,2,6-trisphosphate) was from Perstorp Pharma, Lund, Sweden. The α-trinosi-

Table 1
Effect of α-trinositol on interstitial pressure in skin after subdermal injection of 5 μl 1% lambda carrageenan. Saturated potassium chloride was given 5 min after i.v. injection of 10 mg α-trinositol to induce circulatory arrest. Values are mean ± S.D.

Group	Treatment		Interstitial fluid pressure (mmHg)					
			Pre-injection	Time after subdermal injection of carrageenan (min)				
	Subdermal	Intravenous		0–20	21–40	41–60	61–90	Mean (21–60)
1	None	None	–	−0.4 ± 0.8	−1.5 ± 0.8	−0.8 ± 0.5	−0.9 ± 1.2	−1.1 ± 0.5
2	Saline	None	–	+0.6 ± 1.7[f]	−0.6 ± 1.8	+0.4 ± 2.0[f]	+0.2 ± 1.3[a]	−0.2 ± 1.7[f]
3	Carrageenan	None	−0.2 ± 2.4	−2.2 ± 1.7[c]	−2.4 ± 1.6	−2.2 ± 2.0[c]	−2.0 ± 2.0[d]	−2.2 ± 1.8[c]
4	Carrageenan	α-Trinositol (pre)[g]	−1.3 ± 0.9	−1.5 ± 1.3	−1.2 ± 1.5	−0.9 ± 0.7	−1.2 ± 1.5	−1.3 ± 1.3
5	Carrageenan	Saline (post)[h]	−0.5 ± 0.3	−2.0 ± 0.6[b]	−2.7 ± 0.8[b]	−3.0 ± 1.0[c]	–	−2.9 ± 0.7[c]
6	Carrageenan	α-Trinositol (post)[h]	−0.4 ± 0.5	−0.6 ± 0.6	−1.1 ± 0.9	−0.7 ± 0.7	–	−0.9 ± 0.7
ANOVA			0.3532	0.0030	0.0238	0.0002	0.0779	0.0036

[a] $0.05 < P < 0.1$; [b] $P < 0.05$; [c] $P < 0.01$ compared to Group 2; [d] $0.05 < P < 0.1$; [e] $P < 0.05$; [f] $P < 0.01$ compared to Group 3.
[g] Pre-treatment with α-trinositol. [h] α-Trinositol or 0.9% NaCl given 10 min after carrageenan.

tol vial contained 1 g of lyophilized powder which was dissolved in 0.9% NaCl and diluted to a 400 mg ml^{-1} solution which was then frozen in portions. Dilutions of this solution were made fresh for each experiment. Ten milligrams were given i.v. at 100 mg ml^{-1} in 0.9% NaCl, i.e., in 0.1 ml followed by 0.1 ml 0.9% NaCl to flush the infusion catheter P_{if} measurements. In the edema measurement experiments, the animals were given continuous infusions of 20 mg kg^{-1} h^{-1} for 3–5 h at a flow rate of 0.25 ml h^{-1}, using an Orion M361 infusion pump (Sage). The control animals were given infusions of 0.25 ml h^{-1} 0.9% NaCl for 3–5 h.

3. Results

3.1. Interstitial fluid pressure (P_{if})

The data for the P_{if} measurements are presented in Table 1. In agreement with previous results (Rodt and Reed, 1993), carrageenan induced a statistically significant fall in P_{if} to about −2.5 mmHg ($P < 0.05$) (Groups 3 and 5 vs. Group 2). Intravenous α-trinositol abolished the lowering of P_{if} when given both prior to (Group 4) and after carrageenan (Group 6) ($P > 0.05$ compared to Group 3). α-Trinositol was without effect on P_{if} when Groups 1 or 2 and 5 were compared. Subdermal injection of 5 μl saline increased P_{if} transiently by 1 mmHg during the first 20 min and then P_{if} returned to −0.5 to −1.0 mmHg, in agreement with findings of previous studies (Rodt and Reed, 1993). Since the decline in P_{if} occurred during the first 20 min and P_{if} thereafter remained stable during the observation period, data were also averaged for the period of 21–60 min. The same pattern as discussed above was verified, i.e., treatment with α-trinositol prior to as well as after subdermal injection of carrageenan abolished the lowering of interstitial fluid pressure.

3.2. Edema measurements

In animals that received saline i.v., the paw injected with carrageenan increased gradually in volume by approximately 30% during the first 4 h after injection (Table 2). In animals given α-trinositol 60 min prior to carrageenan, the paw volume increased by 20% during the first 4 h. Apart from this, no statistically significant differences were observed between the experimental groups (Table 2).

4. Discussion

The present experiments demonstrate that the lowering of interstitial fluid pressure (P_{if}) induced by carrageenan is abolished by α-trinositol when given either prior to or after carrageenan. These findings are in agreement with the reported effect of this substance on P_{if} in scald injury in rat skin (Lund and Reed, 1994), dextran anaphylaxis (Koller et al., 1997) and neurogenic inflammation in the trachea (Woie and Reed, 1994).

In contrast, a significant reduction in paw edema was observed only when the continuous infusion of 20 mg kg^{-1} h^{-1} α-trinositol was started 60 min prior to carrageenan injection: edema formation was reduced from about 30% to 20% increase in paw volume at 4 h after carrageenan. There was a tendency towards less edema at 1 h in the two groups that received α-trinositol 60 min prior to, and at the same time as carrageenan injection, but this was not statistically significant (Table 2). Thus, in order for α-trinositol to reduce edema at 3–4 h after injection of carrageenan, a therapeutic concentration must have been reached when carrageenan was injected. The present observations suggest that the mechanism of action of α-trinositol differs with respect to the early and the late phases of carrageenan inflammation and that different mechanisms are involved in lowering P_{if} in the initial stage of edema formation and in edema measured at 4 h.

For P_{if} measurements, α-trinositol was given as an i.v. bolus of 10 mg. This results in a plasma concentration of α-trinositol which is very high initially, and probably higher than the plasma level achieved by continuous infusion in the edema experiments. The lowering of P_{if} took place within a few minutes (Reed et al., 1997), and the time between carrageenan and α-trinositol injection (10 min) was evidently sufficient to start a process in which α-trinositol attenuated the lowering of P_{if}. Since α-trinositol did not reverse the edema formation at 4 h when infusion was started simultaneously with carrageenan injection, a concentration difference at the time of injection does not explain the difference between these two groups.

Table 2
Effect of infusion of 20 mg kg^{-1} h^{-1} of α-trinositol on paw volume when infusion was initiated before (pre), at or after injection (post) of 1.5% lambda carrageenan. Values are % increase in paw volume. (Volume increase at time = 0 is 0 for all experiments)

Time (h)	Saline	α-Trinositol, time relative to subdermal injection of carrageenan				
	Control	60 min pre	same time	15 min post	30 min post	60 min post
1	8.9 ± 1.0	7.0 ± 1.4	6.0 ± 1.0	8.7 ± 1.5	8.8 ± 1.8	8.6 ± 1.6
2	20.8 ± 1.4	16.8 ± 2.6	18.2 ± 2.2	22.9 ± 2.6	27.8 ± 2.8	16.5 ± 2.1
3	26.2 ± 1.4	21.2 ± 2.9	25.0 ± 2.0	30.3 ± 3.2	30.6 ± 3.2	23.7 ± 2.1
4	29.7 ± 1.4	19.5 ± 2.1[a]	27.1 ± 1.9	28.3 ± 2.8	30.1 ± 3.4	24.9 ± 1.5
Number of experiments	36	12	17	11	12	19

[a] $P < 0.05$. Analysis of variance with Dunnett's test vs. control group.

The experimental protocol differed in the two series and this needs to be commented upon. In experiments designed for measurement of edema, 100 μl of a 1.5% solution was injected into the rat paw, in agreement with Claxson et al. (1990). The rat paw (below the ankle) weighs about 1.5 g and the injected volume then represents approximately 7% of the total paw volume. The increase in paw volume was at most 30% above control, but was sufficient to allow comparison between the different groups. However, when measuring P_{if}, only 5 μl of a 1% solution of carrageenan was used, in agreement with our previous study (Rodt and Reed, 1993). The volume of the injectate is a compromise between injecting sufficient test substance to produce a biological effect and not flooding the tissue, which would cause manifest edema and increase P_{if} permanently to positive values. Also, in this group, circulatory arrest was induced as part of the experimental protocol in order to measure the full extent of the lowering of P_{if}. The inflammatory reaction increases capillary fluid filtration via the lowering of P_{if}, thus, raising the interstitial fluid volume which potentially attenuates the lowering of P_{if} induced by carrageenan. In agreement with this, in a previous study (Rodt and Reed, 1993), the same protocol as in the present study, resulted a fall in P_{if} from −0.4 mmHg in control to −4.8 mmHg at 10 min: P_{if} returned to control values at 25 min when the circulation was intact and edema developed. With circulatory arrest a similar initial lowering of P_{if} was observed, but P_{if} remained at −4 mmHg throughout the observation period of 90 min. The small amount of carrageenan injected in the experiments for measurements of P_{if} would not have been detectable using the plethysmography technique for measurement of edema formation. Thus, the two series of experiments give information about the ability of α-trinositol to attenuate inflammation at two different stages and up to 60 min after injection (P_{if} measurements) and throughout the first 4 h after injection (edema measurements).

Interstitial fluid volume is normally tightly controlled by a balance between transcapillary fluid flux and lymph flow as described by Eq. (1) (Aukland and Reed, 1993):

$$J_v = \mathrm{CFC}\left(\left(P_c - P_{if} - \sigma\left(\mathrm{COP}_c - \mathrm{COP}_{if}\right)\right)\right) = \left(\mathrm{CFC} \cdot \Delta P\right) = J_L \quad (1)$$

where J_v is the transcapillary fluid flux, CFC is the capillary filtration coefficient and J_L is the lymph flow. P and COP are hydrostatic and colloid osmotic pressures, respectively. Subscripts c and if are capillary and interstitial fluid, respectively, and σ is the capillary reflection coefficient. Edema associated with inflammatory reactions results from increased capillary filtration, caused mainly by a rise in ΔP since CFC is reported to increase to a maximum two to three times control (Arturson and Mellander, 1964; Dietzel et al., 1969; Pitt et al., 1987; Dyess et al., 1992; Korthuis et al., 1992; Williams and Huxley, 1993). Lowering of P_{if} by carrageenan will raise the net capillary filtration pressure from 0.5 to 1 mmHg and by 4.5 mmHg. The lowering of P_{if} in inflammation is thought to involve perturbation of the cell-matrix receptors, the β_1-integrins (Reed et al., 1992, 1997; Rodt et al., 1994), since blockade of the β_1-receptors and in particular $\alpha_2\beta_1$-integrins (Rodt et al., 1996) induces lowering of P_{if} from −1 to between −4 and −5 mmHg.

Our observation of two phenomena in carrageenan-induced inflammation seems to be in agreement with the concept put forward by Vinegar et al. (1969, 1987), by which carrageenan induced inflammation is divided into an early non-phagocytic and a phagocytic phase starting at 60 min and which involves neutrophils. Vinegar et al. (1987) summarized the different stages of carrageenan edema, and importantly pointed out that in the non-phagocytic phase, the edema developing between 2 and 10 min was independent of the dose of carrageenan. High doses of cyclooxygenase inhibitors reduced the edema in this period by 60%, which made the authors suggest that the remaining part of the edema was due to serotonin since methysergide also attenuated edema formation. The edema developing between 10 and 50 min could not be inhibited by steroidal and non-steroidal anti-inflammatory drugs, anti-histamines or anti-serotonins. It is tempting to suggest that this edema is generated by lowering of P_{if}, and is inhibited by α-trinositol. Cycloheximide, but also steroidal drugs, such as prednisolone and betamethasone, inhibit the late phagocytic phase of edema development, without affecting the early phase. The late events may be associated with activation of collagenases and gelatinases (Nakagawa and Sakata, 1986), several inflammatory mediators and degradation of mast cells (Vinegar et al., 1987). Furthermore, there is evidence that bradykinin is involved since edema induced by carrageenan develops only to a minor extent in kininogen-deficient rats and after treatment with bradykinin antagonists (Oh-ishi et al., 1987; Damas and Remacle-Volon, 1992).

The main conclusion from the present study is that α-trinositol attenuates events associated with carrageenan-induced inflammation in the early (non-phagocytic) phase, when given prior to or after carrageenan. However, the late events associated with carrageenan inflammation can be attenuated only when α-trinositol is given prior to carrageenan.

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