

Increased sensitivity to toluene diisocyanate (TDI) in airways previously exposed to low doses of TDI

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Summary

Repeated airway exposures to toluene diisocyanate (TDI) may cause sensitization and asthma. This study has examined the acute inflammatory response to TDI in guinea-pig tracheobronchial airways, the development of increased sensitivity to TDI and the effects of xanthines and a glucocorticoid on these responses to TDI. A restricted surface area of the tracheobronchial mucosa of Ketalar-Xylazin anaesthetized guinea-pigs was exposed to TDI, dissolved in olive oil, by means of 1 min infusions through an oral catheter. The TDI-induced inflammatory process was quantified by determination of airway luminal entry of plasma. Already 3 nl (~ 20 pmol) of TDI produced a significant and sustained exudation response ($P < 0.001$ to $P < 0.01$, 5 and 17 hr after exposure). Pretreatment with intravenous enprofylline (25 $\mu\text{mol/kg}$) reduced ($P < 0.05$) the acute response measured at 5 hr whereas budesonide (116 $\mu\text{mol/kg}$ intraperitoneally or 26 $\mu\text{mol/kg}$ by tracheal superfusion) was without effect. Two repeated exposures to TDI 3 nl (on days 1 and 8) made the animals hyperresponsive to TDI so that on day 15 a previously subthreshold dose of TDI (0.3 nl) produced significant exudation both at 5 and 17 hr after exposure ($P < 0.001$ to $P < 0.01$). Similarly, two repeated dermal exposures to a large dose of TDI (20 μl) lowered the threshold for tracheal provocation with TDI. Budesonide (2.6 $\mu\text{mol/kg}$ orally) given daily during the topical airway 'sensitization' regimen (days 1–14) significantly reduced the response to the subsequent 0.3 nl challenge dose of TDI ($P < 0.05$). The effects of daily treatments with either theophylline (100 $\mu\text{mol/kg}$) or enprofylline (50 $\mu\text{mol/kg}$) were not significant. We conclude that two separate airway exposures to a low inflammatory dose of TDI increased about tenfold the airway mucosal sensitivity to this agent. Only the acute inflammatory response was reduced by xanthines. The development of increased responsiveness but not the acute inflammatory response to TDI was reduced by glucocorticoid treatment.

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Introduction

Diisocyanates are some of the most important low molecular weight chemicals associated with occupational asthma [1]. These compounds are often volatile and they are highly reactive on mucous membranes especially conjunctivae and the respiratory tract [2]. The isocyanates, particularly toluene-diisocyanate (TDI), have the potential for sensitizing subpopulations of exposed workers [1,3]. Sensitized subjects may develop early and/

or late asthmatic reactions after exposure to low concentrations of TDI [4–6]. Asthmatic sensitization can be induced not only by inhalation but potentially also by dermal exposures to TDI [1,7,8].

TDI is a potent contact sensitizer also in animals. After single or dual applications of high doses, amounting to 1–25 μl of TDI onto the skin of rats and mice, a single topical challenge with a lower dose of TDI produced a delayed type hypersensitivity reaction (DTH-reaction) characterized by a marked ear- or foot-swelling [9–11]. However, little appears to be known about increased sensitivity to TDI in animal tracheobronchial airways.

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This study examines whether the tracheobronchial airways of guinea-pigs can be sensitized by repeated dermal applications of large doses of TDI and alternatively, by repeated exposures of the tracheobronchial mucosa to low doses of TDI. Furthermore, the effects of pretreatment with xanthines or a glucocorticoid were examined both against the acute TDI-induced airway inflammation and against the development of increased sensitivity to TDI. The inflammatory action of topical TDI was recorded by determination of plasma exudation into the tracheobronchial airways. This mucosal exudation may be a quantitative index of ongoing airway tissue inflammation irrespective of which cellular mechanism is involved [12–15]. In accordance we have previously shown that single exposures to TDI produce immediate and sustained late phase plasma exudation into the airway lumen. This response is dose-dependent and occurs already at doses of TDI in the nanolitre range [14].

Material and methods

Guinea-pigs (≈ 350 g, male, D-H) starved for 3–4 hr were anaesthetized with a 3:2 mixture of ketamin (50 mg/ml) and xylazin (20 mg/ml) intramuscularly (1 ml/kg). For intratracheal superfusions of solutions the animals were inclined to a 45° position (head up) and a thin plastic catheter (OD 0.61 mm) was introduced via the mouth and placed into the tracheal lumen about 0.5–1 cm below the larynx. During mucosal superfusion (at a rate of 0.02 ml/min) the catheter was connected to a Sage servo pump [15–17].

Single exposure to TDI

Ten minutes after intubation TDI 0.005–0.15 μ l/ml dissolved in olive oil or olive oil (control) was superfused onto the tracheal mucosa. (Total amount of TDI superfused 0.1–3 nl = ≈ 0.7 – ≈ 20 pmol.) After extubation the animals were maintained in the inclined position for 5 min and then placed in a heated chamber (≈ 28 – 30°C ; 50% humidity) until recovery from the anaesthesia.

Repeated dermal exposures to TDI

A group of animals were shaved on the right side of their backs. The following day (day 1) 100 μ l of a 20% TDI solution in olive oil and ethyl-acetate 1:1 was administered on the shaved back (area 5 cm²) by gently rubbing with a glass rod. On day 7 the left side was shaved and on day 8, 100 μ l of the TDI solution was applied onto the skin. After each application the animals were kept single for 24 hr.

Repeated tracheobronchial exposures to TDI

On days 1 and 8 the animals were anaesthetized and orally catheterized (see above). TDI 0.15 μ l/ml dissolved in olive

oil or olive oil (control) was superfused onto the tracheobronchial mucosa at a constant rate of 0.02 ml/min. The total amount of TDI at each occasion superfused was 3 nl (≈ 20 pmol). Control animals were exposed to olive oil 0.02 ml/min. The sensitization procedure was performed in two separate studies (studies I and II; see Tables 3 and 4). After extubation the animals were maintained in the tilted position for 5 min and then placed in the heated (28–30°; 50% humidity) chamber until recovery from anaesthesia.

Provocation of pre-exposed animals

On day 15 the animals that previously had received TDI dermally or on the tracheal mucosa were prepared for tracheal superfusion and TDI 0.005 or 0.015 μ l/ml was administered (0.02 ml/min). Total dose of TDI 0.1 or 0.3 nl (≈ 0.7 – ≈ 2 pmol). Control animals received vehicle (olive oil; 0.02 ml/min). After extubation the animals were treated as described above.

Drug pre-treatment

Single administration. Enprofylline 25 μ mol/kg (10 mg/ml in saline pH 9.4) was given intravenously 5 min before a single provocation with TDI 3 nl (≈ 20 pmol). Budesonide 116 μ mol/kg (45 mg/kg; 10 mg/ml suspension in saline) was given intraperitoneally 17 hr before provocation ($n=6$ each group). Control animals received saline intravenously 5 min before TDI 3 nl. Budesonide 26 μ mol/kg/ml was also superfused (0.02 ml/min) onto the tracheal mucosa 17 hr prior to exposure to TDI 3 nl ($n=6$).

Repeated drug administration. On days 1–14 (during the sensitization regimen) groups of animals ($n=8$) were given enprofylline 50 μ mol/kg, theophylline 110 μ mol/kg or budesonide 2.6 μ mol/kg orally (gavage). Control animals ($n=8$) received saline (0.5 ml).

On day 15 the animals were challenged with TDI 0.3 nl (≈ 2 pmol) and the experiments were terminated 5 and 17 hr later. Drug administrations were performed in a standardized way from 8 a.m. to 9 a.m.

Exudation of circulating radiolabelled albumin. Twenty minutes before terminating the experiments, ¹³¹Iodine-labelled human serum albumin (0.3 mg/ml; ~ 1 MBq 0.1–0.2 ml) was administered intravenously via an ear marginal vein, to the conscious animals. Thus, data were obtained for ¹³¹Iodine-albumin exudation over a period of 20 min which encompassed the period 5 hr and 17 hr after the commencement of TDI challenge (superfusion).

Termination of experiment

Five hours or 17 hr after the end of TDI or olive oil exposure pentobarbital (60 mg/ml) was given intravenously.

ously, the chest was opened and an incision was made in the still beating heart. A sample of mixed arterial-venous blood was collected in heparinized tubes and centrifuged (500 g, 6 min) for separation of plasma.

Tracheal lavage

The technique of sampling the luminal fluid without disturbing the baseline mucosal barrier function has been validated in previous work [e.g. 14]. The airways (including larynx and lungs) were excised *in toto*. After a brief rinse in saline the lung lobes were tied off at the hilar regions. The heart and larger vessels and oesophagus were removed. After removal of the lymph nodes a small incision was made in the left main stem bronchus and a slightly bent piece of tubing (PE 50) was gently introduced and secured. The preparation was mounted vertically (larynx upwards). The lower portion (intrathoracic) of the trachea and the adjoining bronchi were immediately rinsed with 0.25 ml saline 3–4 times during 1 min. The area lavaged was estimated to $\approx 1 \text{ cm}^2$ (ID $\sim 2.1 \text{ mm}$). The tracheal (luminal) fluid was put in a tared tube and so was a section of the previously lavaged tracheal tissue.

Analyses

Tracheal tissue samples and plasma samples as well as the tracheal lavage (luminal) fluid were weighed ($0.001 \pm 0.0001 \text{ g}$). The activity (counts per minute; c.p.m.) was counted in a Compugamma (Pharmacia-Wallac, Uppsala, Sweden). All samples from individual animals were counted for 1 min in one sequence within 3 hr after completion of the experiments. We have previously demonstrated an excellent stability of the radioiodine binding to albumin during circulation and exudation into the airway lumen [14,15].

Calculations and statistics

The plasma volume (μl , corresponding to intravenous ^{131}I -albumin) was calculated by dividing the obtained activity in lavage fluid, tracheal tissue (50 mg) by the activity in 1 ml (g) plasma. Data are given as mean $\pm$ s.e.m. The difference between means was analysed for significance by Wilcoxon–Mann Whitney's test or Student's *t*-test. In the comparisons between single drug administration a Bonferroni correction was made. In the study with repeated drug administration comparisons between groups were made using analysis of variance (Bartlett's homogeneity test showed no significant variance of s.d. between groups) followed by *t*-test.

Drugs and chemicals

Anaesthetics. Ketalar (Ketamin®, Parke-Davis, Detroit, U.S.A.), xylazine (Rompun® vet 20 mg/ml, Bayer, Leverkusen, Germany), pentobarbital (Mebumal® vet 60 mg/ml, Nordvacc, Skärholmen, Sweden).

Macromolecular tracers. ^{131}I -Iodine-labelled human serum albumin (Institut for energiteknikk, Kjeller, Norway).

Toluene diisocyanate (TDI): (80% 2,4 TDI + 20% 2,6 TDI, Merck, Darmstadt, Germany).

Drugs. Enprofylline 10 mg/ml, ASTRA DRACO, Sweden. Theophylline (Theo-Dur® 20 mg/ml), ASTRA DRACO, Sweden. Budesonide (micronized), ASTRA DRACO, Sweden.

Results

Single exposure to TDI

Five hours after TDI 3 nl exposure there was a significant (ongoing) exudation of plasma (corresponding to ^{131}I -albumin) into the airway tissue ($P < 0.05$) and airway lumen ($P < 0.001$; Table 1, Fig. 1). Seventeen hours after exposure the exudative response had declined but was still significantly increased compared with control (Table 1). Six days after exposure to TDI 3 nl the luminal response had returned to baseline values (0.09 ± 0.03 ; $n = 5$). The response to TDI 0.1 and 0.3 nl was not different from control (olive oil) whereas TDI 1 nl induced a slight but non-significant response at the 5 hr time point (Table 1, Fig. 1). (Olive oil, control, was without effect, see [14].)

Pretreatment with enprofylline 25 $\mu\text{mol/kg}$ significantly attenuated the luminal entry of plasma ($P < 0.05$). Neither treatment with budesonide reduced the effect of TDI (Fig. 2).

Effect of repeated dermal exposures

Superfusion of the tracheobronchial mucosa with TDI 0.1 nl ($\sim 0.7 \text{ pmol}$) produced a significant exudation of plasma (^{131}I -albumin) into the airway lumen 5 hr after exposure ($P < 0.05$) in animals that previously had received the two dermal challenges. Dermal treatment with olive oil followed by the local airway exposure to TDI 0.1 nl was without effect (Table 2).

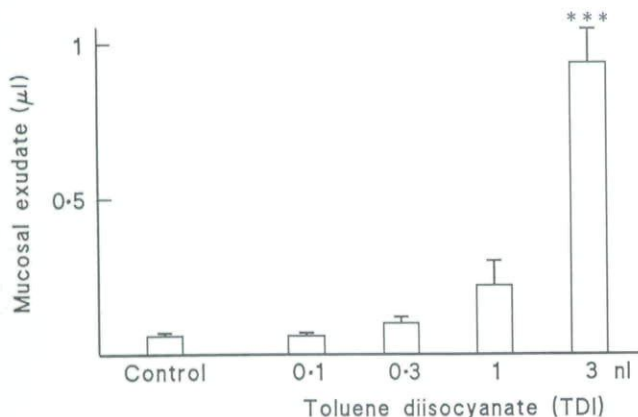
Effect of repeated airway exposures to TDI. Effect of daily drug treatments

The sensitization procedure was performed at two separate occasions (Studies I and II) during which the condi-

Table 1. Dose-related and time course effects of TDI 0.1–3 nl superfusion. ^{131} Iodine-albumin in lavage fluid and tracheal wall at 5 and 17 hr after TDI or vehicle olive oil exposure

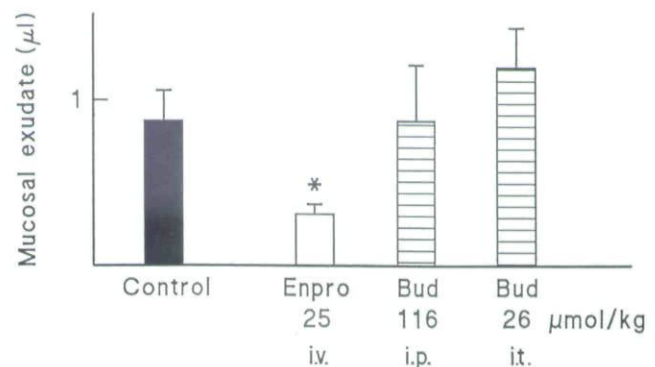
Number of animals	TDI treatment	Time after exposure (hr)	Luminal plasma (μ l)	Tracheal tissue plasma (μ l/50 mg)
18	Control	5	0.06 ± 0.01	1.9 ± 0.1
12	(olive oil)	17	0.06 ± 0.01	1.6 ± 0.1
6	0.1 nl	5	0.06 ± 0.01	1.8 ± 0.1
6	(0.67 pmol)	17	0.05 ± 0.02	1.9 ± 0.1
6	0.3 nl	5	0.10 ± 0.02	1.9 ± 0.3
6	(2 pmol)	17	0.08 ± 0.03	2.0 ± 0.3
6	1 nl	5	0.22 ± 0.08	2.2 ± 0.4
6	(6, 7 pmol)	17	0.10 ± 0.04	1.9 ± 0.3
12	3 nl	5	$0.94 \pm 0.11^{***}$	$3.4 \pm 0.3^{***}$
8	(20 pmol)	17	$0.19 \pm 0.03^{***}$	$2.9 \pm 0.2^{**}$

** $P < 0.01$. *** $P < 0.001$; significant difference from control; Student's *t*-test.

**Fig. 1.** Plasma volumes (μ l) in tracheal lavage fluids sampled 5 hr after single exposures to TDI 0.1–3 nl dissolved in olive oil ($n=6-8$ in each group). Control ($n=18$) received olive oil. *** $P < 0.001$ compared with control (Student's *t*-test).

tion and weight of the animals were controlled. There was no significant difference in weight gain between control (non-exposed), olive oil (control) and TDI 3 nl exposed groups (Table 3). In the study with daily oral drug treatments the mean weight gain was significantly less in both control (saline) and active groups showing that the gavage procedure itself had an adverse effect. The xanthines, possibly due to the known nauseatic effect of these drugs, reduced the weight gain more than could be accounted for by the gavage procedure.

TDI 0.3 nl (2 pmol) produced a significant exudation of

**Fig. 2.** The effect of drugs on the luminal entry of plasma (nl) 5 hr after a single exposure to TDI 3 nl. Control ($n=12$) received saline i.v. or i.t. prior to TDI exposure. Enprofylline (Enpro) was given 5 min prior to TDI ($n=6$). Budesonide (Bud) was administered 17 hr prior to TDI ($n=6$ in each treatment group). * $P=0.02211$ (Student's *t*-test and Bonferroni correction).

plasma (^{131} Iodine-albumin) into the airway lumen 5 hr after exposure ($P < 0.001$) in animals that previously had received the two TDI challenges. Also 17 hr after challenge the luminal exudation caused by TDI 0.3 nl was still significantly increased (Table 4, Fig. 3). Olive oil (control) exposed animals or olive oil challenged sensitized animals were unaffected (Table 4, Fig. 3).

Pretreatment with oral budesonide (2.6μ mol/kg) during the sensitization regimen significantly attenuated the

Table 2. Exudative response to TDI 0.1 nl superfused onto the tracheobronchial mucosa in animals previously exposed to TDI dermally

Dermal exposures on days 1 and 8	Challenge treatment on day 15	Luminal plasma (μ l)	Tracheal tissue plasma (μ l/50 mg)
Control:			
Vehicle <i>n</i> =6	TDI 0.1 nl	0.094 $\pm$ 0.025	1.82 $\pm$ 0.15
TDI 20 μ l <i>n</i> =6	TDI 0.1 nl	0.270 $\pm$ 0.074*	2.83 $\pm$ 0.52

* $P < 0.03737$ (Wilcoxon-Mann-Whitney's test)

subsequent TDI induced luminal exudation response whereas pretreatment with the xanthines produced an insignificant effect ($P > 0.05$; Fig. 4).

Discussion

This study has demonstrated that both dermal applications and, more interestingly, topical tracheobronchial applications of TDI can increase the airway inflammatory response to TDI. Furthermore, exceedingly low tracheobronchial doses (3 nl) of TDI were required to produce the increased responsiveness. In previous studies, we have demonstrated that single exposures to low doses of TDI, dissolved in olive oil, produced prompt, sustained (≥ 17 hr) and dose-dependent plasma exudation in the

guinea-pig tracheobronchial airways [14,16]. At all instances, including threshold inflammatory responses, the plasma exudation effect was evident in both airway tissue and lumen, as also demonstrated in the present study [14,16]. Luminal entry of plasma proteins was, accordingly, selected for evaluation of TDIs ability to produce a significant inflammatory response in this study. Irrespective of what cellular mechanism that may fuel the airway inflammation, plasma exudation and particularly the luminal entry of plasma may tell about the intensity of the ongoing process. More specifically, the exudative activity reflects the degree of involvement of the airway tissue itself in the inflammatory process [12].

We have previously observed that the number of mucus cells (PAS-positive) was significantly increased on days 6 and 21 after a single exposure to TDI 30 nl applied topically by the present method [17]. Also, already 5 hr after the superfusion of TDI 30 nl a significant increase in number of leucocytes (particularly eosinophils and neutrophils) and histamine levels was observed [16, and unpublished data]. TDI-induced increases in airway leucocytes have previously been demonstrated in guinea-pigs subjected to an acute exposure to a high dose of TDI (corresponding to an inhaled body burden of approximately 50 μ l TDI), which also produced loss of cilia and epithelial shedding in tracheal tissue samples [18]. However, 5–17 hr after application of a much lower dose of TDI 30 nl by the present technique, the epithelial lining appeared non-injured [17] and the number of epithelial cells in the tracheobronchial lavage fluid was unchanged, consistent with an intact epithelial lining [16]. Miller *et al.* [19] also found an intact epithelium in guinea-pig main

Table 3. Mean weight gain ($\pm$ s.e.m.) at two different (I and II) sensitization periods

	Oral treatment during days 1–14.	Tracheal superfusion on days 1 and 8	Weight gain (mean $\pm$ s.e.m.)
I		Non-exposed (<i>n</i> =6)	124 $\pm$ 6 g
		Olive oil (<i>n</i> =6)	129 $\pm$ 4 g
		TDI 3 nl (<i>n</i> =6)	119 $\pm$ 5 g
II		Non-exposed (<i>n</i> =6)	123 $\pm$ 5 g
	Saline (0.5 ml)	Olive oil	102 $\pm$ 4 g
	Saline (0.5 ml)	TDI 3 nl	97 $\pm$ 5 g**
	Budesonide (2.6 μ mol/kg)	TDI 3 nl	90 $\pm$ 4 g***
	Enprofylline (50 μ mol/kg)	TDI 3 nl	72 $\pm$ 7 g***
	Theophylline (100 μ mol/kg)	TDI 3 nl	77 $\pm$ 7 g***

** $P < 0.01$, *** $P < 0.001$; significant difference from non-exposed control (Student's *t*-test).

Table 4. Exudative response to TDI 0.3 nl in previously 'sensitized' animals (studies I and II).

Study	Treatment (sensitization) on days 1 and 8	Challenge on day 15	Time after exposure	Luminal plasma (μ l)	Tracheal tissue plasma (μ l/50 mg)
I					
<i>n</i> = 6 each group	Olive oil	TDI 0.3 nl	5	0.08 $\pm$ 0.01	1.6 $\pm$ 0.2
			17	0.09 $\pm$ 0.01	1.8 $\pm$ 0.1
	TDI 3 nl	Olive oil	5	0.09 $\pm$ 0.01	1.5 $\pm$ 0.1
			17	0.09 $\pm$ 0.01	1.7 $\pm$ 0.1
	TDI 3 nl	TDI 0.3 nl	5	0.40 $\pm$ 0.04***	3.0 $\pm$ 0.5*
			17	0.41 $\pm$ 0.13***	2.9 $\pm$ 0.5*
II					
<i>n</i> = 8 each group	TDI 3 nl	Olive oil	5	0.12 $\pm$ 0.02	2.2 $\pm$ 0.3
			17	0.06 $\pm$ 0.03	1.9 $\pm$ 0.3
	TDI 3 nl	TDI 0.3 nl	5	0.63 $\pm$ 0.12***	3.0 $\pm$ 0.4
			17	0.22 $\pm$ 0.06***	2.1 $\pm$ 0.1

* $P < 0.05$; *** $P < 0.001$. Significant difference from control (Student's *t*-test)

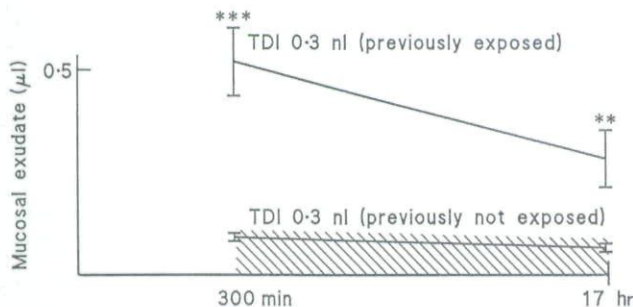


Fig. 3. This figure shows plasma volumes (from studies I and II) in tracheal lavage fluids from guinea-pigs previously exposed twice to TDI 3 nl or olive oil (on days 1 and 8). TDI 0.3 nl given on day 15 produced significantly increased plasma volumes in animals previously exposed to TDI both 300 min and 17 hr after exposure compared with not exposed (olive oil) animals. The striped area shows data from control (olive oil on day 15) on previously TDI exposed animals. ** $P < 0.01$; *** $P < 0.001$ significantly differed from control (Student's *t*-test).

bronchi after the animals had been exposed daily for 10 days to an aerosol of TDI (30–260 p.p.b.; total daily body burden inhaled TDI $\approx 10 \mu$ l). Hence, the present low doses of TDI cannot be expected to have induced major structural damage to the airway mucosa. However, the number of mucus cells may have increased as previously demonstrated [17].

Repeated local tracheobronchial superfusions of TDI in this study made the animals more sensitive to this agent. Thus, TDI 0.3 nl, previously shown to be without

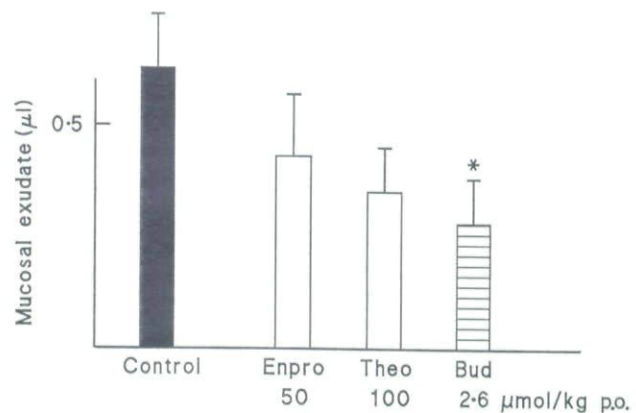


Fig. 4. This figure shows the effects on plasma volumes in tracheal lavage obtained 5 hr after exposure to TDI 0.3 nl in animals previously exposed twice to TDI 3 nl. During the sensitization regimen the animals received daily (14 days) enprofylline (Enpro), theophylline (Theo) or budesonide (Bud) orally (gavage). Control animals received saline ($n=8$ in each treatment group). * $P=0.0357$ significantly differed compared with control (ANOVA) followed by *t*-test).

effect (Table 1, Fig. 1), caused a significant response that also lasted for at least 17 hr after application (Fig. 3). The sensitization procedure did not cause any clinical signs of reactions and the growth of the animals was unchanged compared with the untreated control animals. Also the present dermal sensitization had no effect on animal weight, and macroscopic observations of the skin exposed

to TDI did not show any signs of irritation. The increased tracheobronchial sensitivity to TDI caused by previous animal exposures to either dermal or tracheobronchial TDI has not been explained. Delayed type skin hypersensitivity (DTH) reactions obtained by repeated dermal applications of very large doses of TDI may be T-cell mediated since it has been demonstrated that lymphocytes from sensitized animals administered to non-sensitized animals produced a DTH reaction [9,10]. It cannot be excluded that a similar mechanism is involved in the present dermal application experiments. However, the increased tracheobronchial response to minute amounts of TDI after exposing the airways to TDI may be of greater relevance than our findings that large dose dermal TDI exposures could produce a similar effect.

Xanthines are bronchodilators but may also exert a range of 'anti-inflammatory' effects in the airways. Indeed, it has been suggested that the latter actions may be the more important anti-asthma effects of xanthines such as theophylline and enprofylline [20]. We have previously demonstrated that intravenous enprofylline 5 mg/kg given 5–10 min prior to local superfusions with capsaicin, bradykinin, and PAF-acether significantly attenuates the immediate and, after PAF-acether, the sustained (5 hr) exudative response in guinea-pig tracheobronchial airways [21–23]. As now shown with enprofylline also the exudative response to a single dose of TDI can be reduced by xanthines (Fig. 2). The mechanism of action behind this effect is unknown, except that adenosine antagonism can probably be excluded [20]. The effect may relate to a general property of enprofylline and theophylline, to reduce immediate and late phase exudation responses in guinea-pig airways [21–24]. It may be more an anti-inflammatory effect than a direct vascular antipermeability effect. The latter action is potentially induced by topical β_2 -agonists in guinea-pig airways [22] but these drugs do not reduce the present acute response to TDI (unpublished observations by Erjefält and Persson). In previous work on guinea-pigs sensitized to produce IgE and IgG antibodies Andersson and Bergstrand [25] have shown that prolonged treatment with daily theophylline doses is remarkably effective to inhibit allergy (ovalbumin)-induced airway obstruction also after cessation of the drug treatment. However, in this study the daily treatment with either theophylline or enprofylline during the 14 days of 'sensitization' produced small effects on the subsequent response to TDI challenge. Thirty years ago Gandevia [26] examined respiratory symptoms in man exposed to isocyanate vapour. He observed that theophylline was an effective treatment when given before and during the shift. However, the isocyanate-induced decrease in ventilatory capacity was not reversible by an isoprenaline aerosol [26].

Budesonide is a potent anti-asthma glucocorticoid which reduces allergen-induced exudation in guinea-pig airways [27]. When the allergen (ovalbumin) is given topically to only the large airways, as in this study, both immediate and late phase (5 hr) exudative responses are induced and the latter is inhibited by budesonide (5 mg/kg intratracheally) given just prior to challenge [28]. Half the dose of budesonide that was employed in the present study has previously been shown to inhibit the acute IgE-mediated bronchoconstriction in guinea-pigs. Both immediate and late phase responses were thus inhibited when 5 mg/kg of budesonide was administered intratracheally 6–20 hr prior to allergen provocation, whereas administration 2 hr before challenge only attenuated the late phase (24 hr) lung resistance response [27]. However, budesonide (10 mg/kg intratracheally) given 17 hr before challenge in this study was without effect on the exudation induced 5 hr after challenge with a single TDI (3 nl) dose. Even a large intraperitoneal dose of budesonide was without effect in this study though this latter treatment has been shown to attenuate capsaicin induced exudation in guinea-pig airways [21]. It is unclear what the lack of inhibition by glucocorticoid treatment means. It is possible that in a species that is more sensitive to these drugs than guinea-pigs, the result could have been different. It is further possible that TDI acutely produces an inflammatory response that involves glucocorticoid insensitive pathways. For example it has previously been demonstrated that prolonged budesonide treatment failed to reduce opsonized zymosan triggered LTB_4 production in human alveolar macrophages nor did the treatment reduce the superoxide radical generation triggered by calcium ionophore in these cells [29]. In this study the daily treatment with budesonide during the time period when 'sensitization' to TDI was being induced produced a significant inhibitory effect. Since glucocorticoids potentially influence several immunoinflammatory mechanisms that may have contributed to the increased sensitivity to TDI this inhibitory effect is not very helpful in delineating the pathways by which increased responsiveness to TDI was produced in this study.

Occupational inhalation of TDI may be considered a common 'sensitization' regimen in human subjects. The threshold limit value for TDI is 5 p.p.b. for up to a 10 hr daily work shift. Roughly estimated an airway exposure to TDI (daily body burden) would be $\sim 10 \mu\text{g/kg}$ [1]. Sensitized subjects exposed acutely to about $1 \mu\text{g/kg}$ TDI develop immediate and/or late phase asthmatic reactions measured as decreased FEV_1 and increased levels of albumin in lavage fluids [6,30]. The possibility that the present observations may be relevant to human asthma now remains speculative but intriguing. The mechanisms involved in human TDI-induced asthma have not been

fully revealed although both IgE and T-cell mediated mechanisms have been discussed [1]. Furthermore, the similarities of TDI-asthma to other forms of asthma has recently been emphasized [31]. Favouring a relevance of the present work only low doses of TDI were required and the increased responsiveness was clearly induced by the topical tracheobronchial application of TDI. Indeed, the very low doses of TDI effective in this study suggest that the presently allowed exposure levels of TDI are too high (see above). Alternatively, man is less sensitive than guinea-pigs. As demonstrated by the duplicate experiments included in this study the present repeat challenge regimen produces reproducible results with little spread in both base-line values and challenge data. This aspect is crucial for its further use in studies of TDI-induced mucosal immunoinflammatory mechanisms as well as in studies aimed at finding effective treatment of TDI-induced inflammation and responsiveness.

In conclusion, we have demonstrated that two repeated tracheobronchial exposures to 3 nl of TDI, which is a threshold exudative dose in naive animals, increase the sensitivity of the airway mucosa to this agent, so that even 0.3 nl of TDI produced inflammatory exudation. The increased responsiveness is reduced by daily treatment with a glucocorticoid at a dose level that, when given singly, had no effect on the acute exudative effect of TDI in non-sensitized animals. Enprofylline exhibited a different pattern of effects reducing the effect of single TDI-exposures but, similarly to theophylline, being without significant effect on the development of increased sensitivity to TDI. The present repeated airway exposure regimen to TDI is forwarded as a tentative model for studying TDI-asthma.

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