

Evaluation of the Protective Effect of a New β -Agonist, KWD 2131, on Allergen-Induced Bronchospasm

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The selective anti-allergic effect of a new β -agonist, KWD 2131, in inhibiting allergen-provoked asthma was compared with that of placebo in a double-blind crossover study. Non-bronchodilating doses were given by inhalation to 12 asymptomatic patients with extrinsic asthma. KWD 2131 showed a marginal anti-allergic effect. A significantly better effect than that with placebo was obtained at only one time of measurement after challenge ($P < 0.05$). The reproducibility of the allergen challenge was also evaluated. The mean maximum decrease in FEV_1 during three different allergen-challenge procedures was 37, 31, and 38%, respectively. The difference was not statistically significant.

Key words: allergen challenge; anti-allergic agent; β -agonist; KWD 2131

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The anti-allergic property, i.e. inhibition of mediator release, has been demonstrated in *in vitro* and *in vivo* animal models with DSCG (11, 14, 18, 30, 37 and a series of newly synthesized cromoglycate-like compounds (10).

Sympathomimetic agents also inhibit the mediator release in a variety of experimental models, such as chopped guinea pig lung (4, 35), human lung (3, 38), and leucocytes (25). Contrary to DSCG and its oral analogues, β -agonists show both anti-allergic and bronchodilating properties. Such substances as, e.g. isoproterenol, orciprenaline, and terbutaline, show inhibition of the anaphylactic histamine release in guinea pig lung in the same concentration range as they exert tracheal muscle relaxation (35). This causes difficulties in proving the clinical significance of the anti-allergic effect in asthma of available β -agonists. However, some investigators have found an

inhibiting effect of β -agonists against allergen-induced asthma (5, 16, 24). It is impossible to interpret whether or not an anti-allergic effect is apparent in these studies because of the effect-dualism of the substances used.

KWD 2131, 1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethyl-2-hydroxyethyl)-amino] ethanol sulphate is a new β -agonist structurally related to terbutaline (Fig. 1). KWD 2131's profile of action is changed compared with terbutaline. Whereas terbutaline shows anti-allergic effect and tracheal smooth muscle relaxation in the same concentration range in *in vitro* studies in guinea pigs (35), KWD 2131 shows appreciable anti-allergic effect in concentrations where the tracheal smooth muscle relaxation is negligible (36).

KWD 2131 should therefore provide a tool to prove the value of the anti-allergic property of a β -agonist in the prophylactic treatment of patients with extrinsic asthma.

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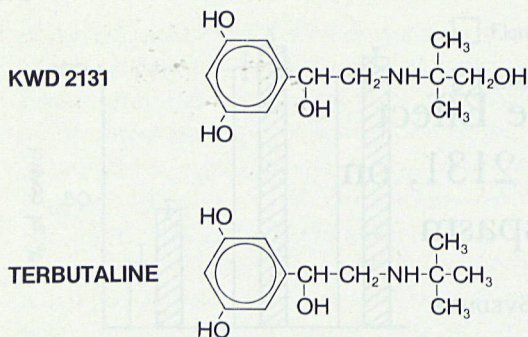


Fig. 1. Chemical structures of terbutaline and KWD 2131

In an earlier histamine provocation study performed in the same patients as in the present study, the highest individual non-bronchodilating doses of KWD 2131 (0.5 and 0.25 mg) have been established (20).

The present study evaluated the protective effect of these doses in allergen-provoked asthma.

The study was approved by the Ethical Committee of University of Göteborg. Informed consent was obtained from all patients.

PATIENTS AND METHODS

Twelve patients, eight female; four male, mean age 33 years (range 24–37—Table 1) with extrinsic bronchial asthma, based on a history of allergy, positive skin test, RAST, and bronchial allergen provocation test, were studied. Of the 12, 11 had earlier taken part in an allergen challenge reproducibility study (26, 27, 29).

At the time of the study, all patients were subjectively and objectively free from asthma symptoms, and the FEV₁ was within normal range of the predicted values. None of the patients had respiratory infection and had not been exposed to relevant allergens for at least 24 h, during which time they had also avoided exercise. Some patients used β_2 -stimulants by aerosol (salbutamol or terbutaline) and/or occasionally DSCG, but no other drugs. Drugs, when used, were not allowed during 12 h before the allergen challenge procedure.

No patient was being treated with immunotherapy. Three were moderate smokers. Smoking was not allowed 4 h before the study. All had earlier taken part in similar studies and were familiar with inhalation and measuring procedures.

The individual allergen dose was determined by titration before the study and defined by means of two variables; allergen extract concentration (range 1:10–1:10 000 weight/volume) and inhalation time (range 1–5 min); these were to give a decrease in FEV₁ of 20–50% from prechallenge value within 30 min after provocation. All patients were also tested 5 min before allergen challenge with inhalation of physiological saline.

The trial design

Part I. This study was designed as a crossover double-blind comparison between nebulized KWD 2131 and placebo (physiological saline) with random allocation. The patients were pretreated with KWD 2131 or placebo before the allergen challenge with the earlier determined dose with approximately 1–2 weeks' interval. The active drug or placebo was given 30 min before challenge during a nebulization time of 15 min (Fig. 2).

Part II. At completion of the double-blind study, a fourth allergen provocation was performed, after a further 1–2 weeks, with a

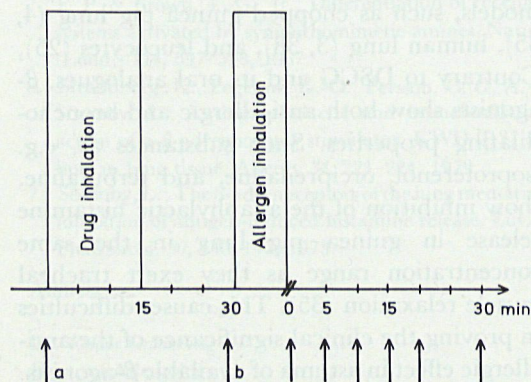


Fig. 2. Trial design. $\uparrow$ = ventilation measurement. a) = initial value. b) = base line value.

single-blind pretreatment placebo control (physiological saline). This procedure was performed to evaluate the importance of a crossover double-blind, randomized trial design vis-à-vis a single-blind non-randomized procedure, where psychological factors and/or time might influence change in allergen response.

The experiment in both parts of the trial started at the same time every day and was performed according to a strict standardized procedure described by Löwhagen & Lindholm (29). The allergen was nebulized and inhaled during quiet tidal breathing for a total of 1–5 min. The allergen, KWD 2131, and placebo were administered via nebulization from a Pari Inhalierboy Nebulizer (Paul Ritzau Pariwerk GmbH und Co., Starnberg, Germany; airflow, 15 l/min, particle size, 0.5–5.5 μ and a continuous nebulizer output of 0.8 ml/min). The same nebulizer was used throughout the study.

The allergen extracts used during the study were taken from the same individual batch and are given for each patient in Table 1.

Forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁), peak expiratory flow rate (PEFR), and maximum expiratory flow rate at 50% of vital capacity (MEF_{50%}) were measured with the Monaghan

M 403 spirometer (Monaghan Co., Denver, USA) with attached recorder. The spirometer had been calibrated against Bernstein Spirometer. The measurements were made in duplicate. The highest value was used in the statistical evaluation.

Ventilation was measured before (initial value) and 30 min after pretreatment with KWD 2131 or placebo (base line value) and 0, 5, 10, 15, 20 and 30 min after allergen provocation (Fig. 2). If the values were still low at 30 min, a further recording at 40 min was made. If needed, bronchodilator spray was thereafter given. The mean per cent fall in FVC, FEV₁, PEFR, and MEF_{50%} at different times and also mean maximum decrease were calculated from the value obtained immediately before provocation (base line value). The degree of maximum percentage protection was calculated by the method of Grieco (19): % protection =

$$\frac{\left(\begin{array}{c} \% \text{ max. decrease} \\ \text{after allergen} \\ \text{challenge} \end{array} \right) - \left(\begin{array}{c} \% \text{ max. decrease} \\ \text{after pretreatment} \\ \text{with drug} \end{array} \right)}{\left(\begin{array}{c} \% \text{ max. decrease after} \\ \text{allergen challenge} \end{array} \right)}$$

Statistical analysis was done with Student's *t*-test.

Table 1

Clinical data

Patient	Sex	Age (years)	Diagnosis	Allergen	Prick test	RAST
E. J.	F	39	ex. asthma	hamster	3+	—
L. A.	F	30	ex. asthma	birch	4+	2
B. T.	F	29	ex. asthma	birch	4+	2
J. B.	M	31	ex. asthma		6+	—
K. S.	M	26	ex. asthma	cat	4+	2
E. R.	F	28	ex. asthma	cat	3+	3
L. N.	M	30	ex. asthma	cat	4+	3
A. F.	F	24	ex. asthma	cat	4+	4
U. S.	F	30	ex. asthma	birch	3+	0
I. S.	F	37	ex. asthma	cow	5+	0
B. A.	F	42	ex. asthma	dog	1+	3
J. S.	M	47	ex. asthma	horse	3+	2

RESULTS

The results are presented as changes in FEV_1 , because FVC, PEFR, and $MEF_{50\%}$ do not add any further substantial information except in a few reported instances.

During saline inhalation (before the double-blind study) all the tested patients showed less than 4% changes in FEV_1 from the initial value.

Part I. Double-blind comparison between KWD 2131 and placebo

Mean initial values in FEV_1 on the 2 test days showed no significant difference. During inhalation of KWD 2131 and placebo, a slight mean increase in FEV_1 of $3.2\% \pm 1.4$ (mean $\pm$ s.e.m., $P < 0.05$) and $1.6\% \pm 1.7$ (mean $\pm$ s.e.m., N.S.), respectively, was obtained. The difference between KWD 2131 and placebo was not significant.

Fig. 3 illustrates the mean per cent changes in FEV_1 after allergen challenge on the various test days.

The difference in FEV_1 between KWD 2131 and placebo at all times of measurements was non-significant, except at the 0-min value ($P < 0.05$) (i.e. immediately after the end of a 1–5-min allergen provocation). In $MEF_{50\%}$ and FVC, significant differences were also found at 10 and 20 min, respectively ($P < 0.05$).

The allergen protection produced by KWD 2131 and placebo (calculated according to the method of Grieco) and based on FEV_1 was $23.2\% \pm 10.1$ and $12.8\% \pm 10.1$ (mean $\pm$ s.e.m.), respectively (Fig. 4). The difference was not significant.

Part II. Single-blind not randomized comparison between KWD 2131 and placebo

Fig. 3 shows the effect of KWD 2131 on allergen-induced asthma compared with single-blind administered placebo (allergen-control) performed after completed double-blind study. Significant differences in FEV_1 were found at 0 ($P < 0.05$) and 15 min ($P < 0.05$). In $MEF_{50\%}$ and FVC, significant differences were found at all measurement

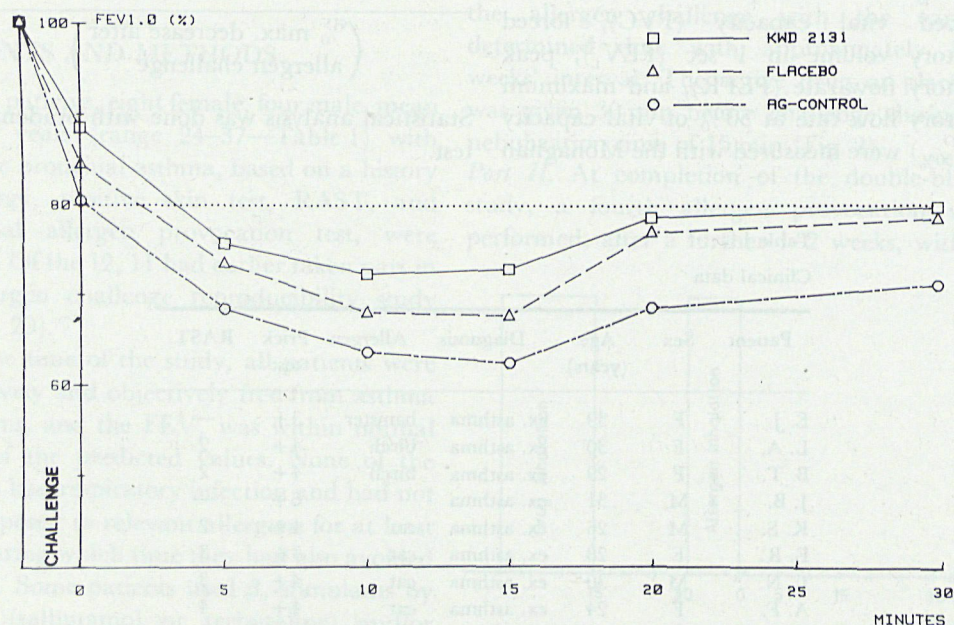


Fig. 3. Mean per cent decrease from base line on the various test days. $N = 12$ patients except at 20- and 30-min. points of measurement ($n = 8$ patients). In relation to base line value the decrease was statistically significant at all points of measurements ($P < 0.001$). Significant difference was also found between KWD 2131 and placebo (at 0-time) $P < 0.05$, and between KWD 2131 v. Ag-control (at 0 and 15 min) $P < 0.05$.

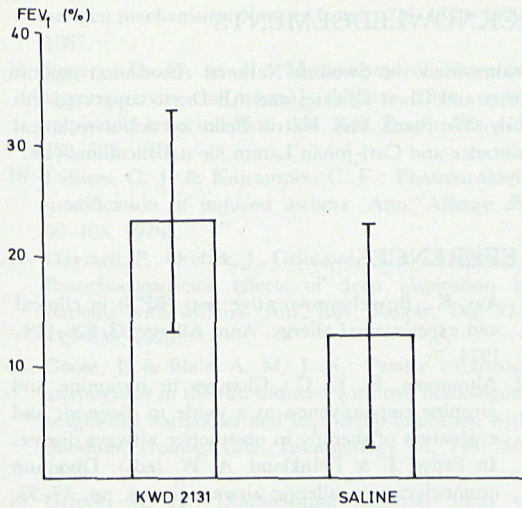


Fig. 4. Per cent protection against allergen challenge.

times (P range < 0.001 – 0.05) except at the 30-min and 10-min values, respectively.

Reproducibility of the allergen response

The response to inhaled allergen was compared on three different occasions: 1) the dose-titration day, 2) the placebo-day in the double-blind study, 3) the placebo-day in the single-blind study. The mean maximum per cent decrease in FEV₁ was 37 ± 3.9 , 31 ± 4.6 , and 38 ± 4.0 (mean $\pm$ s.e.m.), respectively, with a 14% coefficient of variation.

DISCUSSION

A necessary base for evaluation of anti-allergic drugs is a good reproducibility of repeated allergen challenges (1, 7, 9, 34). This requires a strict standardization of the procedure and a careful selection of patients. Studies on drug evaluation with standardized allergen procedures in selected patients were performed by Booij-Nord et al. (6), Cockcroft et al. (13) and Löwhagen & Lindholm (26, 28). Löwhagen & Lindholm (26, 27, 29) in an earlier study, partly in the same patients as in the present study (11/12 patients), described the reproducibility of our allergen challenge

procedure under standardized conditions. In the present study, we followed this procedure to exclude or minimize factors known to negatively influence the reproducibility of the allergen challenge response. These factors are partly patient-related and include abnormal or large day-to-day variation in lung function (13, 29), respiratory infection (15), simultaneous medical treatment (9, 13, 29), natural allergen exposure (2), and inadequate interval between the allergen challenge (7, 8, 12, 29). Another factor of major importance is the technique used in inhaling drugs or allergens. Chai et al. (9) used inhalation from functional residual capacity to vital capacity for five breaths. Cockcroft et al. (13) and our group delivered the nebulized solution for a fixed time during quiet tidal breathing. Tidal volume breathing was chosen as this manoeuvre is easily done and minimizes influence on lung mechanics (17). The particles should also be more evenly distributed (32). As a result of this strict standardization, the reproducibility of the response to inhaled allergen was good, the mean maximum decrease in FEV₁ at three challenges being 37, 31 and 38% with a coefficient of variation of 14%. Our result agrees well with that of Cockcroft et al., who found the coefficient of variation after two separate allergen challenges to be 16%. In the present double-blind study, nebulized KWD 2131 in an individualized, not broncho-dilating, dose (0.5 mg or 0.25 mg) only occasionally gives significantly better protection than placebo ($P < 0.05$) at some points of measurement. The effect was small and probably of no clinical relevance. The importance of a double-blind randomized placebo comparison (Part I) is obvious, since in the single-blind comparison (Part II), KWD 2131 vs. placebo gave better protection than in Part I, which could lead to a faulty conclusion. The reason for this discrepancy could be that the double-blind randomized design minimizes bias and the time factor. In a subcutaneous study that we performed (23), we also found that KWD 2131 gave signifi-

cantly better protection than placebo. This was not found by Pegelow (33) and Pauwels (31).

As the study aimed to prove the anti-allergic effect of a substance which also had a bronchodilating capacity, it was vital to ensure the highest dose without bronchodilating effect in each extrinsic asthmatic. Therefore we had to find an individual sub-threshold dose for bronchodilatation (0.5 and 0.25 mg) (20), since a previous study (21) had shown that the mean sub-threshold dose for bronchodilatation (1 mg) was too high in four of eight individual patients with intrinsic asthma.

In the present study, a non-bronchodilating dose of a new β -agonist, KWD 2131, showed a marginal anti-allergic effect. The relation in anti-allergic effect between terbutaline and KWD 2131 is 1:1 in *in vitro* animal lung studies (36) and *in vivo* studies on human skin (22) and nose (39). If the same relation exists in the human lung, the same dose of terbutaline as that of KWD 2131 used in the present study (0.5 mg) should produce a similar marginal protective effect against allergen challenge. However, as terbutaline is 30 times more potent as a bronchodilator than KWD 2131 in human *in vivo* (21) and *in vitro* studies (38), a significant bronchodilating effect is already shown at 0.05 mg nebulized terbutaline (21). This suggests that the effect seen with therapeutic doses of terbutaline is mainly due to bronchodilatation and not inhibition of mediator release. However, in an *in vitro* study on human lung (38), the relation in anti-allergic effect between terbutaline and KWD 2131 was found to be 7:1. This indicates a possible species difference and also a difference between mast cells in human skin and nose vis-à-vis mast cells in the human lung tissue, which in turn indicates the importance of performing studies on the appropriate organ in the human. Studies with measurements of mediator release and lung function are in progress to evaluate whether higher doses of KWD 2131 show a better anti-allergic effect than terbutaline in equipotent bronchodilating doses.

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