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ZENON SIERGIEJKO, ZIEMOWIT ZIĘTKOWSKI,
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Bronchial hyperreactivity in asthmatics during combined therapy with β_2-agonist and muscarinic receptor blocker

Nadreaktywność oskrzeli u chorych na astmę w czasie skojarzonego leczenia antagonistą receptorów β_2-adrenergicznych i blokerem receptorów muskarynowych

INTRODUCTION

The β_2-agonists are the most effective bronchodilators used for reversing and preventing acute airway obstruction in patients with asthma. Since their introduction in the 1960s, β_2-agonists have been prescribed to many millions patients throughout the world. Their chronic, regular use, especially in high doses might give adverse effects [36]. Some authors have associated asthma exacerbation and mortality with regular β_2-agonist inhalations [8, 17, 29,31]. The following adverse effects have been noted during β_2-agonist application: direct cardiovascular effect, hypokaliemia, down regulation of β-receptors, increased bronchial reactivity, rebound decrease in FEV_1 after cessation of short-acting β_2-agonists and the possibility of increased allergen inhalation after bronchodilatation [30, 38].

Recently many papers have appeared describing attempts to answer the question whether β_2-agonists are beneficial or harmful [7, 10, 15, 16]. Because several recent studies have shown a poorer control of bronchial asthma and an increase in BHR when short acting β_2-agonists are inhaled regularly [3, 6, 13, 18, 24], we attempted to assess the effect of combined therapy with low doses of a β_2-agonist with a muscarinic receptors blocker on bronchial hyperreactivity. Many papers have shown a preventive effect of single doses of β_2-agonists on BHR [4, 23]. It is known that simultaneous therapy with inhaled corticosteroids prevents an increase in BHR during long-term, regular β_2-agonist consumption [2, 12, 27]. We expected similar effects using a muscarinic receptor blocker simulatneously with a β_2-agonist.

The aim of our study was to evaluate the effect of 6 weeks' combined therapy with low doses of a β_2-agonist and a muscarinic receptor blocker (Berodual — aerosol

Table I. Schedule of the study

Succesive days of the study						
1	2	3	4 ... 45	46	47	48
*BPT-histamine *serum ECP level	* BPT metacholine	* BPT-Dpt	Therapy studied	*BPT-histamine *serum ECP level	* BPT metacholine	* BPT-Dpt
No medication before the tests			Berodual 3x2puffs from MDI and more if require	No medication before the tests		

containing 0.05 mg of fenoterol and 0.02 mg of ipratropium bromide in one dose) on specific and non-specific bronchial reactivity, spirometric lung function parameters and serum ECP levels.

MATERIAL AND METHODS

The studies were carried out on 16 mild atopic mite asthma patients found to be sensitive to house dust mite by the case history and positive skin tests with D.pteronyssinus. All the patients were treated with Berodual (Boehringer Ingelheim, Germany) only in a dose of 2 puffs 3 times daily and more if required for a period of 6 weeks. The spirometry, metacholine and histamine bronchial provocation tests and specific bronchial provocation test were performed before and after the therapy. Venous blood samples for determining the serum ECP levels before and after therapy were taken. The study schedule is presented in Table I.

Bronchial provocation tests were performed according to the Ryan et al. method using jet De Villbiss 646 nebulisers connected to a Rosenthal French dosimeter powered by compressed air [22, 25]. As provoking stimuli, Histamine dichloride (Serva, Germany), Acetyl-β-methylcholine chloride (Aldrich, Germany) and mite allergen of Dpt — Aguagen SQ 100,000 (ALK, Denmark) were used. Spirometric parameters were evaluated by a Pneumoximeter (ArtMed, Kraków). The results of non-specific provocation were expressed as $PC_{20}FEV_1$ (that is, the metacholine or histamine concentration in mg/ml causing a decrease in FEV_1 equal to 20%). After the allergen challenge, the early and late asthmatic response were observed. The results of EAR were expressed as $PD_{20}FEV_1$ (that is, the allergen dose in SQ-U causing a decrease in FEV_1 equal to 20%). The spirometric parameters were also estimated hourly over an 8 hour period at the end of the allergen provocation. A reduction in FEV_1 greater than 20% of the baseline value during this period was considered a positive LAR.

Measurement of ECP was made using a ECP RIA kit (Pharmacia CAP System, Sveden) [19].

The study results were analysed using the Wilcoxon match-paired test.

RESULTS

The results obtained are presented in the following tables and Figure 1.

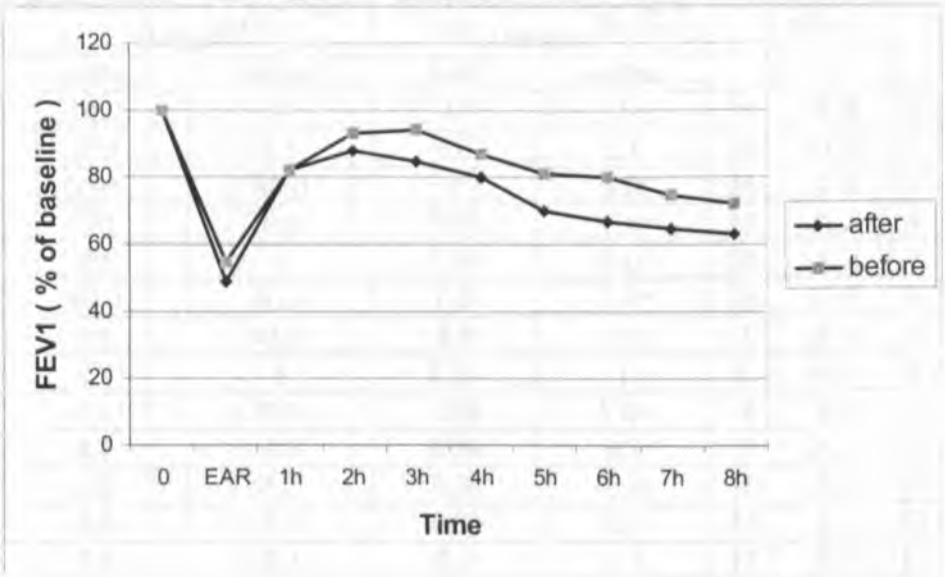

Fig. 1. Effect of simultaneous therapy with ipratroprium bromide and fenoterol on changes in FEV_1 observed for a period of 8 hours after the allergen challenge

In Table II the results showing the effect of the combined therapy on bronchial response to histamine and metacholine are presented. The therapy significantly diminished the bronchial response to metacholine, but enhanced the response to histamine.

In Table III the effect of the therapy with fenoterol and ipratropium bromide on specific bronchial provocation is presented. We can see that the mean value of the $PD_{20}FEV_1$ Dpt after provocation was lower than before. The difference is statistically significant. After the therapy, positive LAR were observed in some patients in whom only an EAR had occurred earlier.

In Figure 1 curves of the changes in FEV_1 observed for a period of 8 hours after bronchial challenge before and after combined therapy with a β_2-agonist and a muscarinic receptor blocker are presented. In patients with a positive LAR, after the combined therapy, the 20% fall below the baseline value occurred earlier and was greater than before the therapy.

The effect of the combined therapy with fenoteral and ipratropium bromide on spirometry is presented in Table IV. No significant changes in spirometry were observed in the patients treated with Berodual.

In Table V the serum ECP levels measured in the patients before and after therapy are given. ECP levels in most of the patients before and after the therapy were within the normal range. A slight but statistically significant increase in these levels was observed after the therapy.

Table II. The effect of 6 weeks' combined therapy with a β_2-agonist and a muscarinic receptors blocker on non-specific bronchial hyperreactivity in asthma patients (SE — standard error)

	Patients	Sex	$logPC_{20}FEV_1$-histamine (mg/ml)		$logPC_{20}FEV_1$-metacholine (mg/ml)	
			before	after	before	after
1	K.P.	M	1	-1	1	0.63
2	K.D.	M	1.5	0.3	1.5	1.07
3	R.T.	M	0.4	1	0.48	-0.05
4	K.A.	M	0.3	0.05	0.3	0.3
5	S.A.	M	0.9	-0.3	1	1.5
6	P.K.	M	0.4	-0.1	-0.4	0.08
7	W.E.	F	0.8	0.6	0.9	1.5
8	Ł.W.	F	1	-0.7	1	1.2
9	C.K.	M	-0.7	0.5	-0.7	1.4
10	S.A.	F	0.6	0.25	0.6	1.4
11	S.J.	F	1.5	-1.5	1.5	1.5
12	A.J.	M	0.5	-0.04	0.4	0.8
13	H.W.	M	1.4	1.2	1.3	1.5
14	S.R.	F	0.08	0.5	0	0.4
15	P.R.	F	1.4	0	1.3	1.5
16	M.W.	F	0.08	0	-0.1	0.5
X ± SE			0.69±0.15	0.04±0.17	0.63±0.16	0.95±0.14
Z			2.25		1.87	
p			0.02		0.06	

DISCUSSION

Our results have shown the possibility of an increase in bronchial hyperreactivity after combined therapy with fenoterol and ipratropium bromide, measured by the BPT using histamine as provoking stimulus. If metacholine was used as a provoking substance, a decrease in bronchial response after above mentioned therapy was observed. It is known that muscarinic agonists such as metacholine and acetylocholine cannot be used as stimuli during non-specific BPT assessing the effect on BHR of anti-muscarinic drugs, because there is a competitive antagonism between them [32]. Anti-muscarinic drugs reduce bronchial response to metacholine very well [1, 5, 20, 21, 28]. In acute studies these drugs caused a small decrease in the dose response curve to histamine [26, 33]. Histamine seems to be a more objective stimulus for checking the bronchial hyperreactivity.

Table III. The effect of 6 weeks' combined therapy with fenoterol and ipratropium bromide on bronchial response to specific allergen (EAR — early asthmatic response, LAR — late asthmatic response)

	EAR – log $PD_{20}FEV_1$- SQ-U		LAR	
	before	after	Before	after
1	4.88	4.26	-	+
2	4.7	5.08	+	+
3	4.51	3.47	+	+
4	5.02	4.86	-	-
5	3.85	3.54	+	+
6	3.7	3.91	-	-
7	2	2.7	+	+
8	4.43	3.9	+	+
9	3	2.78	+	+
10	4.36	3.43	+	-
11	4.11	3.84	-	+
12	3.45	3.23	+	+
13	4.72	4.66	-	+
14	4.66	4.12	-	+
15	3.43	2.78	+	+
16	3.7	3.18	+	+
X ± SE	4.03 ± 0.2	3.73 ± 0.1		
Z	2.25		10 (+), 6 (-)	13 (+), 3 (-)
p	0.02			

Table IV. The effect of 6 weeks' combined therapy with fenoterol and ipratropium bromide on spirometry (%PV — % of predicted value)

Spirometry parameters	X ± SD		Statistical significance
	before	after	
FEV_1 (% PV)	89 ± 19	92 ± 10	NS
FVC (% PV)	105 ± 14	102 ± 13	NS
FEV_1/FVC (%)	72 ± 9	77 ± 10	NS
FEF_{25-75} (% PV)	62 ± 24	67 ± 18	NS
PEF (% PV)	92 ± 22	101 ± 17	NS

Table V. The effect of combined therapy with fenoterol and ipratropium bromide on serum ECP level in asthma patients

	Serum ECP level – mcg/ml	
	before	after
1	16.80	15.36
2	21.00	27.50
3	8.63	11.20
4	35.06	48.49
5	11.31	15.22
6	9.33	12.60
7	37.14	49.40
8	22.49	23.90
9	12.42	17.76
10	23.95	24.16
11	6.62	8.85
12	7.68	10.17
13	13.92	17.44
14	12.70	16.69
15	9.48	16.24
16	16.69	23.56
X ± SD	16.6 ± 9.3	21.2 ± 12.0
Z	3.34	
p	0.00085	

There are several reports on the profitable effect of single doses of β_2-agonists on EAR after allergen provocation [11, 35]. Our results have indicated that a long-term application of β_2-agonists, even in low doses, can enhance bronchial reactivity to a specific allergen. The LAR occurred in more patients after the therapy combined therapy with fenoterol and ipratropium bromide. Usually it was connected with an inhalation of lower doses of the allergen during the second specific bronchial provocation after the therapy. This is in contrast to the reports of some authors suggesting a connection between a higher dose of an inhaled allergen after bronchodilatation and the more frequent appearance of the LAR [14].

An increase in bronchial hyperreactivity lasting 1–3 days after the β_2-agonist cessation has been shown and some authors have tried to explain the enhancement of

bronchial hyperreactivity after long-term β_2-agonist application on the basis of this observation [37].

Trigg et al. have suggested a pro-inflammatory effect of salbutamol, with increased numbers of activated eosinophils in the bronchial mucosa after regular treatment which is in an accordance with our results [34].

Some authors have reported an anti-inflammatory effect of long acting β_2-agonists [9, 39, 40]. Formoterol could reduce serum ECP level, but did not diminish the number of eosinophils in the blood or sputum [40]. It is possible that a long-acting β_2-agonist could have anti-inflammatory properties.

Ipratropium bromide did not prevent the increase in BHR caused by β_2-agonists.

Drugs used in asthma therapy do not always have the desired effects on bronchial reactivity. Long-term, regular treatment even with low doses of short-acting β_2-agonists can increase the bronchial response to specific and non-specific stimuli.

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STRESZCZENIE

Leki β_2-agonistyczne bez wątpienia należą do najbardziej skutecznych środków bronchodilatacyjnych stosowanych w terapii astmy oskrzelowej, jednakże przewlekłe, regularne ich przyjmowanie może dawać objawy niepożądane, do których między innymi można zaliczyć zwiększenie reaktywności oskrzelowej. Jednoczesne stosowanie wziewnych glikokortykosteroidów zapobiega temu zjawisku. Celem naszej pracy była ocena wpływu regularnej, sześciotygodniowej kuracji Berodualem (lekiem zawierającym w jednej dawce 0,05 mg fenoterolu i 0,02 mg ipratropium bromide) na parametry spirometryczne, nieswoistą i swoistą nadreaktywność oskrzeli oraz stężenie ECP w surowicy.

Badania przeprowadzono w grupie 16 chorych cierpiących na łagodną postać astmy atopowej, uczulonych na roztocza kurzu domowego. Wszyscy pacjenci uczestniczący w badaniu przez okres 6 tygodni przyjmowali jedynie Berodual 3 razy dziennie po dwie dawki z inhalatora dozującego (MDI). Dodatkowe dawki tego leku mogli przyjmować w razie potrzeby. Przed rozpoczęciem kuracji oraz po jej zakończeniu wykonywano spoczynkowe badanie spirometryczne, badano nieswoistą reaktywność oskrzeli w stosunku do histaminy i metacholiny oraz odpowiedź oskrzeli na prowokację alergenem Dermatophagoides pteronyssinus (Dpt), a także pobierano krew celem oznaczenia stężenia ECP w surowicy. Badania spirometryczne wykonano za pomocą aparatu Pneumoximeter. Prowokacyjne testy oskrzelowe przeprowadzono wg metody Ryana używając dyszowych nebulizatorów DeVilbiss 646 połączonych z dozymetrem Rosenthala Frencha.

Po 6 tygodniowej kuracji Berodualem zaobserwowano nieznamienny statystycznie wzrost wartości podstawowych parametrów spirometrycznych. Za pomocą testu prowokacyjnego z histaminą wykazano znamienny statystycznie wzrost nadreaktywności oskrzelowej manifestujący się obniżeniem dawki progowej powodującej 20% obniżenie FEV_1 w stosunku do wartości wyjściowej. Jednocześnie badanie reaktywności wykonane za pomocą testu metacholinowego wykazało wzrost wartości $PC_{20}FEV_1$. Ponieważ ipratropium bromide (jeden ze składników Berodualu) i metacholina są związkami wykazującymi kompetycyjny antagonizm w stosunku do receptorów muskarynowch, metacholina nie powinna być używana do oceny nieswoistej nadreaktywności oskrzeli. Zaobserwowano również statystycznie znamienny wzrost reaktywności swoistej oskrzeli manifestujący się obniżeniem dawki prowokującej alergenu Dpt ($PD_{20}FEV_1$) niezbędnej do wywołania wczesnej reakcji skurczowej (EAR), a także częstszym występowaniem późnej reakcji astmatycznej (LAR). U osób wykazujących LAR już przy pierwszym badaniu, po kuracji reakcja ta zaczynała się wcześniej i miała większe nasilenie. Stężenie ECP w surowicy było po leczeniu znamiennie wyższe niż wyjściowe. Bromek ipratropium nie zapobiegał wzrostowi nadreaktywności oskrzelowej w trakcie regularnej kuracji lekiem β_2-agonistycznym o krótkim działaniu.