

Ketotifen does not inhibit asthmatic reactions induced by toluene di-isocyanate in sensitized subjects

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Summary

In order to determine whether treatment with ketotifen inhibits asthmatic reactions induced by toluene di-isocyanate (TDI), we studied six sensitized subjects with previously demonstrated dual or late asthmatic reaction after inhalation challenge with TDI. Ketotifen (1 mg b.i.d., orally) or placebo was administered for 7 days to the examined subjects, according to a double-blind, cross-over, placebo-controlled study design. When the subjects were treated with either ketotifen or placebo, FEV₁ markedly decreased after exposure to TDI. These results suggest that the anti-asthmatic agent ketotifen is not effective in TDI-induced asthma and suggest that it should not be used in the prophylaxis of asthmatic reactions induced by TDI in sensitized subjects.

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Introduction

Isocyanates, a group of low molecular weight compounds, are an important cause of occupational asthma, as they are widely used in industry and are potent sensitizers [1–4]. Sensitized subjects may develop asthmatic symptoms after exposure to isocyanates in their place of work [1–4]. After challenge with isocyanates in the laboratory, these subjects develop early and/or late asthmatic reactions [5]. In previous studies, we found that airway responsiveness increases after a late asthmatic reaction [6,7] and that the time-course of such an increase is similar to the time-course of an acute inflammatory reaction [8], and that indeed late asthmatic reactions are associated with airway inflammation [9,10]. Furthermore, we observed that prednisone prevents both late asthmatic reactions and the associated increase in airway responsiveness and airway inflammation [11–13], and also that inhaled beclomethasone inhibits such toluene di-isocyanate (TDI) effects in a dose-dependent manner [14,15].

Subjects and methods

Study design

Six subjects with a history of sensitivity to TDI and with

previously demonstrated dual or late asthmatic reactions following TDI challenge were studied. The study was conducted according to a placebo-controlled, randomized, double-blind, cross-over design. Each subject was treated with ketotifen (1 mg at 08.00 hr and at 20.00 hr, orally; total dose 2 mg/day) or placebo for 7 days. On the first and sixth days, airway responsiveness to methacholine was determined in each subject. On the seventh day, challenge with TDI was performed 30 min after the administration of 2 mg of ketotifen or placebo. At 8 hr after exposure to TDI, inhalation challenge with methacholine was performed. The same procedure was repeated with the alternative treatment, starting at least 1 week later. All subjects gave written informed consent.

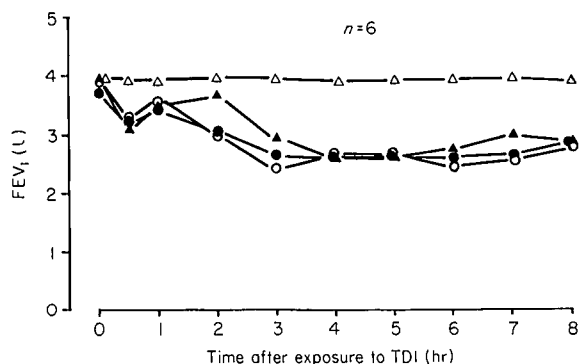
Subjects

All subjects had been free of clinical, symptomatic respiratory infections for at least 8 weeks, or exposure to TDI for at least 2 weeks before the first study day. No subject took sympathomimetics, antihistamines, theophylline, anticholinergic or cromolyn within the days of any study. Normal lung volumes, good cooperation and reproducibility of pulmonary function tests, and a history of sensitization to isocyanates with the demonstration of a dual or a late asthmatic reaction after exposure to TDI were requested for admittance to the study. All subjects underwent detailed medical and occupational history, lung function measurements and intradermal skin tests

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Table 1. Characteristics and baseline pulmonary function of the six examined subjects

Subject (No.)	Age (yr)	Sex	Smoking habits	Atopic status	Height (cm)	Baseline FEV ₁	
						l	% predicted
1	21	M	NS	+	198	4.55	75
2	28	F	NS	—	160	3.30	109
3	20	F	NS	—	158	3.20	109
4	19	F	NS	+	175	4.00	102
5	24	M	NS	—	178	4.45	96
6	31	M	ES	+	170	4.05	103

**Fig. 1.** Mean values of FEV₁ before and after sham exposure (Δ), and exposure to TDI, without treatment (▲), and after 1 week treatment with placebo (○), or ketotifen (●). Abscissa indicates time (hr) and ordinate indicates FEV₁ (l).

with common allergens on a preliminary visit. A subject was considered to be atopic according to the previously described criteria [11]. The characteristics of each subject are reported in Table 1.

Inhalation challenge with methacholine

Airway responsiveness to methacholine aerosol was assessed in each subject according to the slightly modified protocol of Chai *et al.* [17], as previously described [12].

Inhalation challenge with TDI

Inhalation challenges with TDI were performed according to the previously described protocol [12]. Each subject was exposed at the same time of the day, with an interval of more than 2 weeks, and the duration of exposure was the same for each set of experiments.

Data analysis

An immediate asthmatic reaction to TDI was considered to occur when FEV₁ decreased by at least 20% from baseline within 30 min (early component of a dual reaction or early alone reaction) and 1 hr or more (late component of a dual or a late reaction) after exposure to TDI.

In order to test for changes in airway responsiveness during the treatment and after TDI inhalation, the log values for methacholine provocative doses were analysed by use of a paired *t*-test. Mean values for provocative doses are reported as geometric means (*g*) and geometric standard errors of the mean (*g.s.e.m.*). To test for changes of FEV₁ before and after 1 week of treatment, and before and after TDI inhalation, the values of FEV₁ were compared by use of a paired *t*-test, and by one-way analysis of variance. A level of greater than 95% probability for the two-tailed *t*-test was considered to be statistically significant.

Drugs and chemicals

The following chemicals were used: ketotifen (Sandoz, Milan, Italy); toluene di-isocyanate (a mixture of 80% 2,4-isomer and 20% 2,6-isomer) (Montedison, Porto Marghera, Italy); acetyl-β-methacholine chloride (Vetro-tecnica, Padova, Italy).

Results

After exposure to TDI, but not after sham exposure (Table 2), each of the six subjects examined developed a late or a dual asthmatic reaction during no treatment at the screening test (Table 3), and during treatment with ketotifen (Table 4). Five of the six subjects developed a late or a dual asthmatic reaction during treatment with placebo (Table 5). One subject (No. 4), who had developed a dual asthmatic reaction at the screening test and a late asthmatic reaction during treatment with ketotifen, did not develop significant asthmatic reactions during treatment with placebo. Airway responsiveness to methacholine increased at 8 hr after TDI inhalation challenge in five of the six subjects during no treatment at the screening inhalation challenge, and in each of the six subjects during treatment with ketotifen (Table 6). However, airway responsiveness could be measured in all six subjects after ketotifen, but in only three subjects after placebo, the bronchoconstriction being considered too severe by the other three subjects during placebo treatment. Ketotifen did not change FEV₁ and the degree of airway responsiveness measured before exposure to TDI. All subjects tolerated treatment with ketotifen without significant side effects.

Table 2. FEV₁ before and after sham exposure

Subject	Base	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr
1	4.33	4.30	4.35	4.30	4.25	4.15	4.31	4.35	4.30	4.30
2	3.30	3.25	3.27	3.30	3.23	3.17	3.26	3.30	3.25	3.25
3	3.20	3.15	3.13	3.20	3.10	3.15	3.00	3.00	3.10	3.05
4	4.00	4.10	3.90	4.26	4.32	3.96	4.10	4.18	4.12	3.90
5	4.45	4.25	4.50	4.40	4.30	4.55	4.60	4.60	4.50	4.50
6	4.10	4.05	4.30	4.20	4.25	4.20	4.15	4.20	4.30	4.15
$\bar{x}$	3.89	3.85	3.91	3.94	3.91	3.86	3.90	3.94	3.93	3.86
s.e.m.	0.21	0.21	0.24	0.22	0.24	0.24	0.26	0.26	0.24	0.24

One-way ANOVA: $F=0.02$, $P=1$.

Table 3. FEV₁ before and after exposure to TDI at the screening inhalation challenge

Subject	Base	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr
1	4.65	2.20	3.80	4.10	3.20	2.40	2.20	2.65	3.00	2.80
2	3.30	3.10	3.12	3.20	2.45	2.60	2.75	2.80	3.00	2.80
3	3.00	2.80	2.90	2.80	2.30	2.20	2.20	1.92	2.27	2.20
4	4.01	2.00	2.85	4.13	3.20	2.70	2.90	3.10	3.45	3.45
5	4.50	4.20	4.20	4.40	4.00	3.65	3.05	2.95	3.07	3.07
6	4.35	4.18	4.15	3.20	2.40	2.00	2.50	2.90	3.18	3.00
$\bar{x}$	3.97	3.08	3.50	3.64	2.92	2.59	2.60	2.72	2.99	2.88
s.e.m.	0.28	0.39	0.25	0.26	0.27	0.24	0.15	0.17	0.16	0.17

One-way ANOVA: $F=3.64$, $P=0.015$.

Sham *vs* screening: $F=49.9$, $P=0.000$.

Table 4. FEV₁ before and after exposure to TDI during treatment with placebo

Subject	Base	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr
1	4.50	2.61	3.93	4.00	3.12	2.42	2.48	2.07	2.50	3.07
2	3.40	3.20	3.25	2.90	2.05	2.90	2.87	2.70	2.70	2.85
3	3.00	2.80	2.90	2.80	2.30	2.22	2.20	1.92	2.27	2.20
4	3.64	3.87	3.85	3.47	3.55	3.69	3.34	3.33	3.36	3.69
5	4.40	3.70	4.10	3.10	2.55	2.60	2.50	2.40	2.55	2.55
6	4.30	3.70	3.72	1.65	1.12	2.05	2.40	2.20	2.30	2.30
$\bar{x}$	3.87	3.31	3.61	2.98	2.44	2.64	2.63	2.43	2.61	2.77
s.e.m.	0.25	0.21	0.18	0.32	0.35	0.24	0.17	0.21	0.16	0.29

One-way ANOVA: $F=4.42$, $P=0.0003$.

Table 5. FEV₁ before and after exposure to TDI during treatment with ketotifen

Subject	Base	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr
1	4.66	2.98	3.70	4.52	3.50	2.75	2.70	2.70	2.85	3.22
2	3.27	3.10	3.28	2.10	2.00	2.60	2.80	2.65	2.70	2.70
3	3.00	3.15	3.10	3.00	2.50	2.40	2.60	2.50	2.50	2.30
4	4.22	4.05	4.10	4.10	4.07	3.59	3.14	3.45	3.54	3.69
5	3.90	2.90	3.20	2.40	1.90	2.55	2.55	2.65	3.03	3.05
6	3.20	3.00	2.95	2.05	1.90	1.85	1.90	1.93	2.00	2.15
$\bar{x}$	3.71	3.19	3.39	3.03	2.64	2.62	2.61	2.64	2.77	2.85
s.e.m.	0.27	0.17	0.23	0.43	0.38	0.23	0.17	0.20	0.21	0.24

One-way ANOVA : $F=2.08$, $P=0.0036$.

Ketotifen *vs* placebo: $F=0.01$; $P=0.915$.

Table 6. Individual values of PD₂₀ FEV₁ (mg) methacholine before and during ketotifen or placebo treatment, and at the screening inhalation challenge

	Ketotifen			Placebo			Screening			
	Day 0	Day 6	Day 7	Day 0	Day 6	Day 7	Day 0	Day 6	Day 7	Day 8
1	0.38	0.76	0.05	0.86	0.50	0.02	nd	0.08	0.12	0.09
2	0.71	0.35	0.13	0.29	0.40	0.70	nd	2.20	0.27	n.d.
3	0.09	0.02	0.01	0.13	0.07	—*	nd	0.05	0.02	n.d.
4	1.79	0.89	0.03	1.59	0.95	0.42	nd	1.17	0.10	0.33
5	0.19	0.36	0.08	0.36	0.12	—	nd	0.26	0.17	n.d.
6	0.17	0.36	0.02	0.17	0.17	—	nd	0.07	0.03	n.d.
<i>g</i>	0.33	0.29	0.04	0.38	0.25	/	nd	0.24	0.08	/
<i>g.s.e.m.</i>	1.56	1.75	1.47	1.48	1.49	/	nd	1.91	1.51	/

PD₂₀ FEV₁ was measured before treatment, during treatment on the day before exposure to TDI and during treatment at 8 hr after exposure to TDI.

* Not measured because of complaints of bronchoconstriction.

n.d. = not determined.

Day 0 = PD₂₀ FEV₁ (mg) methacholine before treatment.

Day 6 = PD₂₀ FEV₁ (mg) methacholine on the day before TDI inhalation challenge.

Day 7 = PD₂₀ FEV₁ (mg) methacholine 8 hr after TDI inhalation challenge.

Day 8 = PD₂₀ FEV₁ (mg) methacholine 24 h after TDI inhalation challenge.

Paired *t*-test, Ketotifen: Day *vs* Day 7, $t=4.46$; $P=0.006$. Screening: Day 6 *vs* Day 7, $t=2.43$; $P=0.06$.

Discussion

The results of the present study demonstrate that treatment with oral ketotifen (benzocycloheptathiophene), a histamine (H₁) antagonist with anti-allergic properties [16], had no significant effect on baseline FEV₁, or on baseline airway responsiveness to methacholine before exposure to TDI, and did not prevent dual or late asthmatic reactions, and the associated increase of bronchial responsiveness to methacholine induced by exposure to TDI.

TDI-induced asthma is a frequent occupational airway disease [18], potentially fatal [19], with poor prognosis even after cessation of exposure [20–22], and we believe that good prophylaxis and treatment of TDI-induced asthma are important goals. In the strategy of dealing with bronchial asthma, it is important to find a drug with low systemic bioavailability and minimum side-effects. In occupational asthma, cessation of exposure at work is obviously mandatory once the diagnosis is established. However, many sensitized subjects who come to our clinic are self-employed carpenters in their forties, and it is often

impossible, or at least difficult, for them to change their job. They require prophylactic treatment capable of preventing the development of asthmatic reactions in the case of unavoidable exposure to isocyanates. Oral and inhaled steroids completely inhibit late asthmatic reactions induced by TDI [10,11,14,15], but they do not affect early asthmatic reactions and, in the long term, they may have side-effects [23]. Unfortunately, ketotifen, at variance with steroids, had no effect on dual or late asthmatic reactions, thus it is of no use in TDI-induced asthma. Three subjects (Nos 3, 5 and 6) refused to repeat the methacholine inhalation challenge after TDI during treatment with placebo because of breathlessness, whereas they agreed to repeat the methacholine inhalation challenge after TDI during treatment with ketotifen.

FEV₁ was only larger in one subject (No. 5) during ketotifen treatment, and it may be speculated that ketotifen may have a protective effect in the sensation of breathlessness associated with bronchoconstriction induced by TDI. However, post-TDI PD₂₀ FEV₁ during treatment with ketotifen was no different from post-TDI PD₂₀ FEV₁ during no treatment in the screening test, thus the effect of ketotifen, if any, was unlikely to be mediated by an effect on bronchial responsiveness.

It may be argued that the duration of treatment and dose used in the present study were not appropriate. Indeed, although it has been suggested that the maximum effect of ketotifen may not be achieved until 5 weeks after treatment [24], in a recent study ketotifen did not inhibit bronchoconstriction induced by eucapnic hyperventilation in subjects treated for 6 weeks [25]. We planned a 1-week treatment because we assumed that compliance of subjects is better with shorter periods, and also because in previous studies a 1-week treatment with inhaled steroids was sufficient to provide protection [14,15]. Regarding the dose, we gave 1 mg ketotifen b.i.d., as this is the recommended dosage [6]; in addition, as with previous studies with other anti-asthma drugs [14], we doubled the dose of ketotifen before the inhalation challenge with TDI.

Ketotifen has been shown to inhibit allergen-induced bronchoconstriction in subjects with allergic asthma [26]. In our opinion this study had two major biases: firstly, it is an open study; secondly, the inhalation challenges were performed at 1-week intervals, with the sequence no treatment-ketotifen-disodium cromoglycate, so that the effect of the drug could possibly be due to tachyphylaxis [27]. Finally, the dose used (2 mg at 12, 4 and 2 hr before inhalation challenge) was unusually high. Even with these biases a protective effect may be assumed, if that was the case. Thus, it may be that asthmatic reactions induced by TDI are not protected by ketotifen because they are not mediated by an IgE mechanism [28,29].

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