
**A STUDY OF THE POTENTIAL ROLE OF BRADYKININ
ANTAGONISTS AND CANNABINOID AGONISTS IN ATTENUATING
EXPERIMENTALLY INDUCED ALLERGIC AIRWAY
INFLAMMATION IN GUINEA PIGS**

Thesis

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Abstract

Background and aim of work: Bronchial asthma has become a major public health in the world. It affects more than 334 million people worldwide. The present study was designed to evaluate the potential role of bradykinin antagonists (R-715; B1 receptor antagonist and Icatibant; B2 receptor antagonist) and cannabinoid agonists [Arachidonyl-2'-chloroethylamide (ACEA); CB1 receptor agonist and JWH-133; CB2 receptor agonist] in treatment of allergic airway inflammation in comparison to dexamethasone and montelukast.

Experimental approach: Sixty male guinea pigs were allocated into five groups; Group (1): Non-sensitized non- treated, Group (2): Include OVA-sensitized non-treated guinea pigs that were subdivided into the following subgroups: Group (2a): OVA-sensitized saline-challenged non-treated, Group (2b): OVA-sensitized OVA-challenged non-treated. Group (3): Sensitized OVA-challenged vehicle -treated group. Group (4): which was further divided into the following subgroups: Group (4a): OVA-sensitized OVA-challenged R-715-treated group. Group (4b): OVA-sensitized OVA-challenged icatibant-treated: Group (4c): OVA-sensitized OVA-challenged ACEA-treated. Group (4d): OVA-sensitized OVA-challenged JWH-133 treated. Group (5) was further divided into: Group (5a): OVA-sensitized OVA-challenged dexamethasone treated, Group (5b): OVA-sensitized OVA-challenged montelukast treated. Animals were subjected to (a) measurement of airway hyperresponsiveness; (b) cytological (Total & Eosinophil cell counts) & biochemical (albumin, IL-4 & IL-1 β) analysis of bronchoalveolar lavage fluid; (c) measurement of serum OVA-specific IgE level; (d) histopathological (HE & PAS) and immune-histochemical (COX-2 & iNOS) analyses.

Results: The selective bradykinin-1 antagonist R-715 not icatibant (selective B2 antagonist) significantly inhibited airway hyperresponsiveness, significantly decreased peribronchial leukocyte infiltration, goblet cell hyperplasia, COX-2 & iNOS expression, BAL fluid cell count (total and eosinophils), BAL fluid albumin and cytokines (IL-1 β , IL-4) as well as serum OVA-specific IgE level. Attenuation of all parameters was also observed with administration of the selective CB1 agonist ACEA and the selective CB2 agonist JWH-133. The amelioration of airway inflammatory response and the reduction of AHR induced by the tested drugs were comparable with dexamethasone and montelukast.

Conclusion: the current findings revealed that selective bradykinin-1 antagonist may have the therapeutic potential for the treatment of allergic airway inflammation. Cannabinoids agonists also seem to be a promising strategy for a therapeutic approach.

Keywords: asthma, bradykinin, cannabinoid, R-715, Icatibant, ACEA, JWH-133, dexamethasone, montelukast, AHR, bronchoalveolar lavage, albumin, IL-1 β , IL-4, goblet cells, COX-2, iNOS.

Contents

Acknowledgements.....	i
Abstract.....	iii
List of Abbreviations.....	v
List of Figures.....	ix
List of Tables.....	xi
Introduction and aim of the work.....	1
Review of Literature.....	3
• Phenotypes / Endotypes	3
• Etiology and risk Factors	6
• Triggers of asthma	10
• Pathogenesis	10
• Pathology	12
• Airway Hyperresponsiveness	16
• Cells and Mediators of bronchial asthma	18
• Clinical picture	34
• Pharmacotherapy of bronchial asthma	35
• Bradykinin antagonists	52
• Cannabinoid agonists	56
• Animal models of bronchial asthma	59
Materials and Methods.....	62
Results.....	73
Discussion.....	131
Summary.....	156
References.....	160

List of Abbreviations

5-LO	5-Lipoxygenase
AC	Adenylate cyclase
ACEA	Arachidonyl-2'-chloroethylamide
ACE	Angiotensin converting enzyme
Ach	Acetylcholine
AD	Adenosine receptor
AD	Anno Domini
AHR	Airway hyperresponsiveness
ANOVA	Analysis Of Variance
APC	antigen presenting cell
APP	Amyloid precursor protein
ASM	Airway smooth muscle
ATG5	Autophagy protein 5 gene
B1R	Bradykinin 1 Receptor
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage Fluid
BDH	British drug house
BK	Bradykinin
cADPR	cyclic adenosine diphosphoribose
CB1R	Cannabinoid receptor type 1
CBN	Cannabinol
CCL5	Chemokine (C-C motif) ligand 5
CD	Cluster of differentiation
COPD	Chronic obstructive pulmonary diseases
COX-2	Cyclooxygenase type 2
Cyst-LTs	cysteinyl leukotrienes
DC	Dendritic cell
des-Arg9	removal of a terminal arginine residue
Dex	Dexamethasone
DMSO	Dimethyl sulfoxide
EAR	Early asthmatic reaction
EC	Endocannabinoid
ECP	Eosinophilic cationic protein

ELISA	Enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
EPO	Eosinophil peroxidase
ERK	extracellular signal-regulated kinases
ET-1	Endothelin 1 receptor
FAAH	Fatty acid amide hydrolase
FcεRI	high-affinity receptor for the Fc region of immunoglobulin E
FDA	Food and drug administration
FEV1	volume exhaled during the first second of a forced expiratory maneuver
FoxP3	Forkhead box protein 3
FVC	Forced vital capacity
gal	gallus domesticus
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPCR	G-protein coupled receptor
GR	Group
H1	Histamine receptor type 1
HAE	Hereditary angioedema
HDM	house dust mite
Ica	Icatibant
ICAM-1	Intercellular Adhesion Molecule 1
ICS	Inhaled corticosteroid
IFN	Interferon
IL-1R	Interleukin-1 receptor
Inc	Incorporation
iNOS	inducible nitric oxide synthase
IV	Intravenous
JWH	JWH-133
KDa	Kilodalton
LABA	Long acting beta-2 agonist
LAR	Late asthmatic reaction
LTC4	Leukotriene C4
LTRA	Leukotriene receptor antagonist
mAB	monoclonal antibody
MBP	Major basic protein

MC	Mast cell
MCP-1	Monocyte chemotactic protein 1
MHC	major histocompatibility complex
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
Mn	Montelukast
MUC	Mucine
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
NK1	Neurokinin 1 receptor
NO	Nitric oxide
NOS	Nitric oxide synthase
NS	Non-sensitized
NT	Non-treated
O/O-AC	Ovalbumin-sensitized Ovalbumin-challenged ACEA-treated
O/O-DX	Ovalbumin-sensitized Ovalbumin-challenged Dexamethasone-treated
O/O-Ica	Ovalbumin-sensitized Ovalbumin-challenged Icatibant-treated
O/O-JW	Ovalbumin-sensitized Ovalbumin-challenged JWH-133-treated
O/O-Mn	Ovalbumin-sensitized Ovalbumin-challenged Montelukast-treated
O/O-NT	Ovalbumin-sensitized Ovalbumin-challenged Non-treated
O/O-R	Ovalbumin-sensitized Ovalbumin-challenged R-715-treated
O/O-V	Ovalbumin-sensitized Ovalbumin-challenged Vehicle-treated
O/S-NT	Ovalbumin-sensitized Saline-challenged Non-treated
OVA	ovalbumin
OVA/OVA	Ovalbumin-sensitized Ovalbumin-challenged
OVA-Ch NT	Ovalbumin Challenged Non-treated
PAF	Platelet activating factor
PAS	Periodic Acid Schiff
PD200	Provocative dose 200
PDE	Phosphodiesterase inhibitor
PEA	Palmitoylethanolamide
PEFR	Peak expiratory flow rate
PG	Prostaglandin
PI	Phosphatidylinositol
PIP	Peak inflation pressure
PKA	Protein kinase A

PKC	Protein kinase C
PLC	Phospholipase C
pMDI	pressurized metered dose inhaler
PPAR	peroxisome proliferator-activated receptors
PS1	Presenilin 1
RANTES	regulated on activation, normal T cell expressed and secreted
Raw	airway resistance
RhoA	Ras homolog gene family, member A
ROCK	Rho-Kinase
SABA	Short acting beta-2 agonist
SAE	Societe anonyme Egyptienne
SEM	Standard Error of the Mean
Sen	Sensitized
SERCA	sarco/endoplasmic reticulum Ca ²⁺ -ATPase
SHP-2	Src homology region 2 domain-containing phosphatase-2
SR	Sarcoplasmic reticulum
STZ	Streptozotocin
TGF	Transforming growth factor
Th1	T helper cells Type 1
Th2	T helper cells Type 2
THC	Tetrahydrocannabinol
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	regulatory T cells
TRPC	Transient receptor potential cation channel
TRPV	transient receptor potential cation channel subfamily V
VCAM-1	vascular cell adhesion molecule 1
VLA-4	Very late antigen 4

List of Figures

Figure No.	Title	Page No.
1.	Assessment of asthma control in adults.	45
2.	Recommended options for initial controller treatment in adults.	46
3.	Stepwise approach to control symptoms and minimize future risks.	47
4.	Molecular structure of R-715.	52
5.	Molecular structure of icatibant.	54
6.	Molecular structure of ACEA.	56
7.	Molecular structure of JWH-133.	57
8.	Time line of asthma induction and allergen challenge.	65
9.	Interpolating Log dose of acetylcholine at 36 mmHg.	68
10.	Recording of airway peak inflation pressure upon IV administration of increasing doses of acetylcholine (80 – 1280 µg/kg) to a nonsensitized guinea pig under anesthesia.	73
11.	Recording of airway peak inflation pressure upon IV administration of increasing doses of acetylcholine (20 – 320 µg/kg) to a sensitized OVA-challenged nontreated guinea pig under anesthesia.	74
12.	Tracing of airway peak inflation pressure upon IV administration of increasing doses of acetylcholine (40 – 640 µg/kg) to a sensitized saline-challenged guinea pig under anesthesia.	74
13.	Recording of airway peak inflation pressure upon IV administration of increasing doses of acetylcholine (20 – 320 µg/kg) to a sensitized OVA-challenged vehicle treated guinea pig under anesthesia.	75
14.	Tracing of airway peak inflation pressure upon IV administration of increasing doses of acetylcholine (80 – 1280 µg/kg) to a sensitized OVA-challenged dexamethasone treated guinea pig under anesthesia.	75
15.	Airway peak inflation pressure recording upon IV administration of increasing doses of acetylcholine (40 – 640 µg/kg) to a sensitized OVA-challenged montelukast treated guinea pig under anesthesia.	76
16.	A chart demonstrating airway PIP upon IV administration of increasing doses of acetylcholine (40 – 640 µg/kg) to a sensitized OVA-challenged R-715 treated guinea pig under anesthesia.	76
17.	Recording of airway PIP upon IV administration of successive doses of acetylcholine (20 – 320 µg/kg) to a sensitized OVA-challenged Icatibant treated guinea pig under anesthesia.	77
18.	Airway peak inflation pressure recording upon IV administration of increasing doses of acetylcholine (40 – 640 µg/kg) to a sensitized OVA-challenged ACEA treated guinea pig under anesthesia.	77
19.	Airway peak inflation pressure recording upon IV administration of increasing doses of acetylcholine (40 – 640 µg/kg) to a sensitized OVA-challenged JWH-133 treated guinea pig under anesthesia.	78
20.	Effect of increasing doses of acetylcholine 80, 160, 320 µg/kg-1 on the PIP of control and bradykinin antagonists (R-715, Icatibant) treated groups. Results are expressed as means PIP ± SEM.	86

Figure No.	Title	Page No.
21.	Effect of increasing doses of acetylcholine 80, 160, 320 $\mu\text{g}\cdot\text{kg}^{-1}$ on the PIP of control and cannabinoid agonists (ACEA, JWH-133) treated groups. Results are expressed as means $\text{PIP} \pm \text{SEM}$.	87
22.	The mean (\pm SEM) PD200 values of nonsensitized, sensitized treated and nontreated guinea pigs.	90
23.	Hematoxylin & Eosin staining of lung sections taken from non-sensitized (A, B) & sensitized OVA-challenged guinea pigs (C, D):	94
24.	Representative micrographs of lung tissue stained with hematoxylin & eosin obtained from nonsensitized (A), sensitized treated and sensitized nontreated groups (B,C,D,E,F) (x200).	95
25.	Representative lung sections showing periodic acid-Schiff (PAS) staining obtained from nonsensitized (A & B) and sensitized OVA-challenged nontreated guinea pigs (C & D).	100
26.	Representative light micrographs of lung tissue from sensitized OVA-challenged non-treated guinea pigs showing iNOS immunostaining.	103
27.	Illustrative sections of pulmonary tissue (x200 magnification) showing immune-staining of iNOS from nonsensitized nontreated (A) and sensitized treated and nontreated groups (B, C & D).	106
28.	COX-2 immunostaining of lung tissue obtained from sensitized OVA-challenged nontreated guinea pigs (A & B).	107
29.	Representative sections of lung tissue (x200 magnification) showing immunostaining for COX-2 from nonsensitized nontreated (A) and sensitized treated and nontreated groups (B, C & D).	108
30.	Bronchoalveolar lavage fluid obtained from a normal guinea pig stained with Leishman's stain, x400.	111
31.	Bronchoalveolar lavage fluid obtained from a sensitized OVA- challenged non-treated guinea pig (A & B) stained with Leishman's stain, 400x.	112
32.	Effect of tested drugs on the means of total leukocytes in BAL fluid following OVA sensitization and challenge.	113
33.	Effect of bradykinin antagonists (R-715 & Icatibant) and cannabinoid agonists (ACEA & JWH-133) on eosinophil percentage in BAL fluid following OVA sensitization and challenge.	117
34.	The mean (\pm SEM) albumin concentration in BALF of nonsensitized, sensitized treated and sensitized nontreated guinea pigs (n=6).	120
35.	The mean (\pm SEM) IL-4 concentration in BALF of nonsensitized, sensitized treated and sensitized nontreated guinea pigs (n=6).	123
36.	The mean (\pm SEM) IL-1 β concentration in BALF of nonsensitized, sensitized treated and sensitized nontreated guinea pigs (n=6).	126
37.	The mean (\pm SEM) serum level of Ovalbumin-specific immunoglobulin E (IgE) levels of nonsensitized, sensitized treated and nontreated guinea pigs.	130

List of Tables

Table No.	Title	Page No.
1.	Airway resistance expressed as mean PIP values \pm SEM, in response to increasing doses of IV acetylcholine to control and treatment groups.	85
2.	Histopathological score of lung sections obtained from nonsensitized, sensitized treated and sensitized nontreated groups (H&E).	96
3.	The quantification of (PAS) staining of lung sections obtained from animals of different groups.	99
4.	Effect of tested drugs on iNOS cytoplasmic expression score in lung sections obtained from animals of all groups.	103
5.	Effect of tested drugs on COX-2 cytoplasmic expression score in lung sections obtained from animals of all groups.	104
6.	Effect of tested drugs on Eosinophil count in bronchoalveolar lavaged fluid	116
7.	The levels of Serum OVA-Specific IgE (ng-mL ⁻¹) in nonsensitized, sensitized treated and sensitized nontreated groups.	129

Introduction

Bronchial asthma has become a major public health in the world. It represents a unique form of chronic airway inflammation characterized by reversible airway obstruction and airway hyperreactivity (AHR) (**Krieger *et al.*, 2000**).

The cellular response in allergic airway inflammation is controlled by an abroad range of bioactive mediators, including amines, lipid derived mediators, peptides, immunoglobulin E (IgE), cytokines, and chemokines. In asthma, Th2 plays a central role and controls the allergic response through the production of cytokines such as interleukin (IL)-4, IL-5, and IL-13 (**Wills-Karp, 1999**).

One type of peptides is kinins, represented principally by bradykinin, and des-Arg 9 –bradykinin. The leakage of plasma kininogens into the airways and the release of tissue kallikrein from seromucous glands may be the mechanism for kinins generation, since kininogens and kallikrein have been found in the airways of allergic patients affected by asthma (**Christiansen *et al.*, 1992**).

The actions of kinins are mediated by activation of two main bradykinin receptor subtypes, B1 and B2, both of which are members of the seven transmembrane G protein-coupled receptor family (**Leeb-Lundberg *et al.*, 2005**).

The bradykinin B2 receptor is constitutively expressed, while the B1 receptor normally absent in tissues – is highly induced under many inflammatory conditions including experimental endotoxemia, rheumatoid arthritis, hyperalgesia, diabetes and in a model of Sephadex beads-induced lung inflammation in guinea-pigs (**Couture *et al.*, 2001**).

The class of cannabinoids is known to act as immunomodulators, and their potential use as therapeutic has been widely discussed. Cannabinoids have been tested in several experimental models of autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, colitis and hepatitis and have been shown to

protect the host from the pathogenesis through induction of multiple anti-inflammatory pathways (Nagarkatti *et al.*, 2009).

Cannabinoids as well as endocannabinoids bind specifically to G protein-coupled receptors, CB1 and CB2 receptors. CB1 is thought to be mainly expressed in central and peripheral nerve terminals and on a wide range of tissues such as adipose tissue, liver, muscle, gastrointestinal tract, and pancreas (Bouaboula *et al.*, 1993).

CB2 receptor is considered to be restricted to immune-related organs or tissues such as the tonsils, spleen, thymus, and bone marrow with particular high expression levels on B cells and natural killer cells (Howlett, 2002).

Aim of the work:

The present study is designed to evaluate the potential role of bradykinin antagonists (R-715; B₁ receptor antagonist and Icatibant; B₂ receptor antagonist) and cannabinoid agonists [Arachidonyl-2'-chloroethylamide (ACEA); CB₁ receptor agonist and JWH-133; CB₂ receptor agonist] in treatment of allergic airway inflammation.

Male guinea pigs are sensitized and challenged by ovalbumin. Dexamethasone and montelukast are used as standard drugs for comparison. Animals will be tested for airway hyperresponsiveness, measurement of serum OVA-specific IgE, analysis of bronchoalveolar lavage fluid for total and differential leucocytic counts; cytokines (IL-1 β & IL-4) and albumin. After end of experiments lung tissues excised from animals will be examined by light microscopy after staining with haematoxylin and eosin (H&E) and Periodic acid Schiff (PAS). Pathological specimens will be further examined by immuno-histochemical methods to detect inducible nitric oxide synthase and COX2.

Review of Literature