Pulmonary Pharmacology (1989) 2 93–102

0952-0600/89/0002-0093/$10.00

PULMONARY PHARMACOLOGY

Inflammatory Passage of Plasma Macromolecules into Airway Wall and Lumen

I. Erjefält*, C. G. A. Persson*†

**AB Draco, Lund and †Dept of Clinical Pharmacology, University Hospital of Lund, Lund, Sweden*

SUMMARY. **Anaesthetised guinea-pigs received tracer macromolecules 70–340 kDa intravenously and their erythrocytes were labelled in vivo with ^{99m}Tc. Superfusion of tracheal mucosa (via oral catheter) with control solutions and inflammatory agents, was followed by sampling of tracheal surface liquids and tracheal tissue. Under baseline conditions no ^{125}I-fibrinogen (340 kDa) and minimal amounts of erythrocytes, ^{131}I-albumin (70 kDa), and FITC-D (150 kDa) were found in tracheal lavage fluids. Undisturbed baseline conditions with negligible leakage of plasma into airway tissue and lumen were thus obtained with the present provocation and sampling techniques. Superfusion during 2 min with bradykinin 2–10 nmol, histamine 2–8 nmol, capsaicin 0.1–0.4 nmol, PAF 4–8 nmol, ovalbumin 3–6 pmol (in sensitised animals) produced, within 1–10 min, a significant and dose-dependent accumulation of plasma in tracheal tissue and lavage fluids. PAF also induced a late phase plasma leakage response at 5 h. At 10 min PAF given intra-arterially produced a similar leakage into the tissue but less into the lumen compared to topical PAF. Intravenous PAF produced additional effects such as pulmonary oedema. Carbachol 8–16 nmol had only minimal effects on 'leakage' but produced severe bronchoconstriction. Toluene diisocyanate (0.003–0.03 μl) produced dose-dependent and very sustained (17 h) plasma leakage. Recovery of plasma tracers in airway tissue and surface liquids, respectively, was significantly correlated. As examined with capsaicin, absorption of luminal macromolecules increased only slightly during the exudation process. It is suggested that the consistent inflammatory stimulus-induced passage of plasma into the lumen is a consequence of a load on the basal side of the epithelium induced by the extravasated plasma and its derived peptides. An increased interstitial pressure may transiently separate many epithelial cells allowing a mainly uni-directional almost unrestricted flow of large solutes into the lumen.**

INTRODUCTION

We have previously demonstrated that superfusion of the tracheo-bronchial mucosa with mediators or allergen produce concentration-dependent leakage of plasma into the airway tissue.[1,2] This effect was not associated with an increased wet to dry weight ratio of the sampled tracheal specimens.[2] We then discovered that plasma macromolecular tracers, corresponding to a large volume of exudate/transudate, could be recovered at the mucosal surface within minutes after the mucosal provocations.[3] The present work was undertaken to extend our preliminary data and examine whether the further passage across the epithelial barrier of exuded plasma is, indeed, a consistent sign of mucosal inflammation. This would also tell something about the mechanisms involved. How can the normally tight epithelial lining allow a rapid flow of plasma macromolecules into the airway lumen? We have now superfused the guinea-pig tracheal mucosa with different concentrations of a variety of inflammatory stimuli including mediators such as histamine, bradykinin and PAF, a neurogenic stimulus (capsaicin), allergen (in sensitised animals), and an occupational asthma agent. We have particularly validated the sampling of mucosal surface material to avoid artefactual disturbance of the mucosal barriers.

Correspondence: Carl Persson, AB Draco, P.O. Box 34, S-221 00 Lund, Sweden.

METHOD

Animals

Male guinea-pigs, weighing about 350 g, and obtaining a normal weight gain during an observation period of 7–10 days were used. The animals, starved for 3–4 h, were anaesthetised with a mixture of ketamine and xylacin (Ketalar, 50 mg/ml and Rompun, 20 mg/ml, 3:2, 0.1 ml/100 g) intramuscularly. Intravenous administrations were performed via a cannulated branch of the jugular vein or via ear marginal vein.

Sensitisation regimen

A group of animals was sensitised to produce IgE antibodies by one i.p. injection of 0.5 ml saline containing 100 mg Al $(OH)_3$ and 1 μg ovalbumin (OA).[4] The antigen was added to the adjuvant suspension 1 h before injection. These animals were used 5–6 weeks after the

sensitisation and then only in experiments with allergen (ovalbumin) and saline.

Macromolecular tracers

Fluorescein isothiocyanate conjugated dextran (FITC-D Mw 156 kDa) 5% was dissolved in saline. It was given iv in doses 0.2–0.5 ml/100 g. Human serum ^{131}I-albumin, (Mw 69 kDa) and ^{125}I-fibrinogen (Mw 341 kDa) in saline were also used. 0.1–1 MBq of either labelled protein was administered iv.

When the acute effect (within 10 min) of mediators was studied, the tracers were given 10 min prior to the superfusion of the tracheal mucosa.

In studies of a sustained or delayed response (1–17 h after exposure) the tracers were given via the ear marginal vein to conscious animals 15 min before termination of the experiment.

Superfusion of tracheal mucosa

A non-surgical technique of superfusing the tracheal mucosa with mediators and drugs was employed.[1] The anaesthetised animals were tilted backwards and kept resting on their backs in a half upright position (Fig. 1A). A fine lachrymal duct cannula was gently introduced through the mouth. The larynx offered a slightly noticeable resistance to catheterisation. With gentle manoeuvring this was overcome and the cannula moved into the upper trachea. Fine plastic tubing (O.D. 0.61 mm) was then inserted into the cannula and thus introduced into the trachea. The cannula was then withdrawn. By aid of markings on the catheter its tip was placed between 0.5–1 cm below the larynx. After a resting period of 20–25 min, saline or vehicle with or without active agents was infused through the catheter at a constant low rate (0.02 ml/min, Sage servo pump). The infusions usually went on for 2 min (=0.04 ml) but could be extended to 30 min at this low rate, without inducing breathing difficulties due to the volume load.[1] After treatment the animals were kept in the tilted backward position for 5 min and then transferred to cages, which were placed on heating pads to maintain warmth.

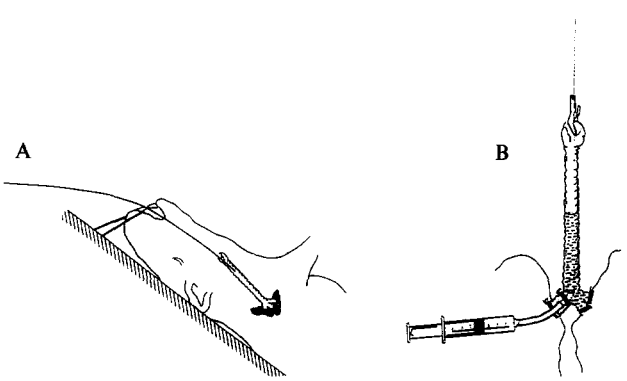

Fig. 1—(A) Catheterisation for superfusion of the tracheal mucosa. (B) Arrangement for retrograde lavage of intrathoracic tracheal mucosa.

Dose regimen

Provocating agents were superfused on the tracheal mucosa. Preliminary studies were performed to guide the selection of threshold effective doses of individual factors (data not shown). The upper dose limit was restricted by signs of respiratory failure of the animals.

Carbachol	8,16	nmol/0.04 ml
Bradykinin	2,5,10	nmol/0.04 ml
Histamine	2,5,8	nmol/0.04 ml
Capsaicin	0.1,0.4	nmol/0.04 ml
Platelet activating factor (PAF)	4, 8	nmol/0.04 ml
Allergen (Ovalbumin)	3,6	pmol/0.04 ml
Toluene diisocyanate (TDI)	0.003–3	μl/0.02 ml

Solutions were made in 0.9% sodium chloride. Stock solutions of capsaicin and PAF were made in 95% ethanol. The vehicle for PAF contained bovine serum albumin, 0.25% in saline. Olive oil was used to dilute TDI.

In one series of experiments, PAF 0.2 nmol/kg was given intra-arterially (via a catheter, PE10, introduced into the right subclavian artery) or intravenously 0.45 nmol/kg (via the left jugular vein). Control animals received vehicle by these routes.

In vivo labelling of red blood cells

About 1 MBq of $^{99m}TcO_4$, dissolved in saline, was given intravenously to animals that had previously received $KClO_4$ and $SnCl_2$ to block tissue uptake of ^{99m}Tc and stabilise its binding to erythrocytes, respectively. A specific labelling of red blood cells with negligible pertechnetate activity in other tissues including plasma, thyroid glands, and intestine was thus obtained.[1]

Passage of ^{131}I-albumin from mucosa into airway tissue

^{131}I-Albumin (1.2 μg human serum ^{131}I-albumin (1 KBq)) in vehicle with or without capsaicin 0.4 nmol was superfused on the tracheal mucosa (0.04 ml/2 min). The experiments were terminated 10 min after exposure. The tracheas underwent the lavage procedure and lavaged tissue was sampled and analysed (see below).

Sampling

The experiments were terminated by administration of 0.15–0.2 ml pentobarbital (Mebumal 60 mg/ml) intravenously. The chest was opened and the animal was exsanguinated by sectioning of the vena cava and

the still beating heart. Mixed arterial venous blood samples were collected in preweighed plastic tubes and in glass hematocrit capillaries. Some of the blood samples (after addition of heparin) were centrifuged (500 g for 10 min) for separation of plasma and red blood cells.

Tracheal lavage

After termination of the in vivo experiment, the trachea, larynx, lungs, and heart were excised in toto. The laryngeal cut edges were dried with a cell cotton cloth. After a brief rinse with saline of excess blood of the lung surfaces the whole preparation was arranged in a tilted position with the larynx upwards. The lobar bronchi were tied off at the hilar levels. A slightly bent plastic tubing (PE50) was gently introduced through a small incision (the edges of which had been blotted with a cell cotton tip) in the left principal bronchus and secured by ligature. The preparation was then mounted vertically (Fig. 1B) and the trachea was slowly rinsed 3–4 times during 1 min using 0.25 ml glucose free Krebs solution (composition in mM: NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15, adjusted to pH 7.4 with carbogen). The lavage fluid was infused to 0.5–1 cm below the level where the tip of the intratracheal catheter had been positioned. About 75–100 μl of the lavage fluid entered the tracheal lumen. The lavaged portion of the trachea could be identified because the fluid level was clearly visible through the thin illuminated tracheal wall.

Tracheal tissue

The trachea was cleaned from external vessels, lymph nodes and connective tissue. A section that had been exposed to superfusion and lavage (intrathoracic part) was put in a preweighed tube for further analysis.

Analyses

The samples of blood, plasma, tracheal tissue and tracheal lavage fluid were weighed on a Mettler AE 166. The activities of the isotopes ^{99m}Tc ^{131}I-albumin and ^{125}I-fibrinogen were counted in a NaI-detector (Harshaw) at 60% energy window, at levels of 162, 375 and 65 kev, respectively. The detector was connected to dual channel analysers (LEAB, Sweden) and the activities were recorded on a computer (LINK). The specificity of simultaneous analyses of more than one isotope was satisfactory for ^{99m}Tc and ^{131}I, as the interference was less than 0.5%. Similarly the propagation of ^{125}I into ^{131}I was less than 0.1%. However, the propagation of ^{131}I into ^{125}I was more than 20%. Hence, ^{125}I activity was counted 20 days later when the ^{131}I had declined considerably (half-lives ^{131}I 8 days; and ^{125}I 60 days). The tissue content of FITC-D was eluted by adding 2 ml sodium phosphate buffer, pH 7.5, to pieces of tracheal tissue followed by shaking for 15 h and centrifugation. Dilution of these supernatants, plasma samples, tracheal lavage fluids as well as FITC-D control solutions were analysed in a fluorimeter (Perkin Elmer LS-5). All dilutions were made with sodium phosphate buffer at pH 7.5. Hematocrit (Hct %) of the mixed blood samples was determined after centrifugation for 10 min (Coupalan, Hematocrit 4). This Hct has previously been shown to correspond to that of the blood-pool remaining in excised tracheas from exsanguinated guinea-pig controls.[1]

Calculations

The blood-pool (g) in the tissue samples and in the lavage was obtained by dividing the ^{99m}Tc counts of these samples with the ^{99m}Tc counts in 1 g blood. The activity of ^{131}I-albumin in the tissue and lavage samples emanates from the blood-pool and from any extravasated plasma. Counts of ^{131}I corresponding to the blood-pool was obtained by multiplying the blood-pool (g) of the samples with counts of ^{131}I measured in 1 g blood. The extravasated plasma volume was then obtained by dividing the surplus counts of ^{131}I (total counts minus blood-pool counts) by counts of ^{131}I in 1 g plasma. Similarly the FITC-D concentrations were used to calculate extravasated plasma.[1]

The blood pool in the tracheal lavage samples and tracheal tissue from both control and exposed animals showed little variation (see results). Hence, the ^{99m}Tc blood-pool determinations were excluded in several experiments and only the total amount of plasma in lavage or tracheal tissue was calculated as follows:

$$\frac{^{131}\text{I, } ^{125}\text{I or FITC-D in lavage or 50 mg tissue}}{^{131}\text{I, } ^{125}\text{I or FITC-D in 1 g plasma}}$$

The density of plasma was set at 1. The plasma volume (total or extravasated) was expressed as 'μl plasma' with reference to the corresponding macromolecular tracer (^{131}I-albumin, ^{125}I-fibrinogen or FITC-D).

Control of ^{131}I binding stability to albumin

Gel filtration with Sephadex G-25 (PD 10 column, Pharmacia) was used to test the binding stability of ^{131}I to the albumin fraction. Samples of lavage fluid and plasma (250 and 10 μl comprising 3200–4400 and 6900–10 200 counts/30 s of ^{131}I, respectively) as well as 1 μl from the stock solution ^{131}I human serum albumin (~36 000 counts/30 s) were added to a sodium phosphate buffer (ph 7.5, containing 0.01% sodium azide) to a volume of 1 ml. Twenty-five 1 ml fractions were collected and counted in the gamma-counter (^{131}I, kev 375). The distribution of ^{131}I activity in the fractions was compared with that obtained with ^{131}I-albumin from the stock solution.

Drugs

Anaesthetics: Ketamin (Ketalar 50 mg/ml Parke-Davis), xylacin (Rompun vet 20 mg/ml Bayer), pentobarbital (Mebumal vet 60 mg/ml ACO, Sweden).

Provoking agents: Bradykinin, capsaicin, carbachol, histamine hydrochloride, ovalbumin (Sigma), platelet activating factor (PAF, Calbiochem), toluene diisocyanate (TDI, 80% 2,4 TDI + 20% 2,6 TDI, Merck).

Tracers: Fluorescein isothiocyanate conjugated dextran FITC-D 156 kDa (Bioflor, Sweden), human serum ^{131}I-albumin (Kemi-intresse, Sweden), human serum fibrinogen (Sigma) labelled with ^{125}I (Amersham).

^{99m}Tc labelling of erythrocytes: Sodium pertechnetate (eluted from a ^{99}Mo-^{99m}Tc generator, Byk-Mallinckrodt), $SnCl_2$ (Merck) and $KClO_4$ (Periodin, POM).

Statistics

All data are expressed as mean ± SEM. Student's t-test for paired or unpaired data was used to evaluate the significance of differences between the means. Linear regression analysis was used to examine correlations between recovery of plasma ^{131}I-albumin in tracheal tissue and lavage liquid.

RESULTS

Stability of ^{131}I binding to albumin

After gel filtration of the ^{131}I-albumin stock solution, 89 ± 2% (n = 3) of total activity was recovered in the albumin fractions, 3–5, and 10.7 ± 0.2% in the iodine fractions, 8–9. In plasma and tracheal lavage samples 92 ± 2% and 91 ± 3% (n = 3) of the total activity was found in the albumin fractions, indicating that the ^{131}I was firmly bound to albumin in plasma and lavage fluid.

Another indication of the firm ^{131}I binding to albumin (during the employed time period 15–25 min) was the lack of ^{131}I accumulation in the thyroid glands, which were taken out and analysed along with the tracheal tissue. The plasma volume in the thyroid glands based on measurements of the ^{131}I-albumin content was 5.6 ± 0.4% (n = 20). Calculation of the thyroid plasma volume from the erythrocyte-bound ^{99m}Tc activities and Hct-values gave a similar value (5.9 ± 0.3%, n = 20). These thyroid plasma values are of an expected magnitude i.e. comparable with those found in tracheal tissue from control animals.

Airway blood-pools

Under control conditions where the plasma tracers were confined to the vascular compartment the blood-pool of the tissue samples could be calculated from the plasma volume corresponding to either tracer and the individual Hct values. The blood-pool of control tissues, as calculated with different plasma tracers (cf Table 1A) did not differ ($p > 0.05$) and the total mean value was 5.9 ± 0.3% (n = 40). The ^{99m}Tc blood-pool in these animals (n = 40) was 5.3 ± 0.4%, which also did not differ ($p > 0.05$) from that in tracheae exposed to capsaicin 0.4 nmol, PAF 4 nmol, histamine 8 nmol and PAF 0.2 nmol/kg i.a. (n = 24). However, when PAF (0.45 nmol/kg) was given i.v. the tissue blood-pool increased from 4.9 ± 0.8% (n = 5) to 8.4 ± 1% (n = 3) ($p < 0.05$).

The ^{99m}Tc blood-pools of lavage fluids obtained from saline and vehicle (0.25% BSA + 0.1% ethanol) exposed tracheas were 0.022 ± 0.005 µl (n = 10) and 0.042 ± 0.01 µl (n = 10), respectively. Erythrocyte counts in the lavage fluids from several experiments (Burcher chamber) corresponded to blood volumes consistently less than 0.04 µl (data not shown). There was no significant difference ($p > 0.05$) between blood-pools (^{99m}Tc) in lavage fluids from mediator-exposed tracheas, except for histamine 8 nmol superfused on

Table 1A. Plasma (µl) recovered in lavage fluid under control conditions as estimated with different plasma macromolecular tracers. Tracers given simultaneously appear on the same line.

Treatment	^{131}I-albumin (µl)	FITC-D (µl)	^{125}I-fibrinogen (µl)
Saline	0.081 ± 0.020 (n = 10)	0.130 ± 0.070 (n = 10)	
Saline + 0.1% ethanol	0.091 ± 0.010 (n = 12)	0.187 ± 0.078 (n = 12)	
		0.241 ± 0.057 (n = 5)	0.000 ± 0.000 (n = 5)
BSA 0.25% + 0.1% ethanol	0.097 ± 0.021 (n = 10)	0.173 ± 0.019 (n = 10)	
	0.150 ± 0.029 (n = 4)		0.000 ± 0.000 (n = 4)

Table 1B. Content of plasma (µl) in 50 mg tracheal tissue under control conditions as estimated with different plasma tracer macromolecules. Tracers given simultaneously appear on the same line.

Treatment	^{131}I-albumin (µl)	FITC-D (µl)	^{125}I-fibrinogen (µl)
Saline	1.72 ± 0.19 (n = 10)	1.68 ± 0.37 (n = 10)	
Saline + 0.1% ethanol	1.88 ± 0.21 (n = 12)	1.78 ± 0.41 (n = 12)	
		1.89 ± 0.28 (n = 5)	1.92 ± 0.11 (n = 5)
BSA 0.25% + 0.1% ethanol	1.86 ± 0.15 (n = 10)	1.50 ± 0.29 (n = 10)	
	1.27 ± 0.12 (n = 4)		1.47 ± 0.11 (n = 4)

the mucosa. Then the blood-pool increased from 0.042 ± 0.005 µl (n=10) to 0.180 ± 0.019 µl (n=6) ($p < 0.001$).

Recovery of tracheal lavage fluid

The amounts of lavage fluid (0.25 ml) recovered from control animals (untreated and vehicle exposed) was 0.240 ± 0.005 ml (mean ± SEM, n=42). The recoveries from bradykinin (2, 5 and 10 nmol), capsaicin (0.4 nmol), PAF (4 and 8 nmol) and ovalbumin (3 pmnol), sensitised animals) treated tracheae were 0.248 ± 0.009 (n=26), 0.231 ± 0.0008 (n=17), 0.245 ± 0.007 ml n=12), and 0.245 ± 0.008 ml (n=8), respectively.

Plasma macromolecules in tracheal lavage under control conditions

Control experiments showed that the lavage fluid of tracheae superfused with saline or the other employed vehicles contained only small amounts of the plasma macromolecular tracers (^{131}I-albumin and FITC-D) corresponding to plasma volumes of about 0.1 µl. The larger protein ^{125}I-fibrinogen (MW 341 kDa), that was examined simultaneously with either FITC-D (MW 156 kDa) or ^{131}I-albumin (MW 69 kDa), was not detectable in the lavage fluid under baseline conditions (Table 1A). Under control conditions the luminal content of FITC-D corresponded to a plasma volume slightly but not significantly ($p > 0.05$) larger than that of ^{131}I-albumin (Table 1A).

Plasma macromolecules in tracheal tissue under control conditions

The tracheal tissue contents of ^{131}I-albumin, FITC-D and ^{125}I-fibrinogen expressed as plasma volumes per 50 mg tissue are accounted for in Table 1B.

Mucosal provocation-induced effects on macromolecular leakage

Carbachol: Carbachol 8 and 16 nmol induced only a marginal leakage of plasma (^{131}I-albumin) into the airway lumen (Table 2). The 2 min superfusion with 16 nmol of carbachol produced respiratory distress and fatal bronchoconstriction (n=3). These animals had highly distended lung lobes.

Histamine and bradykinin: Superfusion of the tracheal mucosa with histamine 2, 5 and 8 nmol or bradykinin 2, 5 and 10 nmol induced a dose-dependent and significant leakage of ^{131}I-albumin into the airway wall and lumen (Table 2).

Capsaicin: Different aspects of the capsaicin-induced plasma exudation were examined, including the acute time course, leakage of different sized plasma tracers, and concomitant tissue uptake of luminal ^{131}I-albumin.

Even at 1 min the capsaicin (0.4 nmol)-induced leakage was significant (Table 3). The lavage fluid contained less plasma than at 10 min ($p < 0.05$), whereas the tissue content of plasma at 1 min was not different ($p > 0.05$) from the 10 min value (Table 3). At 1 and 5 h after provocation with capsaicin there was no significant plasma leakage ($p > 0.05$).

Capsaicin 0.1 nmol (n=5) increased the plasma content (FITC-D) of the lavage fluid and tracheal tissue to 0.60 ± 0.32 µl and 3.60 ± 0.70 µl/50 mg, respectively. With capsaicin 0.4 nmol a further leakage

Table 2. Recovery of plasma (^{131}I-albumin) after local superfusion of the tracheal mucosa with histamine and carbachol.

	Lavage fluid (µl)	Tracheal tissue (µl/50 mg)
Carbachol 8 nmol	0.219 ± 0.052^a (n=5)	2.24 ± 0.40 (n=5)
16 nmol	0.198; 0.280 (n=2)	2.19; 2.43 (n=2)
Bradykinin 2 nmol	0.261 ± 0.070^a (n=6)	1.93 ± 0.33
5 nmol	2.061 ± 0.187^c (n=10)	3.9 ± 0.38
10 nmol	2.678 ± 0.298^c (n=10)	4.56 ± 0.47
Histamine 2 nmol	0.399 ± 0.056^c (n=5)	1.83 ± 0.19 (n=5)
5 nmol	1.561 ± 0.420^c (n=5)	2.51 ± 0.31 (n=5)
8 nmol	2.190 ± 0.640^c (n=6)	5.79 ± 0.92^c (n=6)

a = $p < 0.05$, c = $p < 0.001$. Comparisons were made with saline controls (Tables 1A and 1B).

Table 3. 'Plasma' (µl) recovered in lavage fluid and tracheal tissue after local exposure to capsaicin 0.4 nmol.

Capsaicin 0.4 nmol Plasma tracer	Lavage fluid (µl)	Tracheal tissue (µl/50 mg)
1 min after exposure ^{131}I-albumin	0.577 ± 0.18^c (n=5)	4.73 ± 0.82^b (n=5)
10 min after exposure ^{131}I-albumin	1.46 ± 0.34^c (n=12)	4.84 ± 0.6^b (n=12)
FITC-dextran	3.06 ± 0.7^c (n=12)	6.46 ± 1.1^b (n=12)
	2.43 ± 0.8^c (n=5)	5.13 ± 0.54^b (n=5)
^{125}I-fibrinogen	1.64 ± 0.34^c (n=5)	4.01 ± 0.62^b (n=5)

b = $p < 0.01$, c = $p < 0.001$. Comparisons were made with saline-ethanol controls (Tables 1A and 1B).

was seen and it was demonstrated that fibrinogen (341 kDa) leaked slightly less across the mucosal barrier than FITC-D (156 kDa) (Table 3). In a separate set of experiments it was demonstrated that the still smaller molecule ^{131}I-albumin (68 kDa) also leaked somewhat less into the lumen that FITC-D (Table 3).

Ten minutes after mucosal application of ^{131}I-albumin control animals had 570 ± 37 counts/30 sec in 50 mg tracheal tissue (n = 8), whereas after concomitant capsaicin (0.4 nmol) superfusion the tissue activity of ^{131}I-albumin was slightly but significantly ($p < 0.01$) increased to 852 ± 68 counts/30 s n = 8).

PAF: One min after tracheal superfusion of PAF 4 nmol the recovery of plasma (^{131}I-albumin or FITC-D) in lavage and tracheal tissue was not different from controls ($p > 0.05$) (Fig. 2). Ten min after exposure to PAF-acether 4 and 8 nmol a dose-dependent leakage of plasma into tracheal tissue ($p < 0.05$) and lumen had been induced (Table 4). The 8 nmol dose produced laboured breathing in 3 out of 8 animals. FITC-D was recovered in the lavage in larger amounts than ^{131}I-albumin after 4 and 8 nmol PAF (Table 4).

After tracheal superfusion of PAF-acether 4 nmol experiments were also terminated 1, 3, 5, 7 and 17 h after exposure. Only at 1 and 5 h there was a significantly increased recovery of extravasated plasma (^{131}I-albumin) in both the airway wall and lumen (Fig. 3).

After receiving PAF 0.45 nmol/kg i.v. the animals became pale and the respiration ceased for 30–60 s. The tracheal blood-pool was significantly ($p < 0.05$) elevated compared with control or i.a. administration.

Table 4. 'Plasma' (μl) recovered in lavage fluid and tracheal tissue after local exposure to PAF 4 and 8 nmol.

Plasma tracer	Lavage fluid (μl)	Tracheal tissue (μl/50 mg)
PAF 4 nmol		
^{131}I-albumin	1.99 ± 0.19[c] (n = 8)	7.01 ± 0.4[c] (n = 8)
	2.11 ± 0.81[c] (n = 4)	7.35 ± 1.0[c] (n = 4)
FITC-dextran	3.79 ± 0.79[c] (n = 8)	6.41 ± 1.0[c] (n = 8)
^{125}I-fibrinogen	1.55 ± 0.28[c] (n = 4)	5.98 ± 0.4[c] (n = 4)
PAF 8 nmol		
^{131}I-albumin	3.61 ± 0.89[c] (n = 8)	10.59 ± 1.4[c] (n = 8)
FITC-dextran	9.5 ± 2.78[c] (n = 8)	14.38 ± 1.5[c] (n = 8)

c = $p < 0.001$. Comparisons were made with BSA-ethanol controls (Tables 1A and 1B).

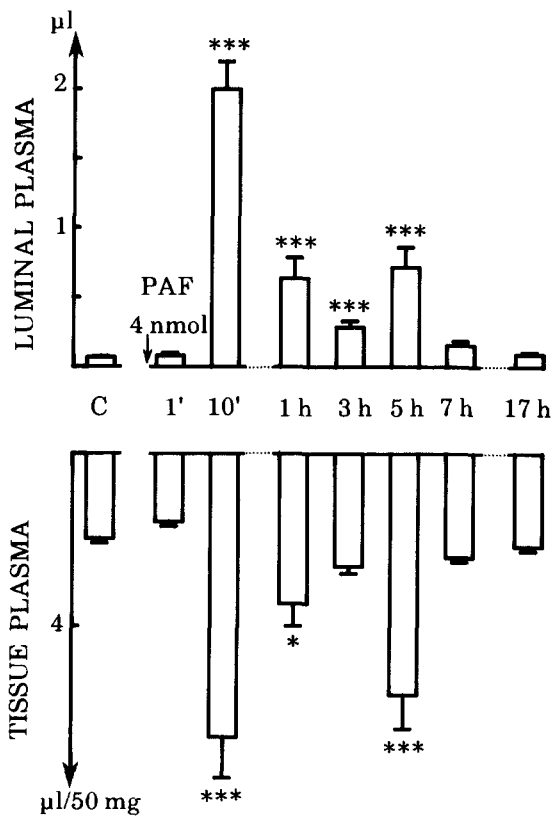

Fig. 2—Recovery of μl plasma (^{131}I-albumin) in lavage fluid (↑) and tracheal tissue (↓) 1 and 10 min and 1, 3, 5, 7 and 17 h after superfusion with PAF 4 nmol (n = 5–8 in each group). C = control, vehicle (n = 38 pooled value) * = $p < 0.005$; *** = $p < 0.001$. Comparisons were made with corresponding control group.

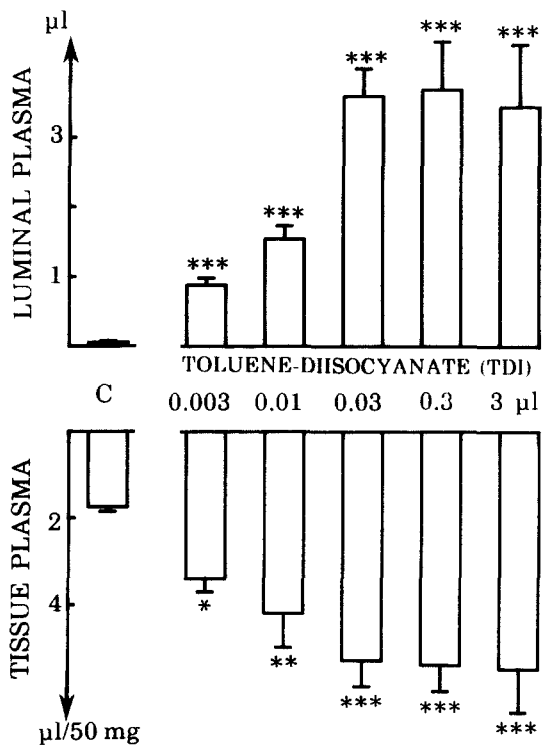

Fig. 3—Recovery of plasma (^{131}I-albumin) in lavage fluid (↑) and tracheal tissue (↓) 300 min after local superfusion of TDI 0.003 – 3 μl (n = 5–12 in each group). C = control, olive oil (pooled values, n = 19) * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Comparisons were made with corresponding control groups (see also Table 6).

However, there was still a significant increase in extravasated plasma in the airway wall as well as in the airway lumen. Iv PAF also produced a pulmonary oedema. It doubled the pulmonary plasma volume from 4.7 ± 0.3 µl/50 mg (vehicle treated n=5) to 8.3 ± 0.6 µl/50 mg. PAF given i.a. was without these pulmonary effects (data not shown).

Allergen (ovalbumin): In the sensitised guinea-pigs, superfusion of ovalbumin 3 pmol produced extensive leakage of plasma (^{131}I-albumin and FITC-D) into airway lumen and tissue (Table 5). Ovalbumin 6 pmol produced fatal respiratory distress within 4 min in 3 animals. The extravasated plasma in these animals amounted to 5–17 µl and 8–18 µl/50 mg in lavage and tissue samples, respectively. Sensitised control did not differ from other control animals ($p > 0.05$) and are included in the pooled control value (Tables 1A and 1B).

Toluene diisocyanate (TDI): The vehicle used for TDI dilutions, olive oil, was without effects on leakage at any of the subsequent sampling times ($p > 0.05$) (see also Table 6). Tracheal superfusions of TDI 0.003–3 µl (0.02 ml/1 min) produced prompt and sustained plasma leakage with a peak at 5 h (Table 6). At this time point there were dose-dependent effects with a maximum reached with 0.03 µl TDI (Fig. 3). At 10 min and 17 h the maximum response was produced by 0.3 µl TDI (Table 6). TDI 3 µl exposure caused respiratory distress and 30% of the animals died within 2 h after provocation. Macroscopic examination showed distended lung lobes in these animals.

In one set of experiments the simultaneous leakage of FITC-D and ^{125}I-fibrinogen was examined 5 h after challenge with TDI 0.03 µl (n=5) or control olive oil (n=5). The recovery of plasma corresponding to FITC-D and ^{125}I-fibrinogen in lavage fluids was 11.85 ± 1.2 µl and 1.49 ± 0.18 µl, respectively. In the airway wall the recovery of FITC-D was 5.85 ± 1 µl/50 mg and that of ^{125}I-fibrinogen 13.1 ± 2.2 µl/50 mg. The recoveries of either tracers in lumen or lavage was significantly increased compared to control ($p < 0.001$).

Table 5. 'Plasma' (µl) recovered in lavage fluid and tracheal tissue of sensitised animals after local exposure to ovalbumin 3 pmol.

Ovalbumin (allergen) 3 pmol Plasma tracer	Lavage fluid (µl)	Tracheal tissue (µl/50 mg)
^{131}I-albumin	2.7 ± 0.4^c (n=8)	4.67 ± 0.9^b (n=8)
FITC-dextran	6.0 ± 0.9^c (n=8)	7.8 ± 1.2^c (n=8)

b=p<0.01, c=p<0.001. Comparisons were made with saline controls (Tables 1A and 1B.)

Table 6. Time course of effects of TDI 0.003–3 µl superfusions. Recovery of 'plasma' ^{131}I-albumin in lavage fluid and tracheal wall at different time points after TDI or vehicle olive oil exposure.

Treatment	Time after exposure	Number animals	Lavage fluid (µl)	Tracheal wall (µl/50 mg)
Control olive oil	10 min–17 h	16	0.05 ± 0.02	1.75 ± 0.11
TDI 0.003 µl	10 min	5	0.31 ± 0.04^c	$2.9\ 0.4^a$
	300 min	16	0.88 ± 0.10^c	3.4 ± 0.3^c
	17 h	4	0.21 ± 0.04^c	2.9 ± 0.2^b
TDI 0.03 µl	10 min	5	0.42 ± 0.09^c	3.4 ± 0.2^c
	300 min	10	3.60 ± 0.40^c	5.3 ± 0.6^c
	17 h	6	0.59 ± 0.10^c	3.0 ± 0.4^a
TDI 0.3 µl	10 min	4	1.22 ± 0.19^c	5.3 ± 2.11^c
	300 min	6	3.70 ± 0.69^c	5.9 ± 0.6^c
	17 h	4	1.15 ± 0.63^c	3.5 ± 0.4^c
TDI 3 µl	10 min	5	0.81 ± 0.19^c	3.3 ± 0.5^b
	60 min	5	0.63 ± 0.31^c	3.1 ± 0.5^b
	300 min	5	3.45 ± 0.90^c	5.5 ± 1.0^b
	17 h	4	0.99 ± 0.29^c	2.6 ± 0.1^c

a=p<0.05, b=p<0.01, c=p<0.001. Note that statistical comparisons were made with corresponding control groups and not the tabulated pool value.

Correlation of exuded plasma in tissue and lumen

Regression analysis showed a variable but significant correlation of recovered plasma (^{131}I-albumin) in tracheal tissue and tracheal lavage fluid at the selected time points for measurement (Table 7).

DISCUSSION

Normally the tracheobronchial vascular endothelium and the mucosal epithelium are effective barriers severely restricting the passage of macromolecules in

Table 7. Correlation between tissue and luminal contents of exuded plasma (^{131}I-albumin)

Mediator	Time (min after exposure)	Correlation coefficient
Histamine 2 nmol	10	0.502
5 nmol	10	0.290
8 nmol	10	0.572
Bradykinin 2 nmol	10	0.580
5 nmol	10	0.689
10 nmol	10	0.892
Capsaicin 0.4 nmol	1	0.967
0.4 nmol	10	0.621
PAF 4 nmol	10	0.736
4 nmol	60	0.111
4 nmol	180	0.836
4 nmol	300	−0.470
8 nmol	10	0.978
Allergen 3 pmol	10	0.332
TDI 0.003 µl	300	0.406
0.03 µl	300	0.458
0.3 µl	300	0.891
3 µl	300	0.921

either direction. Small amounts of albumin (70 kDa) is a normal constituent of airway liquids but much larger plasma molecules such as fibrinogen (342 kDa) may not be present. In this study ^{131}I-albumin as well as FITC-D (156 kDa) were consistently present in minute amounts in the lavage fluid obtained from control tracheae, whereas ^{125}I-fibrinogen was absent. In the saline-exposed tissue all macromolecular plasma tracers were confined to the vascular compartment. Hence the present technique of mucosal provocation[1] and retrieval of mucosal surface liquids did not disturb the airway mucosal and vascular barrier mechanisms. This view is further supported by the fact that negligible amounts of erythrocytes were observed in the lavage liquids. The present technique also provided sampling from a defined area of large airway mucosa with quantitative recovery of the lavage fluids.

Topical application of a wide variety of inflammatory stimuli, histamine, bradykinin, PAF, capsaicin (a neurogenic stimulus), allergen, and TDI (an occupational asthma agent), produced prompt dose-related leakage of plasma in the airway. PAF produced also a late phase leakiness at 300 min. Similar to the action of mediators, the immediate allergen-induced response in the IgE-sensitised guinea pigs, was associated with plasma leakage. The stronger the allergic reaction the more pronounced and rapid was the leakage into the wall and lumen. In single animals it was observed that endothelial-epithelial passage of plasma occurred within 1 min after the allergen provocation. Fick et al[5] studied plasma leakage in atopic asthmatics, but their results are difficult to interpret. After restricted local allergen challenge they observed that the concentration of fibrinogen actually was decreased in the recovered lavage liquids whereas albumin levels rose slightly, by 65%. However, after inhalational allergen provocations all measured proteins in the bronchoaveolar lavage fluids, including fibrinogen, were slightly increased to values 14–95% above the pre-allergen state.[5] More airway specific techniques than bronchoalveolar lavage seem warranted in studies of the plasma exudation that occurs in asthma.[6]

TDI induced a prompt and very sustained plasma leakage into the wall and lumen. The response was dose-dependent starting even at 0.003 μl of TDI. This observation will allow studies in greater detail than previously of the possibility that sensitisation to TDI may be induced in guinea-pig airways.[7] In sensitised subjects with TDI-asthma Fabbri et al[8] have observed that bronchial lavage liquids contained significantly elevated albumin levels prior to and during a late phase reaction to inhaled TDI.

The present findings on airway plasma leakage show that the inflammatory response to several mucosal provocations is rapid in onset. Even biphasic and sustained responses have an inflammatory component that is expressed promptly as leakage of plasma.

Tracheobronchial plasma leakage in experimental studies has previously been measured almost exclusively in the tissue of airways. Perhaps the reputed tightness of airway epithelium has led to an underestimation of the mucosal passage as a potential clearance route of the exuded plasma. The present data on a variety of stimuli extend our previous preliminary findings[3] and support the view that plasma exuded in response to tracheobronchial mucosal inflammatory provocations will not remain in the interstitial space of the airway wall but rapidly enters the airway lumen. This further leakage of plasma may be significant within 1–3 min after provocation and is an important clearance route of extravasated plasma.

There was no difference between the amount of plasma recovered in the tracheal wall 1 and 10 min after provocation with capsaicin, whereas the luminal content rose significantly. These observations are consistent with a brief duration of the vascular leak response. A rapid shut off of extravasation also explains a few observations of relatively larger amounts of plasma tracers in the lumen compared to the wall. This occurred in some animals at 10 min after histamine and at responses occurring 1 and 5 h after PAF. As a consequence a poorer correlation was found between lumen and wall contents of exuded plasma in these groups of animals. In fact, a negative correlation was found between the two methods for plasma exuded around 5 h after provocation with PAF suggesting an unusually rapid clearance into the lumen. Otherwise a good correlation was observed for the immediate responses as well as under conditions of sustained leakiness such as that induced by mucosal provocation with TDI. Even at threshold inflammatory responses, plasma leakage into tissue and lumen was correlated. These correlations demonstrate that the course of an inflammatory process in tracheobronchial mucosa/submucosa can be followed and quantified by analysing plasma tracers in mucosal surface liquids. In contrast to inflammatory cells, which may be present in the airways for rather trivial reasons or for tissue repair as well as for active inflammation, the plasma tracers will indicate that an inflammatory process is ongoing and is, indeed, affecting airway tissue. Furthermore, exuded plasma itself is a multipotential pathogenetic factor in obstructive airway disease.[6]

Complex mucosal/submucosal inflammatory processes seem to play a major role in asthma. Hence, for the purpose of the present investigation mucosal provocations were considered preferable to systemic administrations of inflammatory factors. As exemplified with PAF, it was also demonstrated that the intravascular routes of provocations may produce somewhat different results to those obtained by the present local superfusion technique. After intra-arterial administration of PAF, an accumulation of plasma proteins in the airway wall was associated with only minor further passage of plasma into the lumen.

With i.v. PAF there were additional complications. Compared to the topically effective dose a low intravenous dose was required for the airway inflammatory response but this effect was now associated with a number of other alterations such as an elevated blood-pool in the airway wall as well as the production of a significant protein-rich pulmonary oedema. Intravenous PAF was also associated with signs of severe stress of the animals. Inferentially, asthma-related aspects as well as specificity requirements favour topical mucosal provocations in studies of airway plasma exudation.

Five h after application of TDI, relatively more FITC-D passed into the lumen, whereas a larger proportion of fibrinogen stayed longer in the interstitium. This may have been due to the larger size of fibrinogen and a greater size-selectivity of the epithelial compared to the endothelial barrier. Activation of the coagulation system with fibrin formation in the submucosa might also explain to these data. A strict dependence on size of the tracer was not observed in this study. After capsaicin provocations about equal amounts of albumin and fibrinogen passed into tissue and lumen. Despite its larger size (156 kDa) FITC-D was found to pass more readily than albumin into the airway lumen at a variety of provocations. Hence, in response to mucosal inflammation there may be an almost unrestricted bulk flow of exuded plasma across the epithelial lining into the lumen.

There was only a small (1.5-fold) increase in tissue uptake of luminal macromolecules during exposure to an inflammatory stimulus (capsaicin) that produced marked (20-fold) increase in plasma tracers in the lumen. Such a largely one directional passage could suggest a secretory process. Indeed, the tracheal epithelium has recently been demonstrated in vitro to have a capability to secrete albumin.[9] The quantitative importance of this secretory mechanism is not known. For a variety of reasons secretion would not have contributed to the present findings: 1) Muscarinic stimulants increase the albumin secretion[9] but in the present study even lethal bronchoconstricting doses of carbachol produced only marginal increases in the mucosal surface content of plasma tracers. 2) β_2-receptor agonists increase the albumin secretion[9] but they significantly reduce the plasma exudation (wall and lumen) induced by many of the present mucosal provocations.[6] 3) Finally, FITC-dextran macromolecules are not secreted[9] yet in the present study FITC-dextran passed more readily that albumin into the lumen.

Taken together the present observations suggest a mechanism by which the epithelial passage of plasma can occur in inflamed airways. The fact that plasma appeared promptly on the mucosal surface after all types of inflammatory provocations suggest the involvement of a general epithelial passage mechanism that is coupled to the vascular leakage. At mucosal inflammatory provocations it is likely that the closest microvessels, abundant just beneath the epithelium, will be affected and leak plasma. Hence, the subepithelial interstitium will be endowed with exuded plasma. The negative surface charges and other factors in this milieu activate the exuded plasma protein systems. This activation will produce a large number of inflammatory peptides and the increased number of molecules will increase the osmotic load. Hence, the epithelium will be under attack from the basal side by the exudate and there will likely be a build-up of interstitial pressure. This pressure increase, perhaps together with mediator-induced destabilisation of the epithelium, will cause the epithelial cells to transiently separate thus allowing passage of interstitial plasma along a pressure gradient into the lumen. This hypothesis tallies with our previous suggestion that a paracellular epithelial route must account for the rapid flow of a large volume of plasma exudate/transudate into the lumen.[3] It is supported by the finding of only a small increase in absorption of luminal macromolecules concomitant to the exudation process. The present hypothesis is also supported by the findings of other works. McDonald[10] has demonstrated that inflammatory plasma leakage in rat trachea may preferentially be towards the epithelium. Man et al[11] have demonstrated that an osmotic load produced marked ultrastructural changes in the epithelium, only when that challenge was applied on the serosal side. It is known from experiments with everted gallbladder in vitro that even a slight increase in the serosal pressure may make the epithelium pervious and its cells separated.[12] Finally, Nordin[13] has reported that high interstitial osmotic pressures might occur in the mucosa of the rat trachea that in vivo was subjected to intubation as a kind of inflammatory stimulus.

The passage of exuded plasmsa into the lumen reduces the tendency of submucosal oedema in inflamed airways. The rapid appearance of plasma on the mucosal surface is an important defense reaction, which is even more functional if luminal material at the same time is not allowed to freely pass into the airway tissue. Exaggerated extravasation and mucosal passage of plasma may be pathogenetic in several ways.[6]

Acknowledgement

We thank Mr Z. G-Wagner, Fyzicon, Malmoe, for advice concerning the radioactivity analyses and Mrs I. Källen and Mrs M. Broman for secretarial assistance.

References

1. Erjefält I, G-Wagner Z, Strand S E, Persson C G A. A method for studies of tracheobronchial microvascular permeability ot macromolecules. J Pharm Meth 1985; 14: 275–283.

2. Persson C G A, Erjefält I, Andersson P. Leakage of macromolecules from guinea-pig tracheobronchial micro-circulation. Effects of allergen, leukotrienes, tachykinins and anti-asthma drugs. Acta Physiol Scand 1986; 127: 95–105.
3. Persson C G A, Erjefält I. Inflammatory leakage of macromolecules from the vascular compartment into the tracheal lumen. Acta Physiol Scand 1986; 126: 615–616.
4. Andersson P. Antigen-induced bronchial anaphylaxis in actively sensitised guinea-pigs. Allergy 1980; 35: 65–71.
5. Fick R B, Metzer W J, Richerson H B, Zavala D C, Moseley P L, Schoderbek W E, Hunninghake G W. Increased bronchovascular permeability after allergen exposure in sensitive asthmatics. J Appl Physiol 1987; 63 (3): 1147–1155.
6. Persson C G A. Plasma exudation and asthma. Lung 1988; 166: 1–23.
7. Karol M H. Respiratory effects of inhaled isocyanates. C R C Critical Reviews in Toxicology 1986; 16: 349–379.
8. Fabbri L M, Boschetto P, Zocca E, Milani G, Pivirotto F, Plebani M, Burlina A, Licata B, Mapp C E. Bronchoalveolar neutrophilia during late asthmatic reaction induced by toluene diisocyanate. Am Rev Respir Dis 1987; 136: 36–42.
9. Webber S E, Widdicombe J G. The transport of albumin across the ferret in-vitro whole trachea. J Physiol 1989 in press.
10. McDonald D M. Neurogenic inflammation in the respiratory tract: actions of sensory nerve mediators on blood vessels and epithelium of the mucosa. Am Rev Respir Dis 1987; 136: S65–S72.
11. Man S F P, Thompson A B R, Hulbert W, Park D S K, Hogg J C. Asymmetry of canine tracheal epithelium: osmotically induced changes. J Appl Physiol 1984; 57: 1338–46.
12. Tormey J McD, Diamond J M. The ultrastructural route of fluid transport in rabbit gall bladder. J Gen Physiol 1967; 50: 31–60.
13. Nordin U. The trachea and cuff-induced tracheal injury. Acta Oto-Laryngol 1977 (suppl 345): 1–71.

Date received: 5 September 1988
Date revised: 23 December 1988
Date accepted: 5 January 1989