Phytomedicine

ELSEVIER

Tolerance and effect of an add-on treatment with a cough medicine containing ivy leaves dry extract on lung function in children with bronchial asthma

CrossMark

S. Zeil [a], U. Schwanebeck [b], C. Vogelberg [a],*

[a] Department of Pediatric Pulmonology and Allergology, Technische Universität Dresden, Universitätsklinikum Carl Gustav Carus, 01307 Dresden, Germany
[b] Coordination Centre for Clinical Trials, Technische Universität Dresden, Universitätsklinikum Carl Gustav Carus, 01307 Dresden, Germany

ARTICLE INFO

ABSTRACT

Ivy leaves dry extract is registered as an expectorant in patients with respiratory diseases associated with productive cough. Next to its secretolytical properties, bronchospasmolytical effects are described. However only limited data exist about a possible therapeutical effect in asthmatic patients.

In this double blind, placebo-controlled, randomized cross-over study, 30 children (median age 9.07 years (min–max: 6–11)) suffering from partial or uncontrolled mild persistent allergic asthma despite long-term treatment with 400 μg budesonide equivalent were investigated. After a four week run-in period, patients either received ivy leaves dry extract for four weeks in addition to their inhaled corticosteroid therapy or placebo, followed by a wash-out phase before switching to the other treatment arm. Lung function, FeNO, exhaled breath condensate pH and life quality was analyzed after each treatment period.

There was a significant improvement of MEF_{75-25}, MEF_{25} and VC after treatment with ivy leaves dry extract (MEF_{75-25} change in the mean 0.115 l/s, $p = 0.044$; MEF_{25} change in the mean 0.086 l/s, $p = 0.041$; VC change in the mean 0.052 l, $p = 0.044$), but not after treatment with placebo. For the primary outcome parameters (relative change of FEV_1 and MEF_{75-25} before bronchodilation) no treatment effect could be detected in the cross-over analysis (FEV_1 $p = 0.6763$ and MEF_{75-25} $p = 0.6953$).

This proof-of-concept study indicates that children with mild uncontrolled asthma despite regular inhaled corticosteroid therapy might benefit from an additional therapy with ivy leaves dry extract. However, further studies are needed.

Introduction

Since decades, ivy leaves dry extract is applied for the treatment of respiratory diseases accompanied by productive cough. The tolerance and safety of different ivy leaves extract-containing preparations has been tested and confirmed in several studies (Fazio et al. 2009; Kraft 2004).

The effect of ivy leaves dry extract is based on its secretolytical and broncholytical properties. The main active compounds are saponins (Cioacá et al. 1978; Trute et al. 1997; Bedir et al. 2000), especially alpha-hederin represents the most important saponin molecule responsible for the therapeutic effect (Sieben et al. 2009). Alpha-hederin inhibits the internalization of the β2-receptor leading to an increased adrenergic accessibility of the cells. Subsequently, type II alveolar epithelial cells generate more surfactant yielding to the secretolytic activity by reduction of the mucus viscosity. Similarly, the bronchodilating effect can be related to the increased β2-adrenergic activity resulting in a higher sensitivity to spasmolytics such as adrenalin, the strongest physiological bronchodilator (Hegener et al. 2004). In a double blind comparative study against Ambroxol, the ivy leaves dry extract demonstrated non-inferiority concerning relevant clinical and lung function outcome parameters (Meyer-Wegener et al. 1993).

Bronchial asthma is a chronic inflammatory disease with recurrent obstruction due to bronchospasm and increased mucus secretion. Preliminary clinical studies with asthmatic children treated with an inhaled corticosteroid indicated an improvement of various lung function parameters after three days of add-on therapy with ivy leaves dry extract (Gulyas et al. 1997; Mansfeld et al. 1997; Mansfeld et al. 1998). However, small patient number, the short

* Corresponding author at: Technische Universität Dresden, Department of Pediatric Pulmonology and Allergology, Fetscherstraße 74, 01307 Dresden, Germany. Tel.: +49 351 458 5699; fax: +49 351 458 4334.
E-mail address: christian.vogelberg@uniklinikum-dresden.de (C. Vogelberg).

Table 1
Primary and secondary endpoints of the study.

Primary endpoints	MEF_{75-25} before bronchodilation – relative change
	FEV_1 before bronchodilation – relative change
Secondary endpoints	MEF_{75-25} before bronchodilation – absolute change
	FEV_1 before bronchodilation – absolute change
	FeNO
	Exhaled breath condensate pH
	Peak flow profile
	Lungfunction parameters before and after bronchodilation (RAW, TLC, VC, MEF_{75-25}, FEV_1, FVC, RV, FRC, MEF_{50}, MEF_{25})
	Emergency treatment (beta agonist demand)
	Number of days with asthma symptoms
	PAQLQ result
	ACQ result
	Tolerance

Fig. 1. Study visits took place every 28–30 days. Splitted into two treatment arms, every patient received verum (Prospan® Cough Syrup) and placebo. The treatment and the placebo period were separated by a wash-out phase of 28–30 days. The end of the trial was after a four weeks run-out phase.

duration, the lacking of a placebo control group and the exclusive report of changes of subjective parameters are examples for limitations in further studies (Huntley and Ernst 2000; Hofmann et al. 2003; Clark et al. 2010).

The aim of our study was therefore to investigate the effect of an add-on therapy with ivy leaves dry extract in children with mild persistent asthma treated by an inhaled corticosteroid.

Materials and methods

This double-blind, placebo controlled, randomized cross-over study was conducted in compliance with the ICH-GCP guideline. It was approved by the local Ethics Committee and received consent from Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) and followed the guidelines of the Declaration of Helsinki and Tokyo for humans. A parent or guardian of every child and if possible the child in person provided written informed consent. Primary and secondary endpoints were defined according to Table 1.
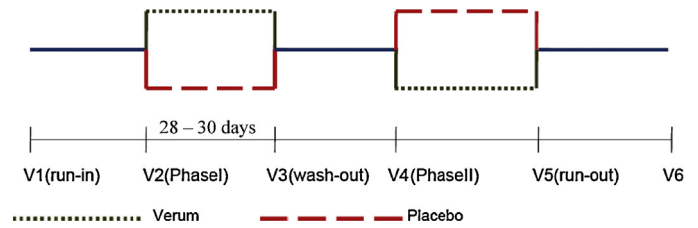
Study subjects

30 asthmatic children aged 6–12 years of both genders suffering from partial or uncontrolled mild persistent allergic bronchial asthma with a grade II severity in terms of the NVL Asthma Version 1.3.2011 (Bundesärztekammer 2009) for at least one year were enclosed in the study. Lack of asthma control was defined as improvement of the $FEV_1 \geq 12\%$ after 2 puffs of 100 μg terbutaline during steroid monotherapy with 400 μg budesonide equivalent/day or an ACT score ≤ 19 (asthma control test). All subjects were included from May 2012 until January 2013. Patients were excluded if they had an acute respiratory infection within the previous four weeks, a history of bronchopulmonary dysplasia, vocal cord dysfunction, gastro-oesophageal reflux, hereditary fructose intolerance or known chronic diseases.

Course of the study and procedures

After initial screening, subjects had to be adjusted to 400 μg budesonide equivalent/day as monotherapy if necessary at the beginning of the run-in phase. The inhaled corticosteroid dosage remained unchanged throughout the whole study. If a pathological lung function (improvement of FEV_1 after bronchodilation of at least 12%) or ACT (less than 20 points) was ascertained at study visit 2, the patient was definitively registered in the trial.

Study treatment was given as Prospan® Cough Syrup (ivy leaves dry extract (5–7, 5:1), extractive agent ethanol 30% (m/m)) in a daily dosage of 2×5 ml, corresponding to 70 mg extract. The extract is well characterized (Landgrebe et al. 1999). An inactive, identical flavored, look-alike syrup was used in the same quantity as placebo. The administration of the trial medication was performed randomized, double-blind, cross-over designed according to Fig. 1 for 28–30 days respectively.

Blinded study medication and placebo were obtained from Engelhard Arzneimittel GmbH & Co. KG. The placebo was manufactured according to GMP standards (good manufacturing practice). The investigational products were packed in an individual way for each patient, who entered the study. All bottles of investigational drug were returned and weighted to supervise the compliance additional to the entry made in the diary. An intake of study medication equal or more than 80% of the total amount was considered acceptable.

In total five to six study appointments took place every 28–30 days. At each visit a bodyplethysmography was conducted, the FeNO and exhaled breath condensate pH (EBC-pH) were measured. Additionally two questionnaires concerning life quality (Standardized Paediatric Asthma Quality of Life Questionnaire (PAQLQ(S)), Asthma Control Questionnaire (ACQ)) were filled in by the participants in absence of the parents. Peak-flow measurements were performed twice daily at home and documented in a diary together with information concerning the intake of the investigational and the permanent medication. Furthermore, asthma symptoms (cough, breathing difficulties) and concomitant treatment had to be documented. Concomitant medication being considered to influence bronchial asthma was not permitted in the trial (leukotriene antagonists, anticholinergics, long acting beta agonists, beta blockers, methylxanthines, anti-IgE treatment, cromoglycin sodium/nedocromil sodium).

The lung function parameters were measured with a bodyplethysmograph (MasterLab, Jaeger, Höchberg, Germany). Parameters were expressed as a percentage of predicted value. FeNO was measured with a chemiluminescence analyzer (CLD 88 sp; Eco Medics, Duernten, Switzerland), expressed in parts per billion (Baraldi and de Jongste 2001). Exhaled breath condensate (EBC) was collected using a modified, commercially available breath condensate sampler (EcoScreen; Jaeger, Höchberg, Germany) and EBC-pH was analyzed as described elsewhere (Vogelberg et al. 2007). All measurements were conducted according to the American Thoracic Society/European Respiratory Society criteria (Beydon et al. 2007).

Statistical analyses

Complete blinding of patients, investigators and statisticians was maintained until all data had been locked. Thereafter data were grouped according to treatment code without revealing their identity. The data were unblinded after completion of the statistical analyses. Data were analyzed based on the intention-to-treat principle. The SPSS 21 and SAS 9.2 software package were used.

The confirmatory data analysis (done for the primary endpoint) was done with aid of the generalized linear model. The secondary outcomes were evaluated and analyzed according to their scale level: For continuous endpoints mean values and confidence

Table 2
Demographic characteristics of the subjects.

Characteristics	Group		Total
	Placebo-Prospan®	Prospan®-placebo	
No.	15	15	30
Male/female	7/8	10/5	17/13
Median age in years (min./max.)	9.07 (6/11)	9.07 (6/11)	9.07 (6/11)

intervals or medians and quartiles were used, for categorical end-points absolute and relative frequencies were applied. Depending on the distribution of the variables the t-test, the U-test and the χ^2-test were used. For intra-individual comparison the paired t-test was used in case of normally distributed variables and the Wilcoxon test in case of non-normally distributed variables. All statistical tests were performed at the significance level $\alpha = 0.05$. Adverse events were evaluated in consideration of frequency, intensity and causality to study drug.

Results

Thirty children were randomized (Table 2). One child withdrew prematurely from the study during the first treatment period because the parents stopped the long-term treatment with inhaled steroids. All other children regularly terminated the study and returned diary cards covering the whole study period. Five subjects showed treatment compliance below 80%. Three of them took the study medication twice daily with too low medication volume.

For the primary outcome parameters (relative change of FEV_1 and MEF_{75-25} before bronchodilation) no significant treatment effect could be detected in the cross-over analysis (FEV_1 $p = 0.6763$ and MEF_{75-25} $p = 0.6953$). However there was a significant period effect concerning FEV_1 ($p = 0.0031$).

Concerning the secondary endpoints significant effects during treatment with ivy leaves dry extract resulted for the absolute change of MEF_{75-25}, MEF_{25} and VC before bronchodilation (MEF_{75-25} change in the mean 0.115 l/s, $p = 0.044$; MEF_{25} change in the mean 0.086 l/s, $p = 0.041$; VC change in the mean 0.052 l, $p = 0.044$). Within the placebo group no treatment effect was detected. The respective differences (between parameters before treatment with ivy leaves dry extract/placebo and parameters after

treatment with ivy leaves dried extract/placebo) did not differ significantly.

For the absolute change of FEV_1 a period effect was detected analogous to the relative change (ivy leaves dry extract median change 0.059 l, $p = 0.16$; placebo median change 0.052 l, $p = 0.021$). The differences did not differ significantly ($p = 0.835$).

The ACQ score decreased significantly with placebo (change in the mean 0.93 points, $p = 0.011$), but not with ivy leaves dry extract.

For the parameter RAW, TLC, FVC, RV, FRC, MEF_{50} before and after bronchodilation, FEV_1, VC, MEF_{75-25} after bronchodilation as well as for FeNO, EBC-pH, peak flow und PAQLQ-Score no significant changes after treatment with ivy leaves dry extract nor with placebo were found (Table 3).

Discussion

The aim of this study was to evaluate a possible benefit of an add on therapy with ivy leaves dry extract in children with partial or uncontrolled mild persistent asthma, treated with inhaled corticosteroids. After four weeks of treatment with ivy leaves dry extract, a partial improvement of lung function compared to placebo was demonstrated. Concerning the primary endpoint of the study, the FEV_1, no significant change was observed. For the parameters MEF_{75-25}, MEF_{25} and VC which are among the secondary endpoints of the study, a significant difference between active treatment and placebo was shown.

Although FEV_1 represents a relatively objective and reproducible parameter and is listed in the current guidelines for asthma as a major factor in assessing asthma severity, it is an ongoing discussion whether FEV_1 is a good parameter for asthma severity in childhood. Several studies demonstrated that children with bronchial asthma, unlike adults, often meet the clinical criteria for severe asthma despite having a normal or only slightly decreased FEV_1 (Paull et al. 2005; The Childhood Asthma Management Program Research Group 2000; Bacharier et al. 2004; Galant et al. 2007). One reason, that FEV_1 remains normal in children, even if already a severe airway obstruction is present, may be due to the altered anatomy of the respiratory tract compared to adults (a relatively large airway diameter in comparison to the lung volume). Additionally there seems to be different disease localizations, for children with all grades of asthma severity show an inflammation of the peripheral airways with only little impairment of the large airways (Tashkin 2002). The associated peripheral airway obstruction remains unreflected by FEV_1. These findings might provide the

Table 3
Change in the means, standard deviation and significance after treatment with placebo and ivy leaves dry extract before bronchodilation for 28–30 days.

	Paired differences					
	Before placebo – after placebo			Before Prospan® – after Prospan®		
	Change in the mean	Standard deviation	Significance	Change in the mean	Standard deviation	Significance
FEV_1	−0.052	0.117	0.021	−0.125	0.107	0.016
MEF_{75-25}	−0.099	0.311	0.093	−0.115	0.294	0.044
MEF_{25}	−0.075	0.231	0.087	−0.086	0.215	0.041
VC	0.001	0.138	0.958	−0.052	0.133	0.044
RAW	0.020	0.158	0.493	0.077	0.246	0.105
TLC	−0.057	0.251	0.211	−0.046	0.308	0.433
FVC	−0.018	0.141	0.499	−0.045	0.140	0.096
RV	−0.065	0.305	0.255	0.013	0.299	0.827
FRC	.0.040	0.229	0.342	0.039	0.241	0.388
MEF_{50}	−0.107	0.316	0.075	−0.111	0.309	0.063
FeNO	5.430	14.891	0.069	3.510	15.456	0.232
EBC-pH	−0.080	1.009	0.671	0.161	0.786	0.287
Peak flow	−15.333	43.225	0.062	−7.890	24.140	0.089
ACQ	0.933	1.893	0.011	0.655	3.588	0.334
PAQLQ	−2.533	6.877	0.053	−2.655	9.994	0.164

evidence, that for children, MEF_{75-25} might be regarded as the most significant parameter for airway obstruction (Lebecque et al. 1993; Alberts et al. 1994; Simon et al. 2010) and should be established as acceptable endpoint in clinical trials concerning asthmatic children (Rao et al. 2012).

In our study, relevant alterations in lung function occurred mainly in parameters reflecting peripheral airways. This peripherally enhanced effect of the study medication, inhibiting the internalization of the β_2-receptor, might be explained by the distribution of this receptor in the lung. In various studies using different investigational techniques it could be demonstrated that the β_2-receptor density increase with increasing airway generation (Johnson 1998; Ueki et al. 1993). Due to the otherwise stable long-term medication during the study, the improvement of lung function might therefore be attributed to the add-on treatment with ivy leaves dry extract.

To avoid a possible overlap between the two treatment periods, a prolonged wash-out period was defined within the study protocol. In addition subjects had to take a standardized baseline therapy to improve comparability between the study groups. Therapy adherence and a correct grading of the asthma severity were guaranteed by the run-in period.

In contrast to changes of the lung function, quality of life was not significantly influenced by the intervention as measured by the PAQLQ(S). However, due to the short duration of the study and the level of lung function changes, this result is not really surprising. In addition, asthmatic children are known to have a bad perception of their asthma severity (Horak et al. 2003).

Within both study arms, a marked period effect in lung function improvement could be demonstrated, especially during the first treatment episode. This effect can be attributed to the significantly better therapy adherence of the patients during the study. Over the entire study period and in all participants a very high adherence was recorded. Next to the high frequency of study visits with the opportunity to control and encourage therapy adherence, an optimal doctor-patient relationship further may account for this effect (Benedetti 2013; Di Blasi et al. 2001). In addition the regular documentation of the treatment within the diary might have improved the therapy adherence. Moreover, the fact that the add-on therapy was performed with a herbal medication, lead to a high acceptance especially from parental side.

In summary, this proof-of-concept study demonstrates a significant improvement in lung function parameters in children with mild persistent, uncontrolled asthma despite regular therapy with an inhaled corticosteroid by an additional treatment with ivy leaves dry extract. Further studies are needed to evaluate whether the supplementary treatment with ivy leaves dry extract might be a treatment option in asthmatic patients.

Conflict of interest

Acknowledgement

References

Alberts, W.M., Ferris, M.C., Brooks, S.M., Goldman, A.L., 1994. The FEF25-75% and the clinical diagnosis of asthma. Ann. Allergy 73, 221–225.

Bacharier, L.B., Strunk, R.C., Mauger, D., White, D., Lemanske Jr., R.F., Sorkness, C.A., 2004. Classifying asthma severity in children. Mismatch between symptoms, medication use, and lung function. Am. J. Respir. Crit. Care Med. 170, 426–432.

Baraldi, E., de Jongste, J.C., 2001. Measurement of exhaled nitric oxide in children. Eur. Respir. J. 20, 223–237.

Bedir, E., Kirmizipekmez, H., Sticher, O., Calis, I., 2000. Triterpene saponins from the fruits of Hedera helix. Phytochemistry 53, 905–909.

Benedetti, F., 2013. Placebo and the new physiology of the doctor-patient relationship. Physiol. Rev. 93, 1207–1246.

Beydon, N., Davis, S.D., Lombardi, E., Allen, J.L., Arets, H.G.M., Aurora, P., Bisgaard, H., Davis, G.M., Ducharme, F.M., Eigen, H., Gappa, M., Gaultier, C., Gustafsson, P.M., Hall, G.L., Hantos, Z., Healy, M.J.R., Jones, M.H., Klug, B., Lødrup Carlsen, K.C., McKenzie, S.A., Marchal, F., Mayer, O.H., Merkus, P.J.F.M., Morris, M.G., Oostveen, E., Pillow, J.J., Seddon, P.C., Silverman, M., Sly, P.D., Stocks, J., Tepper, R.S., Vilozni, D., Wilson, N.M., 2007. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am. J. Respir. Crit. Care Med. 175, 1304–1345.

Bundesärztekammer (BÄK), Kassenärztliche Bundesvereinigung (KBV), Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften (AWMF). Nationale VersorgungsLeitlinie Asthma – Langfassung, 2. Auflage. Version 5. 2009. zuletzt geändert: August 2013. Available from: http://www.versorgungsleitlinien.de/themen/asthma, doi:10.6101/AZQ/000163.

Cioacá, C., Margineanu, C., Cucu, V., 1978. The saponins of Hedera helix with antibacterial activity. Pharmazie 33, 609–610.

Clark, C.E., Arnold, E., Lasserson, T.J., Wu, T., 2010. Herbal interventions for chronic asthma in adults and children: a systematic review and meta-analysis. Primary Care Respir. J. 19, 307–314.

Di Blasi, Z., Harkness, E., Ernst, E., Georgiou, A., Kleijnen, J., 2001. Influence of context effects on health outcomes: a systemic review. Lancet 357, 757–762.

Fazio, S., Pouso, J., Dolinsky, D., Fernandez, A., Hernandez, M., Clavier, G., Hecker, M., 2009. Tolerance, safety and efficacy of Hedera helix extract in inflammatory bronchial diseases under clinical practice conditions: a prospective, open, multicentre postmarketing study in 9657 patients. Phytomedicine 16, 17–24.

Galant, S.P., Morphew, T., Amaro, S., Liao, O., 2007. Value of the bronchodilator response in assessing controller naïve asthmatic children. J. Pediatr. 151, 457–462.

Gulyas, A., Repges, R., Dethlefsen, U., 1997. Konsequente Therapie chronisch-obstruktiver Atemwegserkrankungen bei Kindern. Atemweg. Lungenkrank. 23, 291–294.

Hegener, O., Prenner, L., Runkel, F., Baader, S.L., Kappler, J., Häberlein, H., 2004. Dynamics of β2-adrenergic receptor–ligand complexes on living cells. Biochemistry 43, 6190–6199.

Hofmann, D., Hecker, M., Volp, A., 2003. Efficacy of dry extract of ivy leaves in children with bronchial asthma: a review of randomized controlled trials. Phytomedicine 10, 213–220.

Horak, E., Grässl, G., Skladal, D., Ulmer, H., 2003. Lung function and symptom perception in children with asthma and their parents. Pediatr. Pulmonol. 35, 23–28.

Huntley, A., Ernst, E., 2000. Herbal medicines for asthma: a systematic review. Thorax 55, 925–929.

Johnson, M., 1998. The β-adrenoceptor. Am. J. Respir. Crit. Care Med. 158, 146–153.

Kraft, K., 2004. Tolerability of dried ivy leaf extract in children. Z. Phytother. 25, 179–181.

Landgrebe, H., Matusch, R., Runkel, F., Hecker, M., 1999. Wirkung und Anwendung einer alten Heilpflanze. Pharmaz. Ztg. 35, 11–15.

Lebecque, P., Kiakulanda, P., Coates, A.L., 1993. Spirometry in the asthmatic child: is FEF 25-75 a more sensitive test than FEV1/FVC? Pediatr. Pulmonol. 16 (1), 19–22.

Mansfeld, H.J., Höhre, H., Repges, R., Dethlefsen, U., 1997. Sekretolyse und Bronchospasmolyse. TW Pädiatrie 10, 155–157.

Mansfeld, H.J., Höhre, H., Repges, R., Dethlefsen, U., 1998. Therapie des Asthma bronchiale mit Efeublätter-Trockenextrakt. Munch. Med. Wochenschr. 3, 26–30.

Meyer-Wegener, J., Liebscher, K., Hettich, M., Kastner, H.G., 1993. Efeu versus Ambroxol bei chronischer Bronchitis. Z. Allgemeinmed. 69, 61–66.

Paull, K., Covar, R., Jain, N., Gelfand, E.W., Spahn, J.D., 2005. Do NHLBI lung function criteria apply to children? A cross-sectional evaluation of childhood asthma at National Jewish Medical and Research Center, 1999–2002. Pediatr. Pulmonol. 39, 311–317.

Rao, D.R., Gaffin, J.M., Baxi, S.N., Sheehan, W.J., Hoffman, E.B., Phipatanakul, W., 2012. The utility of forced expiratory flow between 25% and 75% of vital capacity in predicting childhood asthma morbidity and severity. J. Asthma 49, 586–592.

Sieben, A., Prenner, L., Sorkalla, T., Wolf, A., Jakobs, D., Runkel, F., Häberlein, H., 2009. α-Hederin, but not Hederacoside C and Hederagenin from Hedera helix, affects the binding behavior, dynamics and regulation of ß2-adrenergic receptors. Biochemistry 48, 3477–3482.

Simon, M.R., Chinchilli, V.M., Phillips, B.R., Sorkness, C.A., Lemanske, R.F., Szefler, S.J., Taussig, L., Bacharier, L.B., Morgan, W., 2010. Forced expiratory flow between 25% and 75% of vital capacity and FEV1/forced vital capacity ratio in relation to clinical and physiological parameters in asthmatic children with normal FEV1 values. J. Allergy Clin. Immunol. 126, 527–534.

Tashkin, D.P., 2002. The role of small airway inflammation in asthma. Allergy Asthma Proc. 23, 233–242.

The Childhood Asthma Management Program Research Group, 2000. Long-term effects of budesonide or nedocromil in children with asthma. New Engl. J. Med. 343, 1054–1063.

Trute, A., Gross, J., Mutschler, E., Nahrstedt, A., 1997. In vitro antispasmodic compounds of the dry extract obtained from *Hedera helix*. Planta Med. 63, 125–129.

Ueki, J., Rhodes, C.G., Hughes, J.M.B., DeSilva, R., Lefroy, D., Ind, P.W., Qing, F., Brady, F., Luthra, S.K., Steel, C., Waters, S.L., Lammertsma, A.A., Camici, P.G., Jones, T., 1993. In vivo quantification of pulmonary β-adrenoceptor density in humans with (S)-^{11}C-CGP12177 and PET. J. Appl. Physiol. 75, 559–565.

Vogelberg, C., Würfel, C., Knoetzsch, A., Kahlert, A., Range, U., Leupold, W., 2007. Exhaled breath condensate pH in infants and children with acute and recurrent wheezy bronchitis. Pediatr. Pulmonol. 42, 1166–1172.