Characterization of Dextran Sulfate-Induced Guinea Pig Tracheal Plasma Extravasation

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ABSTRACT—We examined the effect of dextran sulfate on guinea pig tracheal vascular permeability. I.v. injection of dextran sulfate (10–100 mg/kg) with captopril (1 mg/kg) induced guinea pig tracheal plasma extravasation. The i.v. administration of bradykinin (0.8–8 μ g/kg) with captopril (1 mg/kg) also induced plasma extravasation. Bradykinin B₂ antagonists, D-Arg[Hyp³-Thi⁵-D-Tic⁷-Tic⁸]-bradykinin (NPC 16731) and D-Arg[Hyp³-Thi⁵-D-Tic⁷-Oic⁸]-bradykinin (Hoe 140), reduced both dextran sulfate (32 mg/kg)- and bradykinin (2.5 μ g/kg)-induced tracheal plasma extravasation in a dose-dependent manner. However, a cyclooxygenase inhibitor, indomethacin (1 mg/kg), and a neurokinin antagonist, {N-[N²-[N-[N-[N-[2,3-didehydro-N-methyl-N-[N-[3-(2-pentylphenyl)-propionyl]-L-threonyl]tyrosyl-L-leucynyl]-D-phenylalanyl]-L-allo-threonyl]-L-asparaginy]-L-serine- ν -lactone} (FK 224) (1 mg/kg), had no effect on tracheal plasma extravasation induced by dextran sulfate and bradykinin. Dextran sulfate (32 mg/kg) with captopril (1 mg/kg) greatly increased the bradykinin level in guinea pig plasma. This evidence suggests that dextran sulfate releases bradykinin in guinea pig plasma and causes guinea pig tracheal plasma extravasation via the activation of bradykinin B₂-receptors.

Keywords: Dextran sulfate, Bradykinin, Bradykinin B₂-receptor, Trachea (guinea pig)

Dextran sulfate is a negatively charged macromolecule that has been shown to activate prekallikrein (1). It is one of a variety of compounds that are able to activate the multifunctional system of proteases, control vasoregulation and amplify the humoral inflammatory response. The essential components of the contact system are zymogen factor XII (Hageman factor), a negatively charged macromolecule that induces a change in the conformation of factor XII, and prekallikrein. In this complex, factor XII and prekallikrein can activate each other in the presence of kininogen, resulting in the cleavage of kininogen by kallikrein and the release of bradykinin (2). Dextran sulfate has a negative charge in its molecule and is considered to activate the contact system through the same mechanism as Hageman factor.

In studies of respiratory disease, there has also been much interest in the inflammatory reaction in the airway. One of the typical clinical pictures of respiratory inflammation is mucosal plasma extravasation. Since the mucosal plasma extravasation in the airway may play an important role in the generation of the chronic disease, it will be very valuable to clarify the mechanism of airway

inflammation.

In this report, we discovered that the administration of dextran sulfate induced guinea pig tracheal plasma extravasation and examined the involvement of endogenous bradykinin on this reaction.

MATERIALS AND METHODS

Tracheal plasma extravasation

Male Hartley guinea pigs (290–410 g; Japan SLC, Shizuoka) were injected i.v. with Evans blue dye (20 mg/kg, dissolved in saline). These guinea pigs were immediately injected with bradykinin or dextran sulfate (2 ml/kg, dissolved in saline). Ten minutes later, the animals were anesthetized by i.p. injection of sodium pentobarbital (10 mg/animal) and killed by exsanguination. The lungs were perfused through the pulmonary artery with 50 ml of saline. The trachea and stem bronchi were dissected out, weighed and then dissolved in 0.25 ml of 1 N KOH at 37°C for 6 hr. After extraction with 2.25 ml of acetone-phosphate solution (0.6 N H₃PO₄: acetone=5:13), the Evans blue dye content of tissues was quantified

colorimetrically at 620 nm. NPC 16731 and Hoe 140 were dissolved in saline and injected i.v. (2 ml/kg) 2 min before Evans blue dye injection. Indomethacin and FK 224 were dissolved in dimethylsulfoxide (DMSO) and injected i.v. (0.1 ml/kg) 2 min before Evans blue dye injection. The increase in the amount of leaked Evans blue dye was calculated by subtracting the value for Evans blue solution in tissue not treated with agonist ($20.3 \pm 2.6 \mu\text{g/g}$ tissue, $N=4$).

Bradykinin release

Male Hartley guinea pigs (290–410 g) were injected i.v. with dextran sulfate (32 mg/kg) and captopril (1 mg/kg). Ten minutes after the administration of dextran sulfate, 5 ml of blood from the carotid artery was collected directly into the plastic tubes that contained 10 ml of absolute ethanol in which *o*-phenanthroline was dissolved (final concentration of $10 \mu\text{M}$). The mixture was heated at 70°C for 10 min. After centrifugation ($1500 \times g$, 4°C , 15 min), the supernatant was evaporated under reduced pressure. The residue was washed three times with 10 ml of diethylether in order to remove lipids, and it was then dissolved in an assay buffer and bradykinin-like immunoreactivity was measured by radioimmunoassay (RIA) using bradykinin RIA kits from Peninsula Laboratories, Inc. (Belmont, CA, USA). A $100\text{-}\mu\text{l}$ aliquot of a

sample or bradykinin was incubated for 24 hr at 4°C with $100 \mu\text{l}$ of anti-bradykinin antiserum. [^{125}I]Bradykinin ($100 \mu\text{l}$) was added and the incubation was continued for a further 24 hr at 4°C . Goat anti-rabbit IgG serum ($100 \mu\text{l}$) and normal rabbit serum ($100 \mu\text{l}$) were added and incubated for 90 min at room temperature. The samples were centrifuged at $1700 \times g$ for 20 min. The supernatant was aspirated off, and the radioactivity of pellet was determined in a gammacounter. The sensitivity of the RIA was 128 pg/tube. Standard curves were constructed, and the results are expressed as ng/ml. Anti-bradykinin antiserum does not crossreact with des Arg-bradykinin. The recovery rate was greater than 90%, as evaluated from measurements of control bradykinin added to the blood of normal, healthy guinea pigs.

Materials

Bradykinin was purchased from Peptide Institute, Inc. (Osaka). Dextran sulfate, captopril and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Evans blue dye and *o*-phenanthroline were from Nacalai Tesque Chemical Co. (Kyoto). [^{125}I]Bradykinin (37 TBq/mmol) and anti-bradykinin antiserum for RIA were purchased from Peninsula Laboratories, Inc. D-Arg[Hyp³-Thi⁵-D-Tic⁷-Oic⁸]-bradykinin (Hoe 140), D-Arg[Hyp³-Thi⁵-D-Tic⁷-Tic⁸]-bradykinin (NPC 16731) and {N-

Fig. 1. Dose-response showing the guinea pig tracheal plasma extravasation induced by dextran sulfate with (+) or without (−) 1 mg/kg captopril. **Significantly different from the control, $P < 0.01$. Values are given as Evans blue dye contents and are the means $\pm$ S.E.M. of four experiments.

[N²-[N-[N-[N-[2,3-didehydro-N-methyl-N-[N-[3-(2-pentylphenyl)-propionyl]-L-threonyl]tyrosyl-L-leucynyl]-D-phenylalanyl]-L-allo-threonyl]-L-asparaginy]-L-serine-γ-lactone} (FK 224) were synthesized in our laboratories.

Statistical analyses

Data are each presented as the means ± S.E.M. of the results from four independent experiments. Statistical analyses were performed by analysis of variance followed by Dunnett's multicomparison test (tracheal plasma extravasation) or by means of the unpaired Student's *t*-test (bradykinin release). *P* < 0.05 was considered as indicative of significance.

RESULTS

Characterization of dextran sulfate-induced guinea pig tracheal plasma extravasation

The i.v. injection of dextran sulfate (10–100 mg/kg) with captopril (1 mg/kg) induced guinea pig tracheal plasma extravasation in a dose-dependent manner (Fig. 1). The i.v. injection of bradykinin (0.8–8 μg/kg) with captopril (1 mg/kg) also dose-dependently induced guinea pig tracheal plasma extravasation (Fig. 2). The responses of dextran sulfate and bradykinin were maximum at the 100 mg/kg and 8 μg/kg dose, respectively.

Fig. 2. Dose-response showing the guinea pig tracheal plasma extravasation induced by bradykinin with (+) or without (–) 1 mg/kg captopril. **Significantly different from the control, *P* < 0.01. Values are given as Evans blue dye contents and are the means ± S.E.M. of four experiments.

Administration of either dextran sulfate (32 mg/kg) or bradykinin (8 μg/kg) without captopril had no effect (Figs. 1 and 2). We examined the effect of bradykinin antagonists on guinea pig tracheal plasma extravasation induced by dextran sulfate (32 mg/kg) and bradykinin (2.5 μg/kg) with captopril (1 mg/kg). The i.v. injection of bradykinin antagonists, NPC 16731 and Hoe 140, reduced dextran sulfate-induced guinea pig tracheal plasma extravasation in a dose-dependent manner (Table 1). These bradykinin antagonists also dose-dependently inhibited bradykinin-induced guinea pig tracheal plasma extravasation (Table 2). The ED₅₀ values of NPC 16731 on dextran sulfate- and bradykinin-induced plasma extravasation were 35 μg/kg and 20 μg/kg, respectively.

Table 1. Effect of bradykinin antagonists on dextran sulfate-induced guinea pig tracheal plasma extravasation

Treatment	Evans blue content (μg/g tissue)	% Inhibition
Control	20.2 ± 4.8	—
NPC 16731 10.0 μg/kg	20.9 ± 3.5	–3.5
NPC 16731 32.0 μg/kg	11.1 ± 5.5*	45.0
NPC 16731 100.0 μg/kg	0.6 ± 6.7*	97.1
Control	30.9 ± 5.6	—
Hoe 140 1.0 μg/kg	28.5 ± 10.1	7.8
Hoe 140 10.0 μg/kg	18.5 ± 5.3	40.2
Hoe 140 100.0 μg/kg	3.7 ± 0.7**	87.9

Dextran sulfate (32 mg/kg) with captopril (1 mg/kg) was intravenously injected in guinea pigs. Each value indicates the mean ± S.E.M. of four experiments. *Significantly different from the control, *P* < 0.05. **Significantly different from the control, *P* < 0.01.

Table 2. Effect of bradykinin antagonists on bradykinin-induced guinea pig tracheal plasma extravasation

Treatment	Evans blue content (μg/g tissue)	% Inhibition
Control	39.2 ± 4.7	—
NPC 16731 3.2 μg/kg	33.3 ± 4.6	14.9
NPC 16731 10.0 μg/kg	24.2 ± 3.1*	38.3
NPC 16731 32.0 μg/kg	14.6 ± 1.4**	62.7
NPC 16731 100.0 μg/kg	5.7 ± 2.7**	85.4
Control	49.6 ± 8.1	—
Hoe 140 1.0 μg/kg	48.8 ± 10.7	1.6
Hoe 140 10.0 μg/kg	24.6 ± 7.0	50.5
Hoe 140 100.0 μg/kg	5.2 ± 3.8**	89.6

Bradykinin (2.5 μg/kg) with captopril (1 mg/kg) was intravenously injected in guinea pigs. Each value indicates the mean ± S.E.M. of four experiments. *Significantly different from the control, *P* < 0.05. **Significantly different from the control, *P* < 0.01.

Table 3. Effect of FK 224 and indomethacin on bradykinin- and dextran sulfate-induced guinea pig tracheal plasma extravasation

Treatment		Bradykinin-induced plasma extravasation		Dextran sulfate-induced plasma extravasation	
		Evans blue content ($\mu\text{g/g}$ tissue)	% Inhibition	Evans blue content ($\mu\text{g/g}$ tissue)	% Inhibition
Control		84.3 ± 17.8	—	40.5 ± 1.8	—
FK 224	1 mg/kg	99.2 ± 17.9	-17.7	46.0 ± 2.4	-13.5
Indomethacin	1 mg/kg	93.5 ± 24.4	-10.9	34.9 ± 4.7	13.8

Dextran sulfate (32 mg/kg) or bradykinin (2.5 $\mu\text{g/kg}$) with captopril (1 mg/kg) was intravenously injected in guinea pigs. Each value indicates the mean $\pm$ S.E.M. of four experiments.

The ED_{50} values of Hoe 140 on dextran sulfate- and bradykinin-induced plasma extravasation were 14 $\mu\text{g/kg}$ and 13 $\mu\text{g/kg}$, respectively. In contrast, the cyclooxygenase inhibitor indomethacin (1 mg/kg, i.v.) and the neurokinin antagonist FK 224 (1 mg/kg, i.v.) had no effect on dextran sulfate- and bradykinin-induced guinea pig tracheal plasma extravasation (Table 3). Injection of the vehicles used to dissolve the drugs had no effect (data not shown). In the experiments on tracheal plasma extravasation, the control reactions in the presence of dextran sulfate (32 mg/kg) or bradykinin (2.5 $\mu\text{g/kg}$) were very discrepant for different treatments. The differences in control extravasation were due to the lack of reproducibility of these techniques. We purchased about 20 guinea pigs for every experiment. Each animal in the same lot shows the same degree of tracheal plasma extravasation. When the lot of animals changes, the degree of the control reaction tends to change. Because of this, we always compared the resulting reaction with the control in the same lot of animals.

Effect of dextran sulfate on the plasma bradykinin level

We examined the bradykinin level in plasma with or without the administration of dextran sulfate. Dextran sulfate (32 mg/kg) with captopril (1 mg/kg) greatly increased the bradykinin level in guinea pig plasma (Table 4). After the injection of dextran sulfate, the bradykinin level increased to about 1000-fold more than that in the vehicle control.

Table 4. Effect of dextran sulfate on bradykinin content in guinea pig blood

Treatment	Bradykinin content (ng/ml blood)
Vehicle	0.004 ± 0.002
Dextran sulfate 32 mg/kg	$3.927 \pm 1.255^{**}$

Each value indicates the mean $\pm$ S.E.M. of four experiments. ** Significantly different from the control, $P < 0.01$.

DISCUSSION

In the present study, dextran sulfate induced guinea pig tracheal plasma extravasation and selective bradykinin B_2 -receptor antagonists, Hoe 140 and NPC 16731 (3, 4), abolished the dextran sulfate-induced response as potently as the bradykinin-induced one. Dextran sulfate caused the release of bradykinin in guinea pig plasma. This evidence suggests that dextran sulfate-induced tracheal plasma extravasation in guinea pigs is mediated by stimulation of bradykinin B_2 -receptors.

In the second part of this study, we examined the effect of a neurokinin antagonist, FK 224, on guinea pig tracheal plasma extravasation induced by dextran sulfate and bradykinin. Bradykinin stimulates sensory nerves in the airways, causing the release of the neuropeptides, substance P and neurokinin A (5). These mediators are potent activators of vascular permeability. Therefore, the stimulation of receptors by these mediators could influence vascular permeability and cause tracheal plasma extravasation. Nakajima et al. (6) reported that the neurokinin antagonist FK 224 inhibited the tracheal plasma extravasation induced by bradykinin instilled into guinea pig airways (7). However, in this report, FK 224 had no effect on guinea pig tracheal plasma extravasation induced by dextran sulfate and bradykinin injected intravenously. This indicates that bradykinin instilled into the trachea, but not bradykinin injected intravenously, stimulates sensory nerves in the airways and causes the release of neuropeptides. In our previous study, we showed that a pungent agent, capsaicin, also induced tracheal plasma extravasation when injected intratracheally. However, intravenous injection of capsaicin had no effect (8). It is possible that both capsaicin and bradykinin injected intratracheally interact with irritant receptors as irritants in guinea pig airways and activate capsaicin-sensitive sensory nerves. This evidence suggests that bradykinin released by dextran sulfate directly interacts with vascular endothelial cells and induces plasma extravasation in guinea pig trachea.

In the third part of this study, we examined the effect of the cyclooxygenase inhibitor indomethacin. Bradykinin was shown to be a potent stimulator of prostaglandin synthesis in various tissues (9), while in the present study, indomethacin had no effect on dextran sulfate- and bradykinin-induced guinea pig tracheal plasma extravasation. In guinea pig airway, bronchoconstriction induced by bradykinin has been shown to be mediated by prostaglandins because it is inhibited by indomethacin (10). This evidence shows that the mechanisms by which plasma extravasation and bronchoconstriction are induced by bradykinin may be different.

Because the contact system combines the activation of the plasma cascade systems with the release of the hypotensive mediator bradykinin, the system has also been implicated in the pathophysiology of septic shock, disseminated intravascular coagulation, and multiple organ failure, including the adult respiratory distress syndrome (11, 12). These syndromes may be characterized by a protein- and cell-rich pulmonary edema due to increased vascular permeability in the lung. Our recent study suggested that a bradykinin B₂-antagonist may be a valuable tool for the treatment of multiple organ failure as a result of the activation of the plasma cascade system, e.g., bacterial shock or adult respiratory distress syndrome.

REFERENCES

- 1 Kluft C: Determination of prekallikrein in human plasma-optimal conditions for activating prekallikrein. *J Lab Clin Med* **91**, 83–95 (1978)
- 2 Cochrane CG and Revak SD: Dissemination of contact activation in plasma by plasma kallikrein. *J Exp Med* **152**, 608–619 (1980)
- 3 Farmer SG, Burch RM, Kyle DJ, Martin JA, Meeker SA and Togo J: D-Arg[Hyp³-Thi⁵-D-Tic⁷-Tic⁸]-bradykinin, a potent antagonist of smooth muscle BK₂ receptors and BK₃ receptors. *Br J Pharmacol* **102**, 785–787 (1991)
- 4 Hock FJ, Wirth K, Albus U, Linz W, Gerhards HJ, Wiemer G, Henke St, Brepohl G, Konig W, Knolle J and Scholkens BA: Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br J Pharmacol* **102**, 769–773 (1991)
- 5 Saria A, Martling CR, Yan Z, Theodorsson-Norheim E, Gamse R and Lundberg JM: Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenylpiperazinium, and vagal stimulation. *Am Rev Respir Dis* **137**, 1330–1335 (1988)
- 6 Nakajima N, Ichinose M, Takahashi T, Yamauchi H, Igarashi A, Miura M, Inoue H, Takishima T and Shirato K: Bradykinin-induced airway inflammation. *Am J Respir Crit Care Med* **149**, 694–698 (1994)
- 7 Morimoto H, Murai M, Maeda Y, Yamaoka M, Nishikawa M, Kiyotoh S and Fujii T: FK 224, a novel cyclopeptide substance P antagonist with NK₁ and NK₂ receptor selectivity. *J Pharmacol Exp Ther* **262**, 398–402 (1992)
- 8 Morimoto H, Fujii T and Senoh H: Plasma extravasation by chemical mediators in rat skin and trachea: A role of neurogenic agents on tracheal edema formation. *Jpn J Pharmacol* **49**, 389–395 (1989)
- 9 Zusmann RJ: Effects of converting enzyme inhibitors on the renin-angiotensin-aldosterone, bradykinin, and arachidonic acid-prostaglandin system: correlation on chemical structure and biologic activity. *Am J Kidney Dis* **10**, Supp 13–23 (1987)
- 10 Arakawa H, Kawikova I, Lofdahl CG and Lotvall J: Bradykinin-induced airway responses in guinea pig: effects of inhibition of cyclooxygenase and thromboxane synthetase. *Eur J Pharmacol* **229**, 131–136 (1992)
- 11 McGuire WW, Spragg RG, Cohen AB and Cochrane CG: Studies on the pathogenesis of the adult respiratory distress syndrome. *J Clin Invest* **69**, 543–553 (1982)
- 12 Kalter ES, Daha MR, ten Cate JW, Verhoef J and Bouma BN: Activation and inhibition of Hageman factor-dependent pathways and the complement system in uncomplicated bacteremia or bacterial shock. *J Infect Dis* **151**, 1019–1027 (1985)