Experimental study of the possible role of statin in bronchial hyperresponsiveness

Thesis

Submitted in partial fulfillment of the Master Degree
in Medical Pharmacology

By

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2015

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List of abbreviations

AAMs:	Alternatively activated macrophages
AHR:	Airway hyperresponsiveness
ALT:	Alanine aminotransferase
AMP:	Adenosine monophosphate
ANOVA:	Analysis of variance
ASM:	Airway smooth muscle
ATP:	Adenosine triphosphate
BAL:	Bronchoalveolar lavage
BHR:	Bronchial hyperresponsiveness
BN:	Brown Norway
cAMP:	Cyclic adenosine monophosphate
COPD:	Chronic obstructive airway disease
CRP:	C-reactive protein
CYP3A:	Cytochrome P3A
Cys LT1:	Cysteinyl leukotriene1
DNA:	Deoxyribonucleic acid
EAR:	Early allergic reaction
ECP:	Eosinophil cationic protein
ELISA:	Enzyme-linked immunosorbent assay
EPO:	Eosinophil peroxidase
EPR:	Early phase response
ERS:	European Respiratory Society

FEV_1	Forced expiratory volume 1
FPP:	Farnesyl pyrophosphate
GDP:	Guanosine diphosphate
GGPP:	Geranylgeranylpyrophosphate
GINA:	Global Initiative on Asthma
GTP:	Guanosine triphosphate
GTPases:	Guanosine triphosphatases
HDL-C:	High density lipoprotein cholesterol
HDM:	House dust mite
H&E	Hematoxylin and eosin
HIV:	Human immunodeficiency virus
HMG-CoA:	Hydroxy-methylglutaryl-coenzyme A
HMGR:	Hydroxy-methylglutaryl-coenzyme A reductase
ICS:	Inhaled corticosteroids
Ig:	Immunoglobulin
IL:	Interleukin
IP_3:	Inositol triphosphate
KHS:	Krebs-Henseleit solution
LABA:	Long acting beta agonist
LAR:	Late allergic reaction
LDL:	Low density lipoprotein
LPR:	Late phase response
MBP:	Major basic protein
MLCK:	Myosin light chain kinase

NFκB:	Nuclear factor kappa B
NSAIDs:	Nonsteroidal anti-inflammatory drugs
OA:	Ovalbumin
OATP:	Organic anion transporting polypeptide
PAF:	Platelet activating factor
PKC:	Protein kinase C
PPARs:	Peroxisome proliferative activated receptors
RSV:	Respiratory syncytial virus
Th2:	T helper 2
TMB:	Tetramethylbenzidine
TNF-α:	Tumour necrosis factor alpha
T reg:	T regulatory

Acknowledgement

FIRST OF ALL, THANKS TO ALLAH

I would like to express my deep gratitude, appreciation and sincere thanks to ***Professor Dr. Fatma Ahmed El Batrawy, Professor of Medical Pharmacology, Faculty of Medicine, Cairo University,*** for her support, meticulous supervision, great valuable remarks, encouragement and assistance until this work was fulfilled.

Deep thanks to ***Professor Dr. Amani Nabil Shafik, Professor of Medical Pharmacology, Faculty of Medicine, Cairo University,*** for her kind help in every step in this work, guidance and for her valuable assistance in completing this work.

I am very grateful to ***Professor Dr. Bahaa Ihab Mounir, Professor of Pathology, Faculty of Medicine, Cairo University***, for his kind assistance in analysis of the pathological findings.

I would like to express my deep thanks to ***Dr. Mohamed Mostafa, Lecturer of Public Health, Faculty of Medicine, Cairo University***, for his time and effort in statistical analysis.

Finally, I would like to express my special deep thanks and gratitude to all members of ***Pharmacology Department, Faculty of Medicine, Cairo University*** for their great help in completing this work.

Abstract

Background: Statins exert favorable effects on lipoprotein metabolism but may also possess anti-inflammatory effects. Several reports have described the ability of statins to suppress acute and chronic inflammation.

Aim: The aim of the present work was to study the possible role of statins in improving bronchial hyperresponsiveness by possible anti-inflammatory, immune-modulatory or smooth muscle relaxant effects. This was conducted by comparison with dexamethasone and bambuterol in ovalbumin sensitized guinea pigs model of bronchial hyperresponsiveness.

Method: Bronchial hyperresponsiveness model was induced in guinea pigs by intraperitoneal injection of 10 mg/kg ovalbumin every other day for a total of three injections (on days 1, 3, 5). After 2 weeks of sensitization, challenge was done by intravenously injecting 0.5 ml of 2% OA per guinea pig. After 3 hours of the challenge the rates of respiration and mortality were measured. Blood samples were collected for measurement of level of Ig E, level of TNF-α and eosinophilic percentage. Animals were sacrificed and trachea was isolated to study the effect of histamine on isolated tracheal ring using power lab. Lung tissue samples were taken for histological assessment of inflammatory cells and degree of fibrosis. **Results:** The 3 tested drugs (simvastatin, dexamethasone and bambuterol) showed significant reduction in serum level of Ig E, TNF alpha, eosinophilic percentage, respiratory rate and response of isolated trachea to histamine. Simvastatin and dexamethasone improved lung pathology.

Conclusion: From the results of the present work, it could be concluded that the tested drug simvastatin, had anti-inflammatory, immune-modulatory and smooth muscle relaxant effects and could improve bronchial hyperresponsiveness.

Key words: simvastatin- anti-inflammatory- bronchial hyperresponsiveness - guinea pigs

Bronchial asthma is a complex syndrome characterized by reversible airway obstruction resulting from allergen exposure and other triggers releasing multiple bronchoconstricting mediators that stimulate airway muscle to contract, thereby further narrowing of airways that are already partially occluded by mucous and edema. Bronchial hyperresponsiveness is the hallmark of bronchial asthma with the symptoms of dyspnea, coughing, exaggerated airway narrowing and wheezing ***(William et al., 2013).***

Bronchial hyperresponsiveness (BHR) is a state characterized by a heightened airway smooth muscle bronchoconstrictor response, measured by bronchoprovocation testing, and airway inflammation with mucous secretion ***(Rubin et al., 2014).***

Bronchial hyperresponsiveness represents the key feature in asthma with variable airflow limitation and airway inflammation. To what extent the mechanisms of airflow limitation, airway inflammation, and BHR overlap are still unclear, and how these 3 components come together probably accounts for the wide variability of asthma as a disease ***(Rohit and Ronina, 2012).***

Commonly used anti-asthma drugs (corticosteroids, B_2 agonists and anticholinergic agents) are applied in asthma management as they have been shown to interfere with processes leading to structural changes of the asthmatic airway wall. However, their ability to prevent or reverse airway remodelling during the natural history of this disease is still controversial ***(Mauad et al., 2007).***

Statins inhibit 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, the proximal rate-limiting enzyme in cholesterol biosynthesis. They effectively lower serum cholesterol but overall benefits exceed that predicted by this outcome alone, suggesting additional cholesterol-independent effects ***(Liao and Laufs, 2005).***

Statins have been proposed as potential new treatment for lung disease ***(Camoretti, 2009)***. Reductions in chronic obstructive pulmonary disease morbidity and mortality, and improvement in lung function, have been attributed to statin use ***(Soyseth et al., 2007).***

According to the information in literature concerning the pleotropic effects of statins, this work was conducted to study the possible role of statins in improving bronchial hyperresponsiveness by possible anti-inflammatory, immune-modulatory or smooth muscle relaxant effects. This was conducted by comparison with dexamethasone as anti-inflammatory drug and bambuterol a B_2 agonist causing smooth muscle relaxation in ovalbumin sensitized guinea pigs model of bronchial hyperresponsiveness.

Bronchial hyperresponsiveness

Bronchial hyperresponsiveness (BHR) is a state characterized by easily triggered bronchospasm (contraction of the bronchioles or small airways). It is a valid measure of the functional airway disturbance typically seen in asthma ***(Yick et al., 2012)***. Bronchial hyperresponsiveness testing has been commonly used in epidemiological asthma studies and in research on respiratory pathophysiology ***(Bossé et al., 2011).***

The Global Initiative in Asthma (GINA) Guidelines suggest the use of BHR testing following a normal spirometry for further examinations of undiagnosed respiratory symptoms. The European Respiratory Society (ERS) Task Force recommends the use of BHR testing also in titration of anti-inflammatory therapy. The use of BHR testing in monitoring asthma treatment has proven to lead to better asthma control, fewer exacerbations, and reduced airway inflammation**,** thus it is included as an objective lung function measure to follow up asthma in many countries ***(Lundbäck et al., 2008)***.

A direct provocative agent, such as histamine or methacholine, acts on airway smooth muscle (ASM) cells, leading to its constriction and narrowing of the caliber typical for airway obstruction. Traditionally, this ASM contraction is believed to be due to the activation of muscarinic receptors after the release of acetylcholine from the cholinergic nerve plates at the neuromuscular synapsis of the parasympathetic axons of the vagal nerves. This approach is in contrast to the indirect methods, which trigger the induced ASM contraction and airway flow limitation by causing an excess release of inflammatory mediators, such as histamine, leukotrienes and prostaglandins, which cause a cascade that determine ASM constriction ***(Anderson, 2010).***

Bronchial hyperresponsiveness has been traditionally divided into two components, the transient and the persistent, which provides the explanation as to why some asthmatics do not show BHR regardless of the findings of other lung function tests, and some asthmatics have BHR regardless of being treated well and being asymptomatic ***(Busse, 2010).***The transient component of BHR is associated with rapid changes and reactions of the bronchus, such as exposure to allergen or occupational sensitizers. It is related to current asthma activity, and is typically the only component present in both the early stages and all through the disease. The persistent component is believed to show features of airway remodeling and is related to both functional and structural changes in the airway due to chronic duration of the disease ***(Cockcroft, 2010).***

Pathophysiology of bronchial hyperresponsiveness

Airway inflammation in asthma is complex and originates from a multi causal pathway in three different processes: acute inflammation, chronic inflammation and airway remodeling. Thus, several pathophysiological determinants are involved ***(Diamant et al., 2010).***

1- Role of respiratory structures and cells in bronchial hyperresponsiveness

Increased ASM mass implicates in the pathogenesis of BHR and remodeling in patients with asthma ***(Borger et al.,2006).***The mechanical changes in the bronchial tree which are typical for asthma's chronic inflammatory process, cause excess ASM contraction and BHR ***(An et al., 2007).***

Increased ASM in asthma, resulting from cell hyperplasia and hypertrophy, has been well recognized. A number of factors affect ASM proliferation, such as growth factors, contractile agonists, extracellular matrix proteins and other mediators, such as lysosomal hydrolase, tryptase, and cytokines ***(Borger et al.,***