Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2022;11(3):1306-10

The anti-inflammatory effects of inhaled steroids and β2-agonist added to inhaled steroids in the treatment of mild asthma

Oznur Guvenc Uzunay[1], Serife Torun[2], Turgut Teke[3]

[1]Beyhekim Research and Training Hospital, Department of Pulmonary Disease, Konya, Turkey
[2]Konya Baskent University, Research and Training Hospital, Department of Pulmonary Disease, Konya, Turkey
[3]Necmettin Erbakan University, Faculty of Medicine, Department of Pulmonary Disease, Konya, Turkey

Received 24 July 2022; Accepted 05 August 2022
Available online 25.08.2022 with doi: 10.5455/medscience.2022.06.147

Abstract

The use of inhaled corticosteroids (ICS) alone may be sufficient for asthma control in mild asthma. We investigated the effect of inhaled steroid and beta-2 agonist combination on airway function and inflammation in mild asthma.We randomized 20 mild persistent asthma patients treated with the 200μg budesonide (10 patients) or budesonide 200μg -formoterol 12μg (10 patients) twice daily for 4 weeks. We investigated the effects of treatment groups on pulmonary function tests by spirometry and eosinophil percentage and eosinophil cationic protein (ECP) levels in serum and induced sputum. We compared values before and after treatment within budesonide and budesonide-formoterol groups and after treatment between groups. We observed that eosinophil percentage and ECP levels in serum and induced sputum decreased significantly after treatment in both treatment groups. Only the decrease in serum eosinophil percentage level was not significant in the budesonide group. On the other that, there was no significant reduction of inflammatory markers between the two treatment groups. The spirometric measurements (FVC, FEV1, FEF25-75) showed a statistically significant increase within the budesonide-formoterol treatment group, but not in the budesonide group. These measurements were not statistically changed between the two treatment groups. In patients with mild asthma, budesonide and formoterol combination therapy did not add to the improvement of airway obstruction and inflammation more than budesonide therapy.

Keywords: Asthma, inhaled steroid, long-acting β2-agonist, induced sputum, eosinophil, ECP

Introduction

Asthma is a common acute illness and ranges in severity from very mild, occasional wheezing to acute, life-threatening airway closure [1,2].

The airway inflammation in asthma is caused by a specific immune response caused either due to a known allergen or any environmental, occupational, and unidentified factors [3].

In previous years, the severity of asthma was classified according to the intensity of the patient's symptoms, the level of respiratory functions, and whether there was an attack, and the treatment of asthma was arranged according to the severity of the patient. At that time, asthma severity and asthma control were confused and often used interchangeably [4] Today, asthma severity is classified retrospectively according to the minimum drug requirement that can keep symptoms and exacerbations under control [5,6,7,8]. Asthma control in a patient who has been under control for at least 3 months at the most appropriate step: If it can be achieved with step 1-2 therapy (eg, low dose Inhale corticosteroid , Leucotrien, etc.), it is diagnosed as "mild asthma"[4].

Eosinophils secrete cytotoxic granule proteins that have the ability to cause tissue damage and dysfunction. Eosinophil granules contain a crystalloid core of major basic protein-1 (MBP-1) and a matrix of eosinophilic cationic protein (ECP), eosinophilic peroxidase (EPO), and eosinophil-derived neurotoxin (EDN). MBP-1 is the main protein of eosinophil granules. MBP-1 is cytotoxic to the epithelium. ECP is highly toxic to the respiratory epithelium. The developing inflammation is eosinophilic in nature as it is brought about by the activation of eosinophils, which is evident by the increase in the ECP concentration in plasma and other body fluids [9-11].

Inhaled steroids are currently the most effective and first-choice

*Corresponding Author: Serife Torun, Konya Baskent University, Research and Training Hospital, Department of Pulmonary Disease, Konya, Turkey
E-mail:serifetor@hotmail.com

potent anti-inflammatory drugs used in the treatment of persistent asthma [12]. Studies have shown the effectiveness of these drugs in reducing asthma symptoms, airway hypersensitivity, inflammation, severity and frequency of attacks, reducing asthma-related mortality, increasing quality of life and lung functions, and ultimately controlling asthma [13,14]. Bronchodilator medications help improve the symptoms of asthma, airflow restriction and airway hypersensitivity without affecting airway inflammation [15,16,17]. Clinical studies in adults showed that the addition of formoterol to budesonide alleviated asthma symptoms and reduced exacerbations [18].

We sought to compare the effects of inhaled steroid and a long-acting β2-agonist added to the inhaled steroid on airway inflammation by assessing the eosinophil count and ECP levels in serum and the induced sputum of mild asthma.

Material and Methods

All patients had a clear history of relevant symptoms for asthma as defined by The Turkish Thoracic Society National Asthma Diagnosis and Treatment Guidelines [15]. All patients attending our hospital had mild asthma with stable symptoms. Twenty patients were included in the study.

- **Inclusion criteria for the study**

1. Patients aged 18 years and over
2. Patients who agreed to participate in the study
3. Pre-study FEV1 measurement >60% of expected predicted value

- **Exclusion criteria for the study**

1. Those who received treatment other than a short-acting inhaled B 2-agonist in the last three months
2. Those with severe persistent asthma
3. Those with a history of smoking
4. Presence of a respiratory or immunological disease other than asthma
5. Having had an upper respiratory tract infection and severe asthma attack in the last 6 weeks

The study was approved by the ethics committee of the Faculty of Medicine of Selcuk University Meram and informed written consent to the study protocol was obtained from all subjects.

Study Design

Before the study was initiated, the patients were evaluated to determine if they met the inclusion criteria by taking a medical history and performing a systemic physical examination, complete blood count, PA pulmonary X-ray, pulmonary function tests, and reversibility. The venous blood samples of the patients included in the study were collected for determining serum ECP level and eosinophil count. Of the blood sample, 5 cc was taken for assessing serum ECP level and was stored at room temperature for 1 h. After coagulation, the sample was centrifuged at 1200 rpm for 10 min at room temperature and the serum was separated; the serum samples were stored at –80˚C until the time ECP was measured. For determining serum eosinophil count, about 5 cc of blood sample was sent to the hematology laboratory. 4.5%-hypertonic saline solution, in fixed concentration, was administered to the patients and sputum was induced. After induction of the sputum, budesonide in a dose of 200 µg was administered to the ten patients, and budesonide and formoterol in a dose of 12 µg/200µg were administered to the other ten patients twice a day (morning and evening). Short-acting β2-agonist (salbutamol) was administered to all of the patients for use in case of necessity. The same procedure was repeated in the patients during the follow-up visit, which was scheduled after four weeks of treatment.

Sputum Induction and Processing

Sputum induction and processing were performed as previously described [19].The subjects inhaled 4.5% hypertonic saline using a ultrasonic nebulizer. The subjects were encouraged to cough deeply and sputum was expecttorated into steril containers. Macroscopically, the viscous part of the sputum was separated from the saliva, transferred into a tube, and weighed. 0.1% dithiothreitol (DTT) solution (in mL) was added in a quantity amounting to two-fold of the sputum amount (in g) was added to it, and was kept under room temperature for 15 minutes. In the meantime, it was agitated several times, using a vortex mixer for 15 min to ensure homogenization. Next, phosphate buffered saline (PBS) solution was added to DTT in equal volume; the filtrate material was centrifuged at 3000 rpm at room temperature for 10 min. The emergent supernatant was set aside for measurement of ECP level and was stored at ¬–80ºC. After the remaining sediment was re-suspended in 2mL of PBS, the total cell count was performed on the obtained cell suspension using a Neubauer Hemocytometer. For each patient, three cytospins were prepared. Cytospins were obtained by cytocentrifuging (Shandon–4 cytocentrifuge; Shandon Southern Instruments, Sewickley, PA, USA) 50µL of cell suspension at 450 rpm for 6min. As they were left to dry, cytospins were stained using wright statin and then differential cell count was performed by counting 200 non-squamous cell for each preparation.

ECP measurement

Sputum and serum ECP levels were measured using fluorescence enzyme immunoassay method by MBL Mesacup ECP test kits in ELX 800 device as a whole at the same time.

Statistical Analysis

Data were presented as mean±standart deviation. All variables have normality distribution by checking Kolmogorov-Smirnov test. Therefore, for the dependent measurements (pre-treatment and post-treatment) dependant sample t-test were used. For the treatment effects multivariate analyses were applied because of correlated measurements. If p-value is less than 0.05, it is considered as significant. Statistical analyses of the trial were performed by using SPSS statistical package software. The power values obtained according to the data obtained and 10 sample volumes are given in the table below. In some parameters (FVC, FEV1 and FEF 25-75) the power seems particularly low.

There may be 2 reasons for this; Larger than expected variance in measurements or small sample size. Since the planned sample volume was taken according to the difference and standard values of previous studies, it is observed that the data in this study show a higher standard deviation value.

Results

The study group consisted of 15 females and 5 males. Their age ranged between 23 and 57 (mean 43.8±9.9) years. The mean age of the patients was 44.0±12.2 years in the budesonide group, and 43.6±7.6 years in budesonide-formoterol group ($p<0.05$). The baseline pulmonary function tests, eosinophil counts, and ECP levels in serum and induced sputum were no significant change between the two treatment groups ($p<0.05$).

All of the patients completed the treatment period without any drug-related side effects. All patients tolerated the sputum induction well and no patient experienced considerable bronchospasm due to the induction. After four weeks of the treatment, none of the patients experienced any attacks necessitating treatment modifications.

The values of pulmonary function test measurements at baseline and 4 weeks after treatment are given in Table 1. There was an increase in all measurements after treatment. FVC, FEV1 and FEF25-75 measurements in after treatment showed a statistically significant increase in within budesonide-formoterol treatment group, but not in budesonide group. All pulmonary function test measurements were not statistically change between the two treatment groups.

Table 1. Pulmonary function tests before and after treatment with budesonide or budesonide-formoterol treatment groups

	Budesonide		Budesonide-Formoterol	
	Before	After	Before	After
FVC (% predicted)	99.5±8.2	101.3±9.8	98.6±21.1	105.0±20.4*
FEV1 (% predicted)	92.8±9.6	96.1±8.6	91.5±17.8	100.0±19.8*
FEF 25-75 (% predicted)	71.3±20.4	76.2±18.5	69.6±15.9	80.2±20.0*

Data are presented as mean ± SD. *$p<0.05$: for comparison between values before and after treatment within groups. FVC, forced vital capacity; FEV1, forced expiratory volume in one second; FEF25-75, mid forced expiratory flow.

The values of mean serum and induced sputum eosinophil percentage, and ECP levels in before and after treatment are shown in Table 2. The decrease in the serum eosinophil percentage was not statistically significant in the budesonide-formoterol group. All other inflammatory markers decreased significantly after treatment in the both treatment groups. There was no statistically significant difference in the reduction of inflammatory markers between the two treatment groups.

Table 2. İnflammatory markers before and after treatment with budesonide or budesonide-formoterol

	Budesonide		Budesonide-Formoterol	
	Before	After	Before	After
Serum eosinophils(%)	3.9±2.1	2.7±1.6*	2.9±1.0	2.8±1.3
Serum ECP(ng/mL)	83.8±48.8	48.1±37.0*	67.0±29.5	38.1±14.4*
Sputum eosinophils(%)	6.6±6.2	3.4±3.1*	8.5±8.3	3.3±3.5*
Sputum ECP (ng/mL)	137.9±55.4	68.8±58.8*	143.7±65.6	76.7±36.7*

Data are presented as mean ±SD.
*$p<0.05$: for comparison between values before and after treatment within groups. ECP, eosinophilic cationic protein

Discussion

It is well accepted that chronic inflammation plays a significant role in the pathogenesis of asthma which is characterized by eosinophilic airway inflammation [15]. In the present study, eosinophil and ECP levels in the blood and induced sputum were investigated in order to compare the anti-inflammatory effects of 200μg/day budesonide (BUD) and 12/200μg/day budesonide-formeterol twice daily in patients with mild asthma. The induced sputum objectively showed the presence of airway inflammation in asthmatic patients and also depicted the attacks and severity of asthma. It is a valuable method used to monitor the disease and treatment.14In the present study, induced sputum method was preferred as it is non-invasive, safe, and reliable. We demonstrated that after treatment in the both treatment groups improved parameters spirometric and inflammation of airways in patients with mild asthma. However, in budesonide-formeterol treatment group, spirometrics measurements statistically significant increased. This proves that adding formoterol to the treatment increases bronchodilator activity.

The treatment guidelines recommend a stepwise treatment approach based on the disease severity, where the first step comprises of anti-inflammatory treatment [12]. While beta-mimetics used in combination with inhaled steroids improve symptoms and pulmonary functions, they are unable to decrease sputum eosinophilia. Mc Ivor et al. have demonstrated that by controlling pulmonary functions and symptoms in asthmatic patients, salmeterol results in a decrease in the dose of inhaled steroid; however, since eosinophilia persists, it signifies that inflammation is uncontrolled and attack may thus occur [20]. In another study, patients with mild asthma were divided into albuterol, budesonide plus, if necessary, albuterol and formeterol budesonide groups and followed for 52 weeks. As a result of this study, in an open-label study involving adults with mild asthma, budesonide-formoterol used when needed was superior to albuterol used when needed for the prevention of asthma exacerbations [21]. Studies have demonstrated that inhaled steroids decrease eosinophil counts in peripheral blood and sputum, and reduce the levels of ECP in sputum and blood [22].Our findings of reduced sputum eosinophil and ECP, serum eosinophil and ECP are in agreement with earlier studies. In contrast to the present study, Hoshino et al., in their investigation comparing SFP combination and FP monotherapy, detected a further decrease in the eosinophil count of the group receiving SFP [23]. Contrary to this finding, similarly, Bacci et al [24]. Did not detect any decrease in the sputum, blood eosinophil and ECP levels with salmeterol treatment, though they found a considerable decrease in these values with treatment using beclomethasone. In the light of these studies, the fact that β2-agonist treatment has no anti-inflammatory effect has been confirmed. Further, it has been determined in the present study that the addition of long-acting β2-agonist to inhaled steroid treatment did not have any added benefit to decrease inflammation. However, the facts that the groups in the present study consisted of very few patients with mild asthma and the short period of treatment, i.e., only four weeks cannot be neglected. Nevertheless, at the end of a 1-year study in which budesonide monotherapy and budesonide/ formoterol combination were used, no statistically significant difference in inflammation markers in induced sputum was detected between two groups [25]. Contrary to these results, in two large and multi-center studies performed on patients,

with uncontrolled asthma, the addition of inhaled salmeterol to the treatment was found to be more effective on the clinical and inflammatory parameters in contrast to increasing the therapeutic dose of inhaled steroid [26,27]. In which patients with asthma were given inhaled budesonide and formoterol and followed up for 12 weeks, in a study comparing FEV1 and sputum ECP and ACT levels, 1 puff b.i.d. Combination medication may be recommended as a standard dose to minimize side effects and achieve desired control of disease compared to 2 puffs b.i.d. [28].

In the present study, while no control group was considered, the serum and sputum ECP levels in the asthma patients were found to be higher than normal and considerably decreased after the treatment in both the study groups. Yin SS et al. in their study consisting of three groups receiving budesonide, salmeterol, and salbutamol, observed a decrease in sputum and serum ECP levels, though, this decrease was found to be more in the group receiving budesonide [29]. Finally, the SIENA study showed that 73% of patients with mild asthma do not have an eosinophilic phenotype and that these patients have a similar clinical response to ICS (mometasone) and antimuscarinic drugs (tiotropium), results that challenge the indication of a drug combination in a newly published study that incorporates ICS as a first option. It was stated that high-dose steroid given even once in mild asthma patients is important in reducing inflammation and subsequent attacks [30,31].

Our study had some limitations. Our study although the number of patients was statistically significant, it was relatively low. The device we used was in capsule form. There are studies conducted between different forms in the literature. The use of bronchodilation in patients may have suppressed the inflammation aspect of the disease and improved PFT parameters. The methodology of our study was limited to a 4-week period in terms of evaluating treatment outcomes. Longer-term patient follow-ups will also be useful to compare the results.

Conclusion

In conclusion, we have determined that the use of inhaled steroid in mild asthmatic patients showed an improvement in pulmonary functions and the anti-inflammatory effect reduced serum and sputum eosinophil counts, and serum and sputum ECP levels. It was demonstrated that treatment using β2-agonist added to the inhaled steroid provides an improvement in pulmonary function parameters, though it has no added benefit on the anti-inflammatory parameters. The findings of the present study appear to support the previous researches suggesting that β2-agonists possess no anti-inflammatory effect.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
The study was approved by the ethics committee of the Faculty of Medicine of Selcuk University Meram and informed written consent to the study protocol was obtained from all subjects approval number (2009/274;29.05.2009)

References

1. Lee J, McDonald C. Review: Immunotherapy improves some symptoms and reduces long-term medication use in mild to moderate asthma. Ann Intern Med. 2018;169:17.
2. Tesfaye ZT, Gebreselase NT, Horsa BA. Appropriateness of chronic asthma management and medication adherence in patients visiting ambulatory clinic of Gondar University Hospital: a cross-sectional study. World Allergy Organ J. 2018;11:18.
3. Nelson H.S. The importance of allergens in the development of asthma and the persistence of symptoms. Dis. Mon. 2001;47:5–15.
4. Astım Tanı ve Tedavi Rehberi 2020 Güncellemesi. https://www.aid.org.tr/wp-content/uploads/2020/12/astim-rehberi-2020.pdf
5. Global Strategy for Asthma Management and Prevention, 2020 (updated). Avaible from: https://ginasthma.org/wp-content/uploads/2020/06/ GINA-2020-report_20_06_04-1-wms.pdf
6. Reddel HK, Taylor DR, Bateman ED, et al. An official American Thoracic Society/European Respiratory Society statement: Asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med 2009;180:59-99.
7. Taylor DR, Bateman ED, Boulet LP, et al. A new perspective on concepts of asthma severity and control. Eur Respir J. 2008;32:545-54
8. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS Guidelines on Definition, Evaluation of Severe Asthma. Eur Respir J. 2014;43:343-73 .
9. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol. 2006;24.147–74.
10. Rothenberg ME. Eosinophils in the new millenium. J Allergy Clin Immunol. 2007;119:132–2.
11. Menzies-Gow A, Robinson DS. Eosinophils, eosinophil cytokines (interleukin–5) and antieosinophilic therapy in asthma. Curr Opin Pulm Med. 2002;8:33–8.
12. Global Initiative for Asthma (GINA) Updated 2021
13. HA Boushey. Effects of inhaled corticosteroids on the consequences of asthma Review > J Allergy Clin Immunol.1998;102:S5-16.
14. Chazan R. Corticosteroid treatment in airways narrowingReview Pneumonol Alergol Pol.2008;76:96-100.
15. Thoracic Society Asthma Diagnosis and Treatment Guide Toraks Journal 2009
16. Asthma Treatment, New targets, New treatments Bavbek S. Başak printing, 2006
17. Gibson PG, Powell H. Initial corticosteroid therapy for asthma. Curr Opin Pulm Med. 2006;12:48-53.
18. Pauwels RA, Lofdahl CG, Postma DS, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. N Engl J Med. 1997;337:1405–11.
19. 19. Gibson PG, Hargreave FE. Evidence from induced sputum. In: Inflammatory mechanism in asthma. Eds. Holgate ST Buse WW Marcel-Deccer Inc NewYork. 1998;75–88.
20. McIvor RA, Pizzichini E, Turner MO,et al. Potential Masking: Effects of salmaterol on airwayinflammation in asthma. Am J Respir Crit Care Med. 1998;158:924–30.
21. Richard Beasley, Mark Holliday, Helen K Reddel, et al. Controlled trial of budesonide-formoterol as needed for mild asthma. N Engl J Med. 2019;380:2020-30.
22. Hargrave FE. Induces sputum and response to glucocoticosteroids. J Allergy Clin Immunol 1997;102:102–5.
23. Hoshino M, Handa H, Miyazawa T. Effects of salmeterol and fluticasone propionate combination versus fluticasone propionate on airway function and eosinophilic inflammation in mild asthma. Allergology Int. 2009;58:357-63.

24. Bacci E, Franco A.D, Bartoli M.L, et al. Comparison of anti-inflammatory and clinical effects of beclomethasone dipropionate and salmeterol in moderate asthma. Eur Respir. 2002;20:66–72.

25. Kips JC, O'Connor BJ, Inman MD, et al. A long-term study of the antiinflammatory effect of low dose budesonide plus formeterol versus high-dose budesonide in asthma. Am J Respir Crit Care Med. 2000;161:996–1001.

26. Woolcock A, Lundback BO, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhale steroids. Am J Respir Crit Care Med. 1996;153:1481–8.

27. Greening AP, Ind PW, Nothfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhale corticosteroid. Lancet. 1994;344:219–24.

28. Hyun-Moon Back, Jong Bong Lee, Anhye Kim, et al. Exposure-response and clinical outcome modeling of inhaled budesonide/formoterol combination in asthma patients. Pharmaceutics 2020;12:336.

29. Yin SS, Liu H, Gao X. Eleveted fractional exhaled nitric oxide (FeNO) is a clinical indicatör of uncontralled astma in children receiving inhaled corticostreoids. Int J Clin Pharmacol Ther. 2017;55:66–77.

30. Christian Domingo, Jordi Rello, Ana Sogo. As-needed ICS-LABA in mild asthma: what does the evidence say?. Drugs. 2019;79:1729-7.

31. Paul M O'Byrne, J Mark FitzGerald, Eric D Bateman, et al. Effect of a single day of increased as-needed budesonide–formoterol use on short-term risk of severe exacerbations in patients with mild asthma: a post-hoc analysis of the SYGMA 1 study. Lancet Respir Med. 2021;9:149-158.