

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**COST Action BM0806****Recent advances in histamine receptor H4R research**

COST Action conference, MC and WG1,2 and 4 meetings held during the European Histamine Research Society 39th Annual Meeting (13 July 2010 to 16 July 2010), **Durham, UK**

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 Main 2010 Action conference was hosted by the Paul L Chazot (Vice Chair of the Action) and took place as an integral part of the 39th Annual EHRS conference held in Durham University, UK. This meeting was postponed from April due to the Ash Cloud incident which affected travel in most of Europe during that month. There were over 110 participants from all over the world, predominantly dominated by EU members. The conference comprised a COST Action WG1 methods workshop, COST Action Public Lecture (delivered by Professor Jean-Charles Schwartz, Bioprojet, Paris), five Plenary Lectures, nine symposia (40 oral presentations) and a dedicated 3 hour peer-reviewed poster session. **Notably, all the symposia and Poster sessions were co-Chaired by ESRs in addition to senior scientists, in order to offer experience in these important roles.** This new initiative was welcomed by the both the ESRs and senior scientists.

COST Action Methods Training School (WG1)

This five hour session attracted approx 40 ESRs and senior scientists from the Action and host University. It included 5 speakers covered a wide range of topics (Professor Rob Leurs - Medicinal Chemistry & receptor modelling; Professor Andras Falus – Genomics & bioinformatics; Dr Abdel Ennaceur – *In vivo* preclinical studies; Dr Paul L Chazot – Antibody production & validation; Professor Emanuela Masini – Inflammation models). Audience participation was encouraged during the session and practical issues were discussed in detail, with participation from a local UK SME company CRB, who specialise in peptide chemistry and antibody development, based in Billingham. This session highlighted the range of expertise and State-of-the-art facilities and model systems available within the Action and encouraged ESRs to approach senior colleagues for

potential STSMs after the session.

COST Action Public Lecture (Professor J-C Schwartz) delivered an impressive historical talk relating the story of the discovery of histamine in the brain and the subsequent path to the present day, where we have the first drugs targeting the brain histamine receptor. This talk was dedicated to the memory of the Nobel Laureate Professor Sir James Black (friend & colleague to many within EHRS and Action) who passed away in April this year. Jean-Charles spent the first part of his talk relating some of his memories of the greatest modern day pharmacologists. At the end of his talk, Jean-Charles reported up-to-date clinical data (expanded in detail in the GB West Plenary session on the following day), which strongly supported the efficacy and utility of H₃ receptor antagonists in a range of clinical settings, notably in narcolepsy and cataplexy for Parkinson's Disease sufferers. Pitolisant (BF2.649) is the first H₃ receptor antagonist/inverse agonist to enter latter stages of clinical development. This talk was filmed and recorded for Public online dissemination *gratis* as two versions (Expert and Public version); the COST Action will be promoted in these recordings.

New Information from Plenary, dedicated COST Oral (Sessions 1, 2, 7) and Poster sessions

The support of the COST Action was acknowledged at the Training School, the EHRS committee meetings, and at the opening session for the EHRS conference. The Plenary lecturers (Professor JC Schwartz (Bioprojet, France), Professor P Blandina (Florence, Italy), Dr N Curruthers (J&J, USA), Dr Marlon Cowart (Abbott, USA) and Dr S Liu (Pfizer, UK) delivered an outstanding array of scientific talks encompassing the basic understanding of the H₃ and H₄ receptor and their growing clinical potential. These set the high standard of the conference acknowledged by many of the delegates. The conference attracted all the major players in the international Pharmaceutical industry who have H₄R drug development programmes, ranging from start-up companies launched during the Action's first year (Griffin Discoveries, The Netherlands) to medium sized companies, Incept, Bioprojet (France), Palau Pharma (Spain) and Evotech (UK) to the large companies, including Pfizer (UK), Johnson & Johnson and Abbott. The H₄R the most recently discovered histamine receptor subtype is linked to a variety of immune and inflammatory disorders, and selective antagonists are reported to be entering the clinic. Dr Steve Liu delivered the COST Plenary Lecture relating to the therapeutic opportunities offered through targeting of the H₃ and H₄ receptors in treating airway and inflammatory diseases. He presented a large body of preclinical evidence which supports the potential for both targets for these therapeutic indications.

The Programme abstracts will be available on the Action website (www.histamineresearch.com) later in 2010.

Break out groups



A number of breakout groups occurred during the conference. A key output from these breakout groups was an agreement from many of our Pharmaceutical colleagues to support an information resource regarding key standard H₄ receptor compounds which will be made **freely** available to the Action and form the basis of a comprehensive compendium for future H₄ receptor research. This unique agreement will be added to further resources (human and animal models and standard compounds) to form the basis of a strategic approach to the Action. Furthermore, support mechanisms were **frankly** discussed to promote future clinical development of H₄ receptor drug candidates. These major outputs were discussed further during the respective WG and MC meetings.

Snapshots of highlights during the COST Action BM0806 oral and poster sessions

S Nijmeijer (ESR, The Netherlands) reported an elegant method based on protein fragment complementation which demonstrated that the hH₄ receptor forms dimers at the surface which upon histamine activation recruits beta-arrestin and internalise; antagonists have no effect. Furthermore, H₄ receptors appear to form higher oligomers and can heterodimerise with chemokine receptors CXCR4. Respective agonists internalise these heterodimers, which offer new insights into H₄ receptor regulation and histamine-chemokine cross-talk.

S Tunde (ESR, Hungary) reported that during dendritic cell (DC) differentiation H₂R and also H₄R expression show a reduction at the protein level. LPS treatment has no significant effect on HR expression, however a tendency was seen in the case of H₄R (increase). Prolonged histamine treatment during DC differentiation causes an elevated migration. The H₄R agonist, 4-MeHis (4-MH) induced a reduction in CCL-19 mRNA level and an enhanced expression of IL-1 β following LPS stimulation which was reversed by a H₄R antagonist. Histamine and 4MH reduce significantly the antigen presentation capacity of DC-s, and H₄R^{-/-} DC-s show an elevated antigen presenting capacity compared to WT DC-s. This adds further information regarding H₄R function.

Abnormal chloride transport is a characteristic feature of cystic fibrosis (CF). Histamine has been shown to activate chloride efflux in other cell types, including epithelial cells, which may prove beneficial in CF. Using HBE and CFBE cell lines and primary human nasal epithelial cells from controls (HNEC) and patients with cystic fibrosis (CFNEC), the presence of both H₃ and H₄ histamine receptors was investigated by **J Stott** (ESR, STSM report). Both H₃ and H₄ receptor proteins were detected in all cell types and this was confirmed by immunocytochemical staining. Addition of histamine to HBE and CFBE cells increased IL-8 release. The presence of H₄ receptors on CF epithelial cells may provide an important route to activating chloride transport in these cells.

Th17 cells are involved in protection against extracellular bacteria and fungi,

are potent mediators of autoimmune diseases, and participate in the pathogenesis of psoriasis and atopic dermatitis. **S Mommert** (ESR, Germany) H₄R is expressed at the mRNA and protein level on human memory Th17 polarized cells. Stimulation of Th17 cells with histamine and 4MH leads to up-regulation of IL-17A mRNA, moderate but significant induction of IL-17 protein on the single cell level and activation of transcription factor AP-1

E Rivera (MC, Argentina) reported that the prototypical H₄R antagonist/inverse agonist, JNJ7777120 markedly prevented radiation injury on Submandibular gland (SMG) mitigating the histological and morphological alterations. JNJ7777120 completely reversed the reduced salivary secretion induced by radiation while conserved SMG mass preserving the gland function, and furthermore, prevented radiation-induced toxicity in SMG by increasing proliferation and suppressing apoptosis of ductal and acinar cells. Therefore, JNJ7777120 clearly protected small intestine against ionizing radiation damage, diminishing the histological alterations and conserving intestinal crypts, which was associated with an increased proliferation. JNJ7777120 enhanced bone marrow repopulation after ionizing radiation exposure, increasing medullar components and reducing adipose replacement of irradiated bone marrow. Based on this strong evidence, a H₄R antagonist/inverse agonist has protective potential in radiation-induced damage in bone marrow, small intestine and SMG, therefore potential clinical value as a radioprotector for patients undergoing radiotherapy

Human malignant melanoma is a highly metastatic cancer that is markedly resistant to conventional therapy. **N Massari** (ESR, Argentina) reported for the first time the presence of H₄R in M1/15 cells (highly metastatic human melanoma cell line) and its associated involvement in histamine-mediated cell proliferation, senescence and differentiation. Accordingly, H₄R is present in highly invasive human melanoma lesions indicating a potential therapeutic application of H₄R ligands.

E Masini (MC, Italy) reported compelling new evidence that JNJ7777120 reduces the inflammatory response of carrageenan-induced pleurisy in the rat, *in vivo*, indicating that selective H₄R antagonists could be useful in treating acute inflammation in humans

B Savall (J&J, USA) reported the development and characterisation of a new high affinity (50 nM v human H₄R) oxime H₄R agonist, JNJ 28610244 and then reported the development of Agonist / Antagonists in a Series of 2-Aryl Benzimidazole H₄ Receptor Ligands. Changes of diamine in this series led from an agonist to an antagonist. Pyrrolpiperidine deconstruction led to heterocyclic series and a Heterocycle scan led to discovery of new selective H₄ agonist. These will be welcome additions to the panel of available selective H₄R agonists.

Crohn's disease (CD) and Ulcerative colitis (UC) are the idiopathic forms of

inflammatory bowel disease (IBD) in humans. **Ashok K Kumawat** (ESR, Sweden STSM ongoing) reported using the G protein α_2 subunit, $G\alpha_2^{-/-}$ mice (model of Crohns disease), in colon, there were a 3-fold reduction of both H_4R and H_2R expression in precolitic mice compared to wild type mice and the H_4R levels were increased with the colitis progression, as they were markedly elevated from pre colitis to late colitis. In the small intestine, preliminary data demonstrate that the H_4R levels were slightly increased in early and late colitis mice compared to wild type mice, whereas there were no difference between precolitic and wild type mice. These are being currently investigated through an STSM in Dr Chazot's laboratory.

Exciting new clinical data was reported for the first time by **Dr Jose Alfon** (Palau Pharma, Spain) on there lead compound, UR-63325 which displays efficacy in a rat asthma model where it reduced both the increase of airway hyperreactivity and the pulmonary inflammation at low doses. The potential risk of the compound in the CNS was assessed regarding general behaviour, locomotion, neuromuscular coordination, and the pro-convulsive threshold in rats. The results obtained showed no significant effect in any of these parameters, finally yielding a very wide safety margin. Therefore, UR-63325 was selected as a clinical candidate for asthma and allergic rhinitis and has entered into Phase I clinical trials. The first Phase I data (reported at this meeting) showed no adverse side effects, including CNS-associated side-effects.

Conclusion

Excellent progress has been made in understanding the functional pharmacology and therapeutic potential of the H_4 receptor since its discovery some 10 years ago, and particularly in the last year since the establishment of the COST Action BM0806. Dr Steve Liu (Pfizer, UK) summarised eloquently the state-of-play for H_4R research in his last slide: **“A big challenge remains on establishing preclinical rationale with existing tools and the significant cross species pharmacology differences”** Availability of new chemical tools and new model systems together with the pooling of scientific resources and expertise will maximise efficacy, and address what are the best therapeutic indications and optimum clinical study designs?

This is where COST Action BM0806 can impact to maximise the chances to meet this major challenge

Dr Paul L Chazot (Organiser & Vice Chair, Action BM0806)

School of Biological & Biomedical Sciences, Durham University, UK