UDC 616.248 – 053.2/5 + 577.17: 616 – 08

A CONCOMITANT ANTIMICROBIAL ACTIVITY OF METHYLATED AND HALOGENATED GLUCOCORTICOSTEROIDS AGAINST MICROORGANISMS ISOLATED FROM THE SPUTUM OF CHILDREN WITH BRONCHIAL ASTHMA

Chernuskiy V. G., Govalenkova O. L., Letyago G. V.
V. N. Karazin Kharkiv National University, Kharkiv, Ukraine

A concomitant antimicrobial activity of glucocorticosteroids (GCS) - prednisolone, methylprednisolone, dexamethasone, polcortolone, beclomethasone dipropionate, fluticasone propionate - for microorganisms isolated from the sputum of 135 children with bronchial asthma (BA) aged 11 ± 0,12 years was studied. In cultures was taken into account the number of isolated strains in the titer of bacteria (S. aureus, S. pyogenes, E.coli, Pr. Mirabilis, Ps. Aeruginosa, C. albicans) 10^3 U/ml and above, as well as yeast micromycetes (C. albicans). The antimicrobial activity of GCS was studied by double serial dilutions method with determining of minimum inhibitory concentration (MIC, mkg/ml) with the addition of these drugs in middle and low doses. Results were measured by nephelometric method according to changes of the optical medium density on the apparatus FEC-M with wavelength of 590 nm. The Antimicrobial activity of GCS was also analyzed depending on availability in their structure the methyl group CH_3 and/or halogens - Cl, F. The greatest antimicrobial activity had fluticasone propionate, which contains in its structure two F atoms and CH_3, and the lowest activity - prednisolone. Low doses of GCS did not demonstrate bacteriostatic action and only average doses had an impact on growth of bacteria in the study. It is concluded that in children with BA should be implemented selectivity in the appointment of inhaled and oral GCS for long-term use in average doses.

KEY WORDS: bronchial asthma, antimicrobial activity, microorganisms, glucocorticosteroids

СУПУТНЯ АНТИМІКРОБНА АКТИВНІСТЬ МЕТИЛ- І ГАЛОГЕНУТРИМУЮЧИХ ГЛЮКОКОРТИКОСТЕРОЇДІВ ПО ВІДНОШЕННЮ ДО МІКРООРГАНІЗМІВ, ВИДІЛЕНИХ З МОКРОТИННЯ У ДІТЕЙ, ХВОРИХ НА БРОНХІАЛЬНУ АСТМУ

Чернуський В. Г., Говаленкова О. Л., Летяго Г. В.
Харківський національний університет імені В. Н. Каразіна, м. Харків, Україна

Вивчено супутню антимікробну активність глюкокортикостероїдів (ГКС) - преднізолон, метилпреднізолон, дексаметазон, полькортолон, беклометазону дипропіонат, флутиказону пропіонату відносно мікроорганізмів, виділених з мокротиння 135 дітей, хворих на бронхіальну астму (БА) у віці 11 ± 0,12 років. У культурах враховувалася кількість виділених штамів в титрі бактерій (S. aureus, S. pyogenes, E.coli, Pr. mirabilis, Ps. aeruginosa, C. albicans) 10^3 Од/мл і вище, а також дріжджових мікроміцетів (C. albicans). Антимікробну активність ГКС вивчали методом дворазових серійних розведень з визначенням мінімальної інгібуючої концентрації з додаванням зазначених препаратів в середніх і низьких дозах. Результати оцінювали за змінами оптичної щільності середовища нефелометрично на апараті ФЕК-М при довжині хвилі 590 нм. Антимікробна активність ГКС аналізувалася також залежно від того, чи входили в їх структуру метильна група CH_3 і/або галогени - Cl, F. Результати показали, що найбільшу антимікробну активність мав флутиказона пропіонат, який у своїй структурі містить 2 атома F і CH_3, а найменша активність спостерігається у преднізолона, що не містить галогенів чи метильної групи. Також встановлено, що низькі дози ГКС не мають бактеріостатичної дії і тільки средньотерапевтичні дози впливають на ріст досліджуваних мікроорганізмів. Таким чином, у дітей з БА слід вибірково підходити до призначення інгаляційних та пероральних ГКС для тривалого застосування в средньотерапевтичних дозах.

КЛЮЧОВІ СЛОВА: бронхіальна астма, антимікробна активність, мікроорганізми, глюкокортикостероїди

СОПУТСТВУЮЩАЯ АНТИМИКРОБНАЯ АКТИВНОСТЬ МЕТИЛ - И ГАЛОГЕНСОДЕРЖАЩИХ ГЛЮКОКОРТИКОСТЕРОИДОВ В ОТНОШЕНИИ МИКРООРГАНИЗМОВ, ВЫДЕЛЕННЫХ ИЗ МОКРОТЫ ДЕТЕЙ, БОЛЬНЫХ БРОНХИАЛЬНОЙ АСТМОЙ

Чернуский В. Г., Говаленкова О. Л., Летяго А. В.
Харьковский национальный университет имени В. Н. Каразина, г. Харьков, Украина

Изучены сопутствующая антимикробная активность глюкокортикостероидов (ГКС) - преднизолон, метилпреднизолон, дексаметазон, полькортолон, бекламетазона дипропионат, флутиказона пропионата - в отношении микроорганизмов, выделенных из мокроты 135 больных бронхиальной астмой (БА) детей в возрасте 11 ± 0,12 лет. В культурах учитывалось количество выделенных штаммов в титре бактерий (S. aureus, S. pyogenes, E.coli, Pr. mirabilis, Ps. aeruginosa, C. albicans) 10^3 Ед/мл и выше, а также дрожжевых микромицетов (C. albicans). Антимикробную активность ГКС изучали методом двукратных серийных разведений с определением минимальной ингибирующей концентрации с добавлением указанных препаратов в средних и низких дозировках. Результаты оценивали по изменению оптической плотности среды нефелометрически на аппарате ФЭК-М при длине волны 590 нм. Антимикробная активность ГКС анализировалась также в зависимости от того, входили ли в их структуру метильная группа CH_3 и/или галогены - Cl, F. Наибольшей антимикробной активностью обладал флутиказона пропионат, который в своей структуре содержит 2 атома F и CH_3, а наименьшей активностью - преднизолон. Низкие дозы ГКС не оказывали бактериостатического действия и только среднетерапевтические влияли на рост исследуемых микроорганизмов. Делается вывод, что у детей с БА следует избирательно подходить к назначению ингаляционных и пероральных ГКС для длительного применения в среднетерапевтических дозах.

КЛЮЧЕВЫЕ СЛОВА: бронхиальная астма, антимикробная активность, микроорганизмы, глюкокортикостероиды

INTRODUCTION

One of leading places in the treatment of bronchial asthma (BA) is given to inhaled glucocorticosteroids (GCS), prescription of which as universal anti-inflammatory drugs is the foundation of basic therapy [1, 2, 3]. GCS provide comprehensive pharmacological effect, which is caused by their influence on the functional activity of the genetic apparatus of cells, suppression of the synthesis and activity of cytokines that stimulate the processes of differentiation, maturation of bone marrow eosinophilic granulocytes and mast cells by blocking the formation of IgE, the suppression of late asthmatic reaction, reduction of bronchial hyperreactivity. However, some studies have shown that in patients receiving inhaled GCS during long time can occur pathogens colonization that can lead to changes in the disease course, the persistence of pathogenic organisms, forming a vicious circle that makes effective treatment in this group of patients very difficult [4, 5, 6].

OBJECTIVE

The aim of this study was to investigate the antimicrobial activity of average and low doses of GCS, the structure of which includes methyl group and halogens, on microorganisms isolated from the sputum of children with BA.

MATERIALS AND METHODS

To determine the antimicrobial activity of GCS (prednisolone, methylprednisolone, dexamethasone, polcortolone, beclomethasone dipropionate, fluticasone propionate) was conducted microbiological examination of sputum from 135 children with BA in the period of exacerbation in all forms of the disease (atopic, non-atopic, mixed). The diagnosis of BA was established according to the recommendations of GINA (2012) [7]. Patients' age was from 5 to 14 years, the average was 11 ± 0,12 years. Sputum culture tests were carried out on a nutrient medium - Mueller-Hinton agar by the standard technique [8]. In cultures was taken into account the number of isolated strains of bacteria in the titer 10^3 U/ml and above, as well as yeast micromycetes (C. albicans). The antimicrobial activity of GCS was studied by double serial dilutions method with determining of minimum inhibitory concentration (MIC, mkg/ml) with the addition of these drugs in average and low doses recommended by GINA (2012) for the treatment of BA with the assessment of their impact on the growth of microorganisms (tab. 1).

Table 1

Doses of GCS which used for determining the minimum inhibitory concentration for isolated microorganisms from the children with BA sputum in the period of exacerbation

Drug	Doses	
	Low doses	Middle doses
Prednisolone	0,1-0,2 mg/kg/day	0,5-1 mg/kg/day
Methylprednisolone	0,1 -0,2 mg/kg/day	0,5-1 mg/kg/day
Dexamethasone	0,01-0,02 mg/kg/day	0,05-0,1 mg/kg/day
Beclomethasone dipropionate	100-250 mkg/day	250-500 mkg/day
Fluticasone propionate	50-100 mkg/day	100-250 mkg/day
Polcortolone	0,02-0,05 mg/kg/day	0,1-0,2 mg/kg/day

Tube containing clean growth medium served as a control. To each tube was added 0.05 ml of saline containing 10^6 ml of the microbial cells. The tubes were incubated for 16-18 hours at the temperature of 37°C (or before the appearance of bacterial growth in the control tube). Results are taken into account by nephelometric method according to changes of the optical medium density on the apparatus FEC-M with wavelength of 590 nm [9]. Antimicrobial activity of GCS was also analyzed depending on availability in their structure methyl group CH_3 and/or halogens - Cl, F (tab. 2)

Table 2

GCS drugs depending on availability in their structure of the methyl group (CH_3) and/or halogens

GCS	The number of methyl groups (CH_3) and / or halogens (F, Cl)
Prednisolone	-
Methylprednisolone	CH_3
Dexamethasone	F
Polcortolone	F
Beclomethasone dipropionate	Cl+CH_3
Fluticasone propionate	2F+CH_3

.

Statistical analysis of MIC results for GCS was conducted with the help of applications Excel, Statgraphics-5 with the definition of the mean value (M), standard error of the mean (m). The reliability between the MIC means of drugs to selected microorganisms in comparison with prednisolone was estimated by parametric statistical methods (Student's t test).

RESULTS AND DISCUSSION

Study showed that in children with BA sputum of in all forms of the disease were determined following organisms: S. aureus, S. pyogenes, E.coli, Pr. mirabilis, Ps. aeruginosa, C. albicans (tab. 3).

Table 3

Microview of sputum from children with BA depending on the form of disease (%)

Microorganism	Total (n=135)	Form of BA		
		Atopic (n=44)	Non-atopic (n=45)	Mixed (n=46)
S. aureus	14,1	9,1	17,9	15,2
S. pyogenes	10,4	6,8	11,1	13,1
E.coli	10,4	11,4	8,9	10,9
Pr. mirabilis	9,6	13,6	8,9	6,5
Ps. aeruginosa	14,8	16,0	13,3	15,2
C. albicans	11,1	18,2	4,4	10,9

In 29.6 % of children with BA in the sputum were seeded associations of S. aureus with S. pyogenes, E.coli, Pr. mirabilis, Ps. aeruginosa.

In determining the antimicrobial activity of GCS it was found that low-dose drugs recommended by GINA for patients with BA do not exert a bacteriostatic action on microorganisms isolated from the sputum of children with BA. Only in average doses appears inhibitory effect of GCS on the growth of microorganisms. As indicated in table 4, the worst antimicrobial effect had prednisolone and the most effective was fluticasone propionate. This drug has the pronounced effect on all types of microorganisms and only one that showed antimicrobial activity against C. albicans. It should be noted that the prednisolone and methylprednisolone didn't show activity against P. aeruginosa.

To a certain extent, the antimicrobial activity of studied GCS may be associated with features of the chemical structure of the drug. Thus, prednisolone, which in its composition does not contain methyl group or halogens, showed minimal antimicrobial activity. In process of chemical structure complication due to the presence of methyl group (CH_3) and/or Cl, F was marked augmentation of GCS antimicrobial activity. Thus, in comparison with antimicrobial activity of prednisolone, methylprednisolone (contains CH3 group) was in 1.7 times more active to S.aureus and S. pyogenes ($p < 0.05$), in 1.5 times - to Pr. mirabilis ($p < 0.05$) and in 1.2 times - to E. coli ($P < 0.05$). Dexamethasone (which includes F) exceeded the activity of prednisolone to S. aureus and S. pyogenes in 2.5 times ($p < 0.05$) to Pr. mirabilis - in 3.7 times ($p < 0.05$), and to E. coli - in 1.2 times ($p < 0.05$). The same level and spectrum of antimicrobial activity was marked for halogen-containing beclomethasone dipropionate and polcortolone.

Table 4

Antimicrobial activity of glucocorticoid drugs against the microflora isolated from the sputum of children with BA in the period of exacerbation in serially diluted minimal inhibitory concentration (MIC), (M ± m) mcg/ml

GCS drugs in average doses	Antimicrobial activity in serial dilutions of MIC (mcg / ml)					
	S. aureus	S. pyogenes	E. coli	Pr.mirabilis	Ps.aeruginosa	C. albicans
Prednisolone	483,2 ± 11,3	491,3 ± 11,9	532,6 ± 12,4	391,2 ± 12,8	-	-
Methylprednisolone	284,6 ± 17,3*	283,2 ± 16,5*	393,5 ± 11,6*	253,9 ± 10,6*	-	-
Dexamethasone	192,5 ± 14,8*	190,6 ± 15,2*	398,2 ± 22,5*	130,7 ± 18,7*	164,3 ± 9,8	-
Polcortolone	228,7 ± 20,2*	245,8 ± 20,9*	386,3 ± 9,8*	167,8 ± 11,2*	170,4 ± 11,6	-
Beclomethasone dipropionate	234,3 ± 18,9*	242,1 ± 17,8*	371,4 ± 14,3*	142,5 ± 16,5*	172,6 ± 13,9*	-
Fluticasone propionate	109,8 ± 7,5*	110,5 ± 8 ,3*	120,5 ± 6,7*	93,4 ± 5,8*	141,2 ± 11,8*	393,6 ± 21,9

Notes: * - $p < 0,05$ - indicators of MIC drugs to isolated microorganisms in comparison with indicators for prednisone

At the same time, fluticasone propionate due to two F atoms and CH_3 exceeded activity of prednisolone to S. aureus, S. pyogenes, P. mirabilis and E. coli, respectively, in 4.4 - 5.2 times ($p < 0.05$).

Our findings are consistent with Derom E. study, which shows that for GCS series (prednisolone, beclomet, dexamethasone) inherent ability to provide bacteriostatic effect to S.aureus and E.coli in adult patients with BA and during prolonged passaging in the presence of GCS minimal dose cultural properties of these organisms was stimulated [10]. However, our results did not show the possibility of GCS low doses to enhance the growth of microorganisms. Furthermore, in conditions of prolonged use, especially irrational schemes, GCS can act as a formation factor of microorganisms' drug resistance [5, 11, 12] The analysis in system «chemical structure - biological effect» demonstrates that concomitant antimicrobial

activity in the studied GCS drugs depends on the availability in their chemical structure methyl groups and halogens. Given this, it is possible, regardless of the clinical form and severity of BA in children, to find the selective approach to the appointment of inhaled and oral GCS for long-term use in average therapeutic doses.

CONCLUSIONS

1. Glucocorticosteroids have the concomitant antimicrobial activity, the severity of which depends on the availability in their chemical structure methyl and halogens compounds.

2. Concomitant antimicrobial potential increases in difluoromethanecontaining fluticasone propionate, in halogencontaining dexamethasone, polcortolone and beclomethasone dipropionate against micro-organisms isolated from the sputum of children with BA.

PROSPECTS FOR FUTURE STUDIES

The study of antimicrobial activity of methyl- and halogencontaining GCS is a promising scientific trend at drug selecting for treatment of BA in children, predicting the development of disease remission and determining the duration of the treatment.

REFERENCES

1. Cincinnati Children's Hospital Medical Center. Evidence-based care guideline for management of acute asthma exacerbation in children // Cincinnati (OH): Cincinnati Children's Hospital Medical Center. - 2010. – №. 16. – p. 35.
2. Global Initiative for Asthma: Global strategy for asthma management and prevention [electronic course] // Global Initiative for Asthma. - 2012. Available at http://www.ginasthma.org/local/uploads/files/GINA_Report_2015_May19.pdf.
3. Drannik G. N. Klinicheskaya immunologiya i allergologiya / G. N. Drannik // Monografiya. – M., Meditsinskoye informatsionnoye agentstvo, 2003. – 603 s.
4. Dimitrijević S. Childhood asthma and its comorbidities / S. Dimitrijević, S. Živanović, L. Šaranac // Scientific Journal of the Faculty of Medicine in Niš. – 2011. - №2. – P. 83-88.
5. Temper E. S. Vliyaniye gormonov na rost i antibiotiko-chuvstvitel'nost' uslovno patogennykh mikroorganizmov / E. S. Temper, V. A. Lisenko // Zhurnal mikrobiologii, epidemiologii i immunologii. – 1988. – № 10. – S. 111-112.
6. Peshkova V. M. Prakticheskoye rukovodstvo po spektrofotometrii i kolorimetrii /V. M. Peshkova, M. I. Gromova. - M.: Izd-vo Moskovskogo universiteta, 1985. - 136 s.
7. Harju T. N. Pathogenic bacteria and viruses in induced sputum or pharyngeal secretions of adults with stable asthma / T. N. Harju // Torax. – 2011. – Vol. 15. – P. 75–79.
8. Anandan C. Is the prevalence of asthma declining? Systemic review of epidemiological studies / C. Anandan, U. Nurmatov, O. C. P. van Schayck, A. Sheikh // Allergy. – 2010. – V.65. – P. 152–167.
9. Derom E. Effects of inhaled ciclesonide and fluticasone propionate on cortisole secretion and airway responsiveness to adenosine 5'monophosphate in asthmatic patients/ E. Derom // Pulm Pharmacol Ther. - 2005. - Vol.18, №5. - P.328-336.
10. Turchína Í. P. Osoblivostí klíníchnogo perebígu bronkhíal'noï astmi u khvorikh z kolonízatsíêyu verkhníkh dikhal'nikh shlyakhív umovno-patogennoyu míkrofloroyu / Í. P. Turchína // Astma ta alergíya. – 2014. - № 3. - S. 31-37.
11. Feshchenko YU. Í. Dinamíka kolonízuyuchoï míkroflori verkhníkh dikhal'nikh shlyakhív u khvorikh na bronkhíal'nu astmu v protsesí líkuvannya / YU. Í. Feshchenko, L. M. Kurik // Astma ta alergíya. – 2011. - № 3. – S. 5-8.
12. Wood L. G. Potentially pathogenic bacteria cultured from the sputum of stable asthmatics are associated with increased 8-isoprostane and airway neutrophilia / L. G. Wood // Eur. Resp. J. – 2010. – Vol. 40. – P. 146–154.