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-1β) 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-1β, 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-1β, IL-4, goblet cells, COX-2, iNOS.

Contents

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 opstructive pulmonary diseases
COX-2	Cyclooygenase 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	Eosinphilic 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	Heriditary 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-κB	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 Ca2+-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

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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; B_1 receptor antagonist and Icatibant; B_2 receptor antagonist) and cannabinoid agonists [Arachidonyl-2'-chloroethylamide (ACEA); CB_1 receptor agonist and JWH-133; CB_2 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-1β & 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