



www.cost.esf.org

Action BM0806

2009 - 2013

Recent Advances in Histamine Receptor H₄R Research

BMBS



Participating countries: AT, CH, DE, DK, ES, FI, FR, GR, HU, IE, IL, IT, NL, PL, SE, SI, UK

Chair of the Action: Ekaterini Tiligada, GR, aityliga@med.uoa.gr

COST Science Officer: Kalliopi Kostelidou, kkostelidou@cost.esf.org

www.histamineresearch.com**CHAIR**

Dr Ekaterini Tiligada
University of Athens
Medical School
aityliga@med.uoa.gr

VICE CHAIR

Dr Paul L Chazot
University of Durham
paul.chazot@durham.ac.uk

SECRETARY

Prof Madeleine Ennis
Queen's University of Belfast
m.ennis@qub.ac.uk

WG1 Leaders

Prof András Falus
Semmelweis University
faland@dgci.sote.hu
Dr Paul L Chazot
University of Durham
paul.chazot@durham.ac.uk

WG2 Leaders

Prof Emanuela Masini
University of Florence
emanuela.masini@unifi.it
Dr Elke Schneider
CNRS UMR 8147
schneider@necker.fr

WG3 Leaders

Prof Rob Leurs
VU University Amsterdam
leurs@mac.com

Prof Gabriella Coruzzi

University of Parma
gabriella.coruzzi@unipr.it

WG4 Leaders

Prof Holger Stark
JW Goethe-Universität
h.stark@pharmchem.uni-frankfurt.de

Prof Madeleine Ennis

Queen's University of Belfast
m.ennis@qub.ac.uk

STSM Coordinators

Dr Evangelia Zampeli
University of Athens
zampevi@yahoo.gr
Dr Aurelio Moya-Garcia
University of Malaga
amoyag@uma.es

CONTACT

Dr Ekaterini Tiligada
Dept of Pharmacology Medical
School
University of Athens
M. Asias 75
11527 Athens, Greece
☎ +30 2107462575
☎ +30 2107462554
aityliga@med.uoa.gr

Dr Kalliopi Kostelidou

Science Officer
COST Office
149 Avenue Louise
1050 Brussels, Belgium
☎ +32 2 5333816
kkostelidou@cost.esf.org

Scientific Report

**BPS Winter Conference 17th December 2009 Queen Elizabeth II
Conference Centre, London**

**COST Action BM0806 Symposium: The histamine H₄ receptor:
new multi-use therapeutic target**

Introduction

The recently identified histamine H₄ receptor (H₄R) has attracted much interest because of its function and potential therapeutic exploitation. Principally expressed on haematopoietic cells, it plays a significant role in immune responses and inflammatory processes. However, more recently, it has been shown also to be expressed on endocrine cells, and to be functionally expressed in the CNS on neurons, indicating an extensive biological role. Key therapeutic arenas in which this receptor is expected to impact were discussed in this symposium, including chronic inflammatory disorders, itch, ulcers, neuropathic pain and cancers. This symposium was a follow-up to the successful EPBAR histamine symposium in 2008 (which formed the basis for a themed edition of the British Journal of Pharmacology published in March 2009, and was sponsored and promoted as part of the ESF COST Action BM0806 entitled "Recent advances in histamine H₄ receptor research (HARR4-EuCOST)" (<http://www.histamineresearch.com>). The main objective of this Action is to support a network of European experts to foster a multidisciplinary approach to H₄R research, and enhance basic understanding and the therapeutic potential of this exciting new drug target. The symposium was chaired by the Paul L Chazot (Vice Chair of the Action) and Dr Katarina Tiligada (Chair of the Action). The speakers within the symposium were either active Action members or affiliates of this Action, from Europe, North and South America. Affiliated BPS peer-reviewed oral communications are also reviewed herein.

Symposia

Professor Rob Leurs (Leiden/Amsterdam Centre for Drug Discovery (LACDR), The Netherlands) introduced the topic by discussing many of the key molecular pharmacological aspects of the receptor. Two new main themes were discussed, one based on recent work relating to mutagenesis and modelling the agonist and antagonist binding sites within the H₄R. H₄R Q7.42 is a key residue for agonist 4-methyl histamine (4-MeHis) deep in the binding pocket, while D3.32 and E5.46 higher in the binding pocket are crucial for JNJ7777120 inverse agonist/antagonist binding. Notably, it is rare to have a large polar residue in the agonist binding site for the GPCR family.



The second theme focussed on very new unpublished data which demonstrated that the H₄R interacts directly with the chemokine US28 receptor to tether both receptors within the cell, yet increase signalling via NKKappaB in a G-protein-mediated mechanism. This novel linkage offers new potential for manipulating inflammatory processes.

Dr Lars Karlsson (Johnson & Johnson Pharmaceutical Research & Development, San Diego, USA) focussed on the role of the H₄R in chronic inflammation in the lung and itch, using JNJ7777120 which is the prototype selective H₄R inverse agonist/antagonist developed by Johnson & Johnson. This compound, despite not being taken forward into the clinic mainly due to its short half-life, has proved to be an invaluable experiment tool for studying the role of the H₄R. The H₄R knockout (KO) mice, produced by the same company, have also proved to be a valuable model system. In these H₄R KO mice, significant reductions in macrophages, eosinophils, lymphocytes as well as changes in cytokine production profile (IL-4, -5, -6, -13 and -17 levels) have been observed compared to wild type mice, establishing the wide ranging role of the H₄R in immune responses. Using an ovalbumin/Alum airway inflammation mouse model, clear positive effects were seen with JNJ7777120 in both the sensitization and challenge phase, with reduction in eosinophils in both. Improved lung function was also seen. Dendritic T cells were suggested as major players in both phases; H₄R activation modulates DC cytokine production, as evidenced using H₄R KO mice and pharmacological manipulations. Furthermore, H₄R activation potentiates Th2 T cell polarization (changes in IL-4, -6 and -17). In terms of itch, histamine induces bouts of scratching which are greatly reduced in the H₄R KO mouse; furthermore, JNJ7777120 (3-100mg/kg po) also significantly reduces bouts of itch in wild-type mice. This strongly implicates the H₄R in the itch phenomena. Sedating H₁R antagonists have small positive effects, but non-sedating antagonists have no significant effect in this model. Interestingly, combining a sedating H₁R antagonist with JNJ7777120, completely ablates the histamine-induced scratching bouts. This was not achievable using non-sedating H₁R antagonists. A similar picture was seen with IgE-induced itch. Importantly, haematopoietic cells do not seem to be involved, as only centrally active H₄R antagonists work; non-CNS permeating compounds have no effect on itch, strongly supporting the idea that these effects are via neuronal H₄R, consistent with recent work from the Abbott group (USA) and Paul Chazot (UK).

Professor Ralf Gutzmer (Hannover Medical School, Germany) discussed the role of the H₄R in atopic dermatitis (AD) and psoriasis. H₄R are expressed antigen-presenting cells (APC) cells (dendritic cells) and are upregulated in both AD and psoriasis versus controls. Human *ex vivo* Langerhans cells (LC) express the H₄R, and upon activation inhibit CLC-I2, increase TNFalpha and IL-12 levels and promotes migration of LCs into deeper areas. These effects were blocked by H₄R antagonists. New data was presented demonstrating the expression of the H₄ receptor on keratinocytes, with higher levels in deeper layers versus outer layers of the skin. Keratinocyte H₄R expression was higher in AD, but not psoriasis or lupus patients. H₄R activation elicited increased keratinocyte proliferation and skin thickening; whether this involves effects upon differentiation and/or apoptosis is as yet unknown.

Sjögren's syndrome (SS) is an autoimmune disease with unknown etiology and unclear pathogenesis. Salivary and lacrimal glands are affected and there is a strong female dominance in this autoimmune disease. It is hypothesised that SS is associated with low androgen levels. Dr Paul Chazot (Durham University, UK) in collaboration with the

group of Professor Yrjo Konttinen (Helsinki University, Finland) reported for the first time the presence of H₄Rs in murine salivary gland samples, with specific staining identified in the both intercalated ducts and in the acini. No expression was seen in the HDC KO mice tissue, where testosterone levels are known to be depleted. Furthermore, H₄Rs were expressed in both Human Submandibular Glandular (HSG) intercalated duct cells and HSGm acinar cells, in a testosterone-dependent manner. This implicates the dysregulation of H₄Rs in the pathology of Sjögren's syndrome.

Professor Elena Rivera (Universidad de Buenos Aires, Argentina) reported exciting new data to support the role of the H₄R in tumourgenesis. Histamine via the H₄R appears to increase apoptosis, while clobenpropit (H₄R partial agonist) and VUF8430 (H₄R agonist) decrease proliferation and increase senescence in a range of tumour cell lines. JNJ7777120 reduces lung metastases (by 80%) in MDA-MB-231 xenograph tumour-bearing mice. JNJ7777120 reduced H₄R in histidine decarboxylase (HDC)-positive cells. Both the H₃ and H₄ receptors are expressed in benign and malignant carcinomas. Higher level of expression of the H₄R was detected in malignant versus benign breast carcinomas. Whether an agonist or an antagonist is the ideal strategy is yet to be determined; both may lead to reduced levels of H₄R activities, via a different time-course.

Professor Gabriella Coruzzi (University of Parma, Italy) discussed recent data relating to the role of the H₄R in gastroprotection. We have recently reported the presence of H₄Rs in the rat gastric mucosa, specifically located on ghrelin-producing cells, in contrast to the H₃R which are expressed on the HDC-positive enterochromaffin-like (ECL) cells. Two ulcer models of differential severity were utilized to determine the role of the H₄R in the stomach. JNJ7777120 (at anti-inflammatory doses of 10-30mg/kg sc) significantly reduced the mild gastric mucosal damage induced by indomethacin (20mg/kg sc.) or indomethacin (30mg/kg sc) plus bethanechol (5mg/kg ip) in both the rat and mouse, respectively. Interestingly, the putative H₄R agonist, VUF10460 (3,10,30 mg/kg) elicited similar gastroprotection to JNJ7777120 in the rat, but not the mouse model. Neither antagonist nor agonist ligands were effective in severe gastric lesion 0.6N HCl model. The species pharmacology of the ligands described in this and the previous study, and the levels of local histamine, may also be of relevance to these apparently paradoxical results. The species differences require further investigation, but the results suggest the potential for H₄R antagonists as gastroprotective anti-inflammatory agents.

Affiliated Oral Communications

Dr Jadwiga Handzlik (Jagiellonian University, Cracow, Poland) describe the design and synthesis of a new series of JNJ7777120 analogues, with an N-methylpiperazine amide motif. The most promising compound was a 3,5-dichlorobenzo[b]thiophene-2-carboxylic acid derivative with a moderate affinity for the H₄R. The reduction in affinity compared to the parent compound related to the lack of –NH– in the heterocyclic element within the new compound series.

Dr Elisabeth Rosethorne (Novartis Institute for Biomedical Research, Horsham, UK) described a pharmacological complication of using JNJ J7777120. As well as acting as an antagonist for the hH₄R in terms of G-protein activation (GTPgammaS assay), JNJ J7777120 also can act as an agonist in terms of recruiting beta-arrestin, with very similar potencies, but the latter independent of G protein activation. This may have implications

in explaining differential effects of this (and other H₄R) compounds in different tissues and/or cell types.

Further to the species issue eluded to previously, Dr Nick Clark (Pfizer Global R & D, Sandwich, UK) reported the detailed species pharmacology of a novel high affinity (K_i = 9.55nM v hH₄R) selective H₄R ligand, PF-2988403. The profile differed dependent on test species with a full spectrum displayed, ranging from neutral antagonist (hH₄R), to partial and full agonist for the rH₄R. Interestingly, the *in vivo* effects of this compound in the rat reflected this agonist pharmacology, with clear pro-inflammatory effects.

Summary

Overall, the information reported in this conference provided further validation for the development of H₄R inverse agonists/antagonists as novel therapeutic agents for a range of clinical arenas. Interpretation of preclinical testing remains problematical due to significant inter-species pharmacological differences. However, a number of international pharmaceutical companies are taking their H₄R drug candidates into the clinic for a growing list of indications.

Dr Paul L Chazot (Organiser)

School of Biological & Biomedical Sciences, Durham University, UK