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Different Nebulized Budesonide Dosing Regimens in a Mouse Model of Chronic Asthma

Kronik Astım Modelinde Farklı Nebulize Budezonid Doz Rejimleri

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Abstract

Objective: The aim of this study was to compare the effectiveness of different inhaled steroid regimens on the lungs and their potential side effects on the bone tissues in chronic asthma model.

Materials and Methods: Thirty-five specific pathogen-free BALB/c mice were divided into five groups. The mice in all of the study groups except the control group were sensitized with chicken egg albumin. After sensitization, the mice in group 2 were treated with saline modeling twice daily, the mice in group 3 were treated with 250 mcg of nebulized budesonide twice daily, the mice in group 4 were treated with 500 mcg of budesonide once daily, and the mice in group 5 were treated with 1000 mcg of budesonide every other day for the last 14 days of the challenge period. After challenge, the mice were sacrificed and lung and tibia samples were histologically examined.

Results: Pulmonary parameters, including subepithelial smooth muscle thickness, goblet cell count, mast cell count and epithelial thickness, were the lowest in group 5 compared to other groups (p<0.01). Lung epithelial basement membrane thickness was the lowest in group 4 (p<0.01). In tibia bone tissue, there were no significant differences among treatment groups (group 3, 4 and 5) in terms of trabecular, cortical and osteoid thickness and number of trabeculae (p>0.01).

Conclusion: The beneficial effect on lung tissue was highest in the treatment group receiving budesonide every other day (group 5) and no further measureable side effects on bone mineralization were observed in this group compared with the other treatment groups. Every-other-day treatment application seems to be the most effective regimen in chronic asthma model.

Öz

Amaç: Çalışmanın amacı kronik astım modelinde farklı inhale steroid doz rejimlerinin akciğerler üzerine etkisinin ve kemikler üzerine olan yan etkisinin araştırılmasıdır.

Gereç ve Yöntemler: Spesifik patojen içermeyen 35 BALB/c fare beş gruba ayrıldı. Kontrol grubu hariç diğer tüm gruplar tavuk yumurtası albumini ile duyarlaştırıldı. Duyarlanmadan sonra, grup 2'deki farelere günde iki kez salin, grup 3'deki farelere günde iki kez 250 mcg budezonid, grup 4'deki farelere günde tek doz 500 mcg nebul budezonid ve grup 5'deki farelere de iki günde bir 1000 mcg budezonid ilaç yükleme döneminin son 14 gününde uygulandı. İlaç uygulaması sonrasında fareler sakrifiye edilerek, akciğer ve tibia dokusu histolojik olarak incelendi.

Bulgular: Akciğer bulgularından subepitelyal düz kas kalınlığı, goblet hücre sayısı, mast hücre sayısı ve epitel kalınlığı grup 5'de en düşük orandaydı (p<0,01). Akciğer epiteli bazal membran kalınlığı grup 4'de en düşüktü (p<0,01). Tibia kemik dokusunda, tedavi grupları arasında (grup 3, 4 ve 5) trabekül sayısı, trabeküler, kortikal ve osteoid kalınlığı ve trabekül sayısı açısından fark saptanmadı (p>0,01).
Sonuç: Akciğer dokusu üzerine yararlı etki en çok günaşırı budezonid nebul alan grupta (grup 5) saptandı ve bu grupta kemik mineralizasyonu üzerine oluşan yan etki diğer tedavi gruplarından daha fazla değildi. Günaşırı tedavi uygulaması kronik astım modelinde en etkili tedavi yaklaşımı gibi görünmektedir.

Introduction

Asthma is a chronic inflammatory disease of the airways (Av) characterized by reversible bronchial obstruction, airway hyper-responsiveness, inflammation, and remodeling (1). During an acute inflammatory response, cellular infiltration, release of various mediators, contraction of bronchial smooth muscle, airway hyper-responsiveness, and airway obstruction occur. In the chronic period of asthma, partially reversible airway remodeling begins. Such remodeling is characterized by epithelial desquamation, intense inflammatory cell infiltration, goblet cell hyperplasia, smooth muscle hypertrophy, angiogenesis, thickening of the basement membrane, and a reduction in airway wall elasticity (2).

Current strategies for the management of asthma largely focus on suppressing airway inflammation and slowing the remodeling process (3). Inhaled corticosteroids are known to be the most effective and preferred drugs in asthma symptom control and for slowing the progression of airway remodeling. So far, asthma control level is under expectations despite huge effort. In the long term, the daily regimen of inhaled corticosteroids that is required, corticosteroid phobia, the risk of emerging systemic side effects due to high doses of corticosteroids, and a decrease or failure in compliance are the main reasons (2,4).

It has been shown in many studies that treatment compliance in patients may be improved by increasing drug bioactivity by prolonging the drug half-life, decreasing the number of daily doses of medication, and by developing different drug combinations (5).

One of the most serious side effects caused by inhaled corticosteroids is osteopenia and osteoporosis. Although it was less frequently observed in women using inhaled corticosteroids than in women using oral corticosteroids, women using inhaled corticosteroids have been shown to have decreased bone density in the distal radius, lumbar region and the hip bones (6). However, the effective doses and periods required for inhaled corticosteroids to affect bone tissue are not fully determined.

The aim of the present study was to compare the effects of long-term treatment with budesonide, when applied twice in a day, once a day, and every other day while maintaining a fixed cumulative daily dose in order to decrease dosage and increase compliance to the treatment, specifically assessing its effects on lung histology and side effects on bone tissue morphology in a mouse model of chronic asthma.

Materials and Methods

Experimental Animals

Specific pathogen-free, 6-week-old female BALB/c mice weighing 18-20 g obtained from the Animal Laboratory at Dokuz Eylül University were used in the present study. The characteristics of these mice at 6 weeks of age were considered to be comparable to those of adolescent humans. The mice were maintained in hygienic macrolane cages and were allowed access to food and water ad libitum on a 12-h light/12-h dark cycle. All experimental procedures were designed according to the requirements of the Animal Care and Ethics Committee of Dokuz Eylül University with a project number of 70/2009. The protocols complied with the standards specified in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources and published by the National Academies Press, National Research Council, Commission of Life Sciences, Institute of Laboratory Animal Resources.

This experimental model developed by Temelkovski et al. (7) replicates many features of human asthma; in contrast to many other experimental asthma models, this model features chronic histopathological changes including intraepithelial goblet cell hyperplasia, mast cell infiltration, epithelial thickening, and subepithelial fibrosis in the lung tissue.

Sensitization and Inhalation Exposure

BALB/c mice are high responders to ovalbumin (OVA), which is the material used for the sensitization

procedures to establish a chronic asthma model (7). Thirty-five BALB/c mice were divided into 5 groups. Group 1 was designated as the control group and group 2 as the chronic asthma group receiving placebo. The mice in all of the study groups except for the control group were sensitized on days 0 and 14 with intraperitoneal injections of 10 μg chicken egg albumin (OVA, grade 5, ≥98% pure; Sigma, St. Louis, MO), with aluminum as an adjuvant. The mice in study groups 2, 3, 4 and 5 were then challenged with an aerosol of 2.5% OVA in 8 cc of saline for 30 min/day, 3 days per week for 8 weeks beginning on the 21st day and proceeding until the 74th day of the sensitization period. The mice in group 1 received normal saline with aluminum on days 0 and 14 of the experiment and 8 cc of aerosolized saline for 30 min/day, 3 days per week for 8 weeks beginning on the 21st day of the study and proceeding until the end of the sensitization period (7). The exposures were carried out in a whole body inhalation exposure system. The OVA was aerosolized by the delivery of compressed air to a sidestream jet nebulizer, and the aerosol was injected into a 220x100x70 cm^3 chamber. The aerosol generated by the jet nebulizer was comprised of >80% particles with a diameter of <4 μm for OVA. The particle concentration was maintained in the range of 10-20 mg/mm^3 (7). The temperature and relative humidity were maintained at 20-25 °C and 40-60%, respectively.

Study Drugs

The mice in group 1 received saline rather than OVA (control group); group 2 did not receive any treatment (placebo) and group 3 received 250 μg of nebulized budesonide twice a day. Group 4 received 500 μg of nebulized budesonide once a day; and group 5 received 1000 μg of nebulized budesonide every other day in the last 14 days of the challenge period. Budesonide (Pulmicort nebule®, 0.25 mg/2 mL, AstraZeneca) exposures were carried out in a whole body inhalation system as OVA. The concentration of budesonide was calculated according to the volume of the chamber, concentration of the drug inside the chamber and the nebulization period. The time period of budesonide nebulization was continued an average of 60 minutes in each dose. The cumulative budesonide particle concentration that the mice exposed was maintained in the range of 6-8 mcg/mm^3/day. The timing of the doses were calculated

according to adapted formulation of median life span of female BALB/c mice and humans which is ranging from 20 to 28 months for female mice corresponding to an average of 65 years of humans.

Sacrifice and Sampling

The animals were sacrificed by an overdose of ketamine 24 h after the last drug administration, which was the 75th day of the study. The histological specimens were evaluated by two histology specialists who were blinded to the identity of the study groups. The thickness of the basement membrane, epithelium, and subepithelial smooth muscle and the number of goblet and mast cells were measured in the histological specimens. The number of trabeculae and the trabecular, cortical and osteoid bone thicknesses were measured in the bone tissues.

Histopathological Analysis of the Lung Tissue

The lung tissues were obtained from the mid zone of the left lung of the mice. The samples were fixed in 10% formalin for evaluation using a light microscope (Olympus BX-51, Tokyo, Japan), and the adjacent lung tissue regions were stored in 2.5% glutaraldehyde for further evaluation using an electron microscope (Libra 120; Carl Zeiss, Oberkochen, Germany). Photomicrographs were taken using a JVC TK-890-E camera (Japan), which was fitted to the microscopes. The histological analysis was performed using the UTHSCSA Image Tool for Windows Version 3.00 software.

After fixation, the samples were embedded in paraffin blocks, and serial sections of 5-μm in thickness were cut and stained using three different dyes for the evaluation of different parameters by light microscopic analysis. The first 10 samples were stained with hematoxylin and eosin (H&E) with the aim of measuring the thickness of the epithelium, basement membrane and subepithelial smooth muscle layers of the medium and small Av. The thickness of both the epithelium and the subepithelial smooth muscle layers were measured at four points (3, 6, 9 and 12 o'clock) for each airway. Each section contained approximately two to three Av; therefore, approximately 20 Av were evaluated for each mouse.

Ten consecutive tissue sections were stained with Periodic acid-Schiff to enumerate goblet cells and with toluidine blue to count mast cells. The goblet cells were counted in 10 sections for which five

randomly selected Av were imaged. The percentage of goblet cells per 100 µm was calculated by dividing the total number of goblet cells by the total airway circumference and multiplying the result by 100. For mast cell counting, a standard transparent counting frame representing an area of 2×10^4 µm² was used and eight fields in each photograph were examined for each mouse.

For the preparation of lung samples for electron microscopy, the tissue was embedded in EPON, sliced in ultrathin sections and stained with uranyl acetate and lead citrate. For each mouse, five to seven ultrathin sections were prepared for the evaluation of the cellular structure of the airway, the epithelium, thickness of the basement membrane and the presence of connective tissue. All of the histological sections prepared for electron microscopy were visualized, eight to ten areas were imaged.

Histopathological Analysis of the Bone Tissue

The bone tissues were obtained by the extraction of the tibia at the time of sacrifice. The tibias of the mice were immersed in 8% formic acid for 6 months to decalcify the bone. When the bone tissue was deemed to be soft enough, it was fixed in 10% formalin and then embedded in paraffin blocks and sectioned at a thickness of 5 µm. The first 10 sections were stained with H&E for histological evaluation of the trabecular bone structure, including the number of trabeculae and the trabecular and cortical thickness. The second

set of 10 sections was stained with Masson's trichrome for evaluation of the thickness of the osteoid bone tissue. The thickness of the trabeculae, the cortex and the osteoid bone were measured at 3, 6, 9 and 12 o'clock, as in lung tissue. For each mouse, eight to ten areas were imaged.

Statistical Analysis

The program SPSS 13 was used for data analyses (SPSS Inc., Chicago, IL, USA). The data were presented as the mean ± standard deviation (minimum-maximum) of seven mice in each group. The comparisons between more than two independent groups were analyzed using one-way analysis of variance (ANOVA). Wherever overall group comparisons were found to be significant, Bonferroni multiple comparisons test was performed for pair-wise comparison by using a Bonferonni correction by setting $p < 0.05/5 = 0.01$ as nominal cutoff. A p value of less than 0.05 was considered statistically significant.

Results

Examination of the Lung Tissue

There was no mortality in any of the groups throughout the study period. In asthma group (group 2), statistically significantly higher basement membrane, subepithelial muscle and epithelial thicknesses and higher goblet and mast cell counts were observed compared to that in the control group (group 1), which confirmed the successful establishment of the chronic asthma model (p<0.01 for each parameter) (Table 1,

Table 1. Comparison of histopathological findings of lung tissue among study groups

	Group 1 control group (n=7)	Group 2 asthma group (n=7)	Group 3 budesonide 2x250 µg/d (n=7)	Group 4 budesonide 1x500 µg/d (n=7)	Group 5 budesonide 1x1000 µg/ every other day (n=7)	p value[a,b]
Basement membrane thickness (nm) (mean ± SD)	271.61±20.61	669.27±15.80	467.43±15.30	354.69±27.51	390.34±10.49	0.004[*]
Subepithelial smooth muscle thickness (n) (mean ± SD)	4.96±0.47	11.83±0.70	6.83±0.58	8.55±0.42	5.78±0.38	0.003[†]
Epithelium thickness (µm) (mean ± SD)	10.05±0.80	21.35±1.95	16.22±0.90	13.24±0.39	12.09±0.73	0.003[‖‡]
Goblet cell count/100 (µm) (mean ± SD)	0.32±0.16	2.91±0.33	2.58±0.80	2.06±0.30	0.65±0.12	0.004[*]
Mast cell count/20.000(µm²) (mean ± SD)	0.87±0.20	2.51±0.30	1.67±0.31	1.39±0.25	1.31±0.18	0.004[Ψ]

d: Day, SD: Standart deviation, nm: Nanometer, µm: Micrometer, µm²: Square micrometer, µg: Microgram
[a]p<0.05 was significant for ANOVA, [b]p<0.01 was significant paired-wise comparisons among study groups by (ANOVA) after Bonferonni correction, [*]p<0.01 between all paired-wise group comparisons, [†]p<0.01 between paired-wise group comparisons, [‖]Comparison between groups 2 and 1, 3, 4, 5 (p<0.01 for each), and groups 1 and 3 were statistically significant (p=0.002), [ψ]p<0.01 between all paired-wise group comparisons, [Ψ]Comparison between groups 2 with 1, 3, 4 and 5 are statistically significant (p<0.01 for each)

Figure 1, 2). All parameters were statistically significantly higher in group 2 compared to treatment groups with budesonide (groups 3, 4 and 5) (p<0.01 for each).

Subepithelial smooth muscle thickness and goblet cell count were significantly lower in group 5 compared to groups 2, 3 and 4 (p<0.01, p=0.008, p=0.003, respectively). Subepithelial smooth muscle thickness was higher in group 4 when compared to group 3 (p=0.006) (Table 1, Figure 2).

Basement membrane thickness was statistically lower in group 4 compared to groups 2, 3 and 5 (p<0.01, p=0.003, p<0.01). There was no significant difference between budesonide treatment groups (groups 3, 4 and 5) in terms of epithelium thickness and mast cell counts (p>0.01 for each) (Table 1, Figure 2).

Examination of the Bone Tissue

When the histological parameters of the bone tissue were compared between the controls (group 1) and the asthmatics (group 2), there was no statistically significant difference in the number of trabeculae, trabecular, cortical or osteoid bone thicknesses (p>0.01).

The number of rabeculae was significantly reduced in groups 3 and 4 compared to group 2 (p=0.003, p=0.003, respectively). However, trabecular number in group 5 was not statistically significantly reduced when compared to group 2 (p>0.01). When asthmatics (group 2, placebo) and treatment groups (groups 3, 4, and 5) were compared, the trabecular, cortical and osteoid thicknesses were significantly higher (p<0.01 for each).

The bone tissue histological parameters, including the number of trabeculae and the trabecular, cortical, and osteoid bone thickness were compared between each of the treatment groups (group 3, 4, and 5), but no statistically significant differences were observed (p>0.01), as shown in Table 2 and Figure 3.
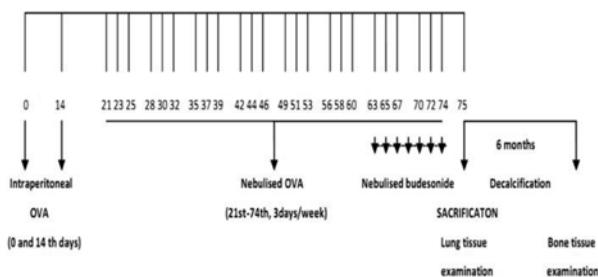
Figure 1. Study algorithm for the establishment of murine model of chronic asthma, treatment with budesonide and sampling of lung and bone tissues

OVA: Ovalbumin

Discussion

In the present study, the overall beneficial effect is the highest in the treatment group receiving nebulized budesonide every other day and no further measureable side effects on bone mineralization were observed in this group compared with the other treatment groups.

To our knowledge, even though there are many studies on the effects of a single daily dose of budesonide on asthma symptoms, there are no published studies investigating the effects of high dose budesonide used every other day. In the present study, we observed that instead of a twice daily approach, a once-daily budesonide application led to important decreases in all pulmonary parameters, except basement membrane thickness. We found that all pulmonary parameters except the basement
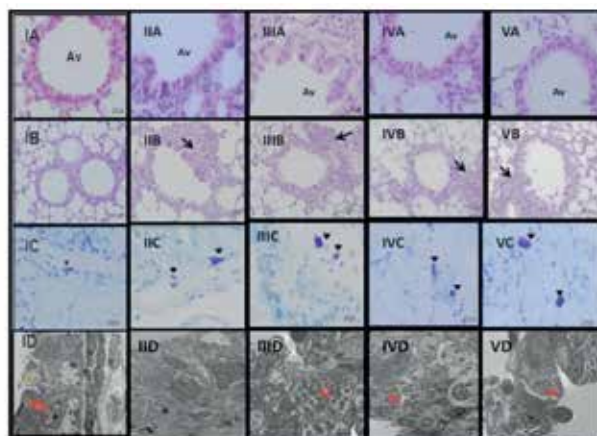
Figure 2. Light and electron microscopic findings of lung tissue of study groups. 1; control, 2; asthma (placebo), 3; group received budesonide of 250 mcg every one hour, 4; group received budesonide of 500 mcg every 2 hours, 5; group received budesonide of 1000 mcg every 4 hours. In light microscopic images, lung tissues were stained with hematoxylin and eosin (A; first row), Periodic acid-Schiff (B; second row), and toluidine blue (C; third row). Microscopic findings of respiratory epithelium and Goblet cells stained with Periodic acid-Schiff were shown in study groups (IA-VA), Light microscopic findings of respiratory epithelium and cellular infiltration stained with hematoxylin and eosin were shown in study groups (IB-VB), touidine blue-stained paranchymal structures and mast cells were shown in study groups (IC-VC), electron microscopic findings of respiratory epithelium with cilia were shown in (ID-VD). Airways, thickened basement membrane (*), increased peribronchial mononuclear infiltration (arrow), mast cells (arrowhead), cilia (C) and goblet cell (Gc)

Gc: Goblet cells, Sm: Smooth muscle, Arrow with tail: Thickened smooth muscle, Arrow without tail: Mast cells, Subepithelial smooth muscle layer (↯), Basement membrane (*), peribronchial mononuclear infiltration (†)

membrane thickness were ameliorated in the group of mice administered high doses of nebulized budesonide every other day when compared with the once- and twice-daily groups. A decrease in the basement membrane thickness was found to be most significantly different in the once-daily group, while it was still lower in the every-other-day group when compared with the other groups. This finding may be explained by the increased intracellular esterification and the longer hydrolysis with high doses of budesonide. In other words, unlike other inhaled corticosteroids, intermittent use of high-dose budesonide, every other day for example, might be considered effective and could increase treatment compliance in asthmatic patients.
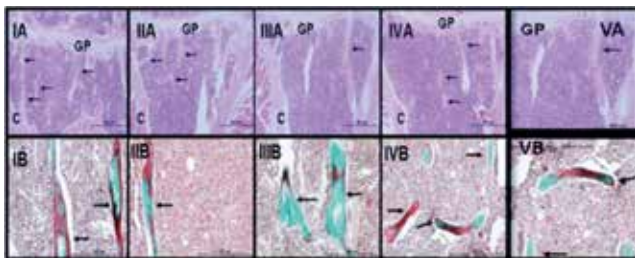

Figure 3. Light and electron microscopic findings of bone tissue of study groups. 1; control, 2; asthma (placebo), 3; group received budesonide of 250 mcg every one hour, 4; group received budesonide of 500 mcg every 2 hours, 5; group received budesonide of 1000 mcg every 4 hours. In light microscopic images, proximal tibia were stained with hematoxylin and eosin (A row) and Masson trichrome (B row). In hematoxylin and eosin stained sections trabeculae number and trabecular and cortical thicknesses were seen. In Masson trichrome stained sections, red-green stained regions are osteoid zone of trabeculaee

GP: Growth plate, C: Cortex, Arrows: Trabeculaee

Budesonide is distinct from other inhaled corticosteroids in that it has a higher affinity for Av, a faster esterification process that allows it to be deposited in the respiratory epithelium and a slower rate of degradation with a lower capacity for systemic distribution (8,9). Experimental studies have demonstrated that the esterification of 70-80% of budesonide occurs in the Av within 20 minutes, and in human nasal tissue, 30% and 50% esterification occurs within a few hours of budesonide inhalation (10,11). It has been shown that the duration of action of budesonide, when compared with other inhaled corticosteroids, is longer when it is esterified. Lexmüller et al. (12) demonstrated that the elimination of budesonide from the trachea and the main stem bronchus was 35-fold slower than that for fluticasone propionate. Moreover, budesonide overall retention in trachea at 3 hours was greater than that of other corticosteroids, and the budesonide-ester pool was 3-fold greater than the cyclesonide ester pool. Budesonide becomes esterified more promptly and to a greater extent than cyclesonide, and budesonide esterification prolongs budesonide airway retention. This phenomenon was explained by the hypothesis that when the concentration of free budesonide within the cell decreases, the inactive deposited forms of budesonide esters are hydrolyzed by intracellular lipases to become activated and are slowly released from the cell within 24 h (13). The results of the present study are consistent with the results of previous trials and suggest that the greatest effects on lung histopathology in the chronic asthma model are achieved with a higher dose of budesonide

Table 2. Comparison of histopathological findings of bone tissue among study groups						
	Group 1 control group (n=7)	Group 2 asthma group (n=7)	Group 3 budesonide 2x250 µg/d (n=7)	Group 4 budesonide 1x500 µg/d (n=7)	Group 5 budesonide 1x1000 µg/every other day (n=7)	p value[a,b]
Trabeculae number (mean ± SD)	11±1.13	10.75±1.10	5.00±0.40	5.00±0.64	6.75±0.94	0.003*
Trabecular thickness (nm) (mean ± SD)	61.38±0.47	63.47±1.43	29.65±3.08	36.90±2.23	35.38±2.61	<0.001[†]
Cortex thickness (µm) (mean ± SD)	151.85±0.16	149.64±4.52	96.71±5.28	99.34±7.14	98.56±5.28	0.003[††]
Osteoid thickness (µm) (mean ± SD)	23.17±0.34	21.08±1.37	15.56±3.98	12.27±1.96	12.69±1.17	0.004[ψ]
d: Day, SD: Standart deviation, nm: Nanometer, µm: Micrometer, µg: Microgram, [a]p<0.05 was significant for ANOVA, [b]p<0.01 was significant paired-wise comparisons among study groups by (ANOVA) after Bonferonni correction, *Comparison between group 1 and groups 3, 4 (p<0.01 for each), and group 2 and 3, 4 (p<0.01 for each), [†]Comparison between groups 1 and 2 (p>0.01), and group 4 and 5 (p>0.01) were statistically insignificant, however, other group comparisons were statistically significant (p<0.01 for each), [††]Comparison between group 1 and groups 3, 4, 5 (p<0.01 for each), and group 2 and groups 3, 4, 5 (p<0.01 for each), [ψ]Comparison between group 1 and groups 3, 4, 5 (p<0.01 for each), and group 2 and groups 3, 4, 5 (p<0.01 for each)						

administered over longer time intervals. Moreover, this finding suggests that histological attenuation is greater in the group that received the higher dose of budesonide across longer time intervals because the esterification of budesonide, which causes a slower release of the drug that depends on the decrease in the concentration of free budesonide, increases its time of effectiveness by maintaining a steady-state drug concentration in the respiratory epithelium.

The prolonged retention of budesonide in the Av contributes to its efficacy (10). Therefore, it is proposed that budesonide has high efficiency and safety in asthma and suitable for once-daily inhalation. Reducing the number of required daily inhalations may increase patient compliance with asthma therapy, so that increases efficiency in long term (12). Many promising studies have shown good results in patient groups administered a single daily dose of budesonide (14,15). To date, one systematic review has concluded that a single daily dose of budesonide is considerably efficacious as the initial therapy in children with mild persistent asthma and that it is generally well tolerated as the maintenance therapy after control was established in patients with moderate asthma. A single daily dose was observed to facilitate the control of asthma and to increase patient adherence to treatment (16). Similarly, a second meta-analysis demonstrated that once and twice daily doses were not different in long-term control and had advantages in terms of patient compliance and satisfaction (17). In the present study, we found that every-other-day dose regiment provided the highest effect of budesonide on the lung tissue. Further studies are needed about the dose regimen as every other day to overcome the major problem of compliance.

Consistent with previous studies, the bone mineralization that was calculated by assessing bone mineral density was determined to be inadequate in children who were treated with high doses of corticosteroids (18). However, there were no clear effects of medium- and low-dose inhaled corticosteroid on the overall and regional bone density in children with asthma. As a result, it was concluded that medium- and low-dose inhaled corticosteroids are safe for use (19). Recently, in a systemic review and meta-analysis, longer use (>12 months) of inhaled corticosteroids in children with asthma was found to

have a limited effect on annual growth velocity and represents a 0.7% reduction in final height compared to non-inhaled corticosteroid users (20).

To our knowledge, there is no study demonstrating the effects of different dose ranges of inhaled corticosteroids on bone demineralization. The most important study evaluating once- and twice-daily budesonide treatments in terms of side effects is the above mentioned START study, which showed no difference in terms of local side effects with different doses of inhaled budesonide (21). In our study, the trabeculae number was lower in twice-daily and once-daily treatment groups and the trabecular thickness was lower in twice-daily treatment group compared to asthma group. However, there was no significant difference in side effects between the groups, irrespective of the frequency of drug administration.

Study Limitation

There were some limitations in our study. In the determination of the effect of different dosage regimens on lung tissue and the side effects on bone tissue, only histopathological changes could be demonstrated. The cytokine levels, biomarkers and airway responsiveness could not be evaluated.

Conclusion

In brief, high-dose budesonide, when applied every other day, seems be more effective on asthma histopathology when compared with other dose regimens, while the side effects were found to be similar among the different groups. Considering the importance of treatment compliance, we suggest that budesonide treatment might be more efficient in an every-other-day dosing regimen due to the mechanisms of budesonide esterification. Well-designed, prospective and controlled clinical studies are needed to further investigate the intracellular mechanisms of action of budesonide and should be conducted to evaluate the efficiency of different dose regimens in clinical practice, particularly in selected patient populations.

Ethics

Ethics Committee Approval: All experimental procedures were designed according to the requirements of the Animal Care and Ethics Committee of Dokuz Eylül University with a project number of 70/2009.

Peer-review: Internally peer-reviewed.

Authorship Contributions

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