

4th INTERNATIONAL
SYMPOSIUM ON
CATHEPSIN C

ISyCatC IV

In honor of Prof. Christine Pham

THERAPEUTIC
TARGETING OF
CATHEPSIN C

from pathophysiology
to treatment

17th > 19th April 2024 | Tours, France

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Symposium organised by



LE STUDIUM

Loire Valley
Institute for Advanced Studies

4TH INTERNATIONAL SYMPOSIUM ON CATHEPSIN C

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Introduction

Program

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Grant



With the support of



Cathepsin C also called dipeptidylpeptidase 1 [DPP1], attracts more and more attention from both scientists and clinicians because of its role in the activation of proinflammatory neutrophil serine proteases [NSPs: elastase, proteinase 3, cathepsin G and NSP-4] implicated in certain chronic inflammatory/auto-immune disorders and cancer. Promising preclinical and clinical data suggest that pharmacological inhibition of neutrophilic serine proteases might ameliorate these conditions. Patients with Papillon-Lefèvre syndrome have a genetically determined deficiency in cathepsin C but, reassuringly, do not exhibit marked immunodeficiency despite the absence of neutrophil serine proteases in immune defense cells. Hence, the pharmacological control of cathepsin C activity in bone marrow precursor cells represents an attractive therapeutic strategy for neutrophil serine protease-mediated disorders including chronic obstructive pulmonary disease COPD, bronchiectasis, cystic fibrosis, ANCA-associated vasculitis, pulmonary arterial hypertension, inflammatory bowel diseases and rheumatoid arthritis. Chronic inflammatory respiratory diseases affect over 1 billion people worldwide, and cause the death of 4 million people every year. A further increase in the number of deaths from lung diseases is predicted until 2030, in particular from COPD. A variety of cathepsin C inhibitors, developed by pharmaceutical companies and academic investigators, are currently being employed and evaluated in preclinical/clinical trials as anti-inflammatory drugs. A review of the therapeutic targeting of cathepsin C resulted from the first International Symposium on Cathepsin C **ISyCatC** [Tours/France, April 2017] and was published in the journal *Pharmacology and Therapeutics*¹. This symposium launched the **International Cathepsin C Consortium (IcatCC)** that we set up in 2016. The consortium obtained LE STUDIUM Loire Valley Institute for Advanced Studies Research Consortium Awards [2021-2022 and 2024-2025].



Positive results from a Phase 2 clinical trial with a cathepsin C inhibitor in patients with non-cystic fibrosis bronchiectasis were announced in 2020². The scientific community and patients are looking forward to the results of Phase 3 clinical trial with the same drug, which will be announced soon. Two other cathepsin C inhibitors are under evaluation in Phase 2 clinical trials for the same indication³⁻⁴. Due to overlapping phenotypes and similar underpinning molecular mechanisms for a number of diseases associated with inflammation, a potential positive effect in bronchiectasis patients could be translated directly to the potential treatment of other neutrophil serine proteases mediated inflammatory diseases. That news is very encouraging for biochemists studying the functionality of cathepsin C, cell biologists studying its maturation and tissue localization, chemists developing specific cathepsin C inhibitors and clinicians managing patients with neutrophil serine proteases-mediated disorders. Lowering the constitutively produced neutrophil serine proteases by pharmacological inhibition of cathepsin C holds great promise for the future therapies. It is gratifying to see that the hard work of all colleagues from academic labs and industry and advocacy in cathepsin C field may have a clinical payoff.

From the perspective of unmet medical needs, drug repurposing for rare diseases offers a great opportunity. Indeed, rare diseases not being economically attractive, is not a priority for the majority of the drug industry. There are more than 7.000 rare diseases, and over 95% of them lack an approved therapeutic agent. Drug repositioning would be a particularly attractive approach for rare diseases for both scientific and commercial reasons. A special issue on the topic: **“Neutrophil serine proteases and cathepsin C in rare diseases”** for Rare Disease and Orphan Drugs Journal was resulted from the 3rd International Symposium on Cathepsin C **ISyCatC III** [Tours/France, April 2022].

Brice Korkmaz
On behalf of Scientific Committee

1. B. Korkmaz, G.H. Caughay, I. Chapple, F. Gauthier, J. Hirschfeld, D.E. Jenne, R. Kettritz, G. Lalmanach, A.S. Lamart, C. Lauritzen, M. Legowska, A. Lesner, S. Marchand-Adam, S.J. McKaig, C. Moss, J. Pedersen, H. Roberts, A. Schreiber, S. Seren, N.S. Thakker. Therapeutic targeting of cathepsin C: from pathophysiology to treatment. *Pharmacol Ther* [2018] 202-236.
2. J.D. Chalmers, C.S. Haworth, M.L. Metersky, M.R. Loebinger, F. Blasi, O. Sibila, A.E. O'Donnell, E.J. Sullivan, K.C. Mange, C. Fernandez, J. Zou, C.L. Daley, W. Investigators. Phase 2 Trial of the DPP-1 inhibitor brenscatib in bronchiectasis. *N Engl J Med* [2020] 2127-2137.
3. S. Kreideweiss, G. Schänzle, G. Schnapp, V. Vintonyak, M.A. Grundl, BI 1291583: a novel selective inhibitor of cathepsin C with superior in vivo profile for the treatment of bronchiectasis. *Inflamm Res* [2023] 1709-1717.
4. Poster abstract, Hou et al., Phase 1 clinical trial results with HSK31858. *Eur Resp J.*, 2023; 62; Suppl. 67, PA2836

wednesday □ April 17th

- 16:00 Registration
- 17:45 Welcome cocktail reception
- 19:00 **OPENING AND WELCOME SPEECH**
- B. Korkmaz** [France]
- International cathepsin C consortium [Icat-CC]
- 19:05 **C. Beaumont** Université de Tours [France]
Vice-présidente en charge de la recherche
- 19:10 **M. Si-Tahar** Inserm [France]
Représentant régional
- 19:15 **S. Gabillet** Le Studium [France]
General Secretary, Loire Valley Institute for Advanced Studies
- 19:20 **B. Korkmaz** [France]
- Cathepsin C inhibition as a potential therapeutic approach in neutrophil-mediated inflammatory disease
 - **Prof. Christine Pham's biography**
Award ceremony
- 20:00 **KEYNOTE LECTURE**
- J. Chalmers** [United Kingdom]
- Clinical trials with cathepsin C inhibitors
Discussion on pathophysiology/treatments

thursday □ April 18th

- 09:00 **Session 1**
- PHYSIOLOGY AND PATHOPHYSIOLOGY OF CATHEPSIN C**
- Chair: G. Lalmanach [France]
- 09:00 **M. Novinec** [Slovenia]
- Exploring the impact of oligomeric state on cathepsin C activity
- 09:20 **A. Aghdassi** [Germany]
- Intracellular vesicle transport and involvement of cathepsin C in acute pancreatitis
- 09:40 **C. Pham** [USA]
- Cathepsin C, a historical perspective
- 10:05 **Session 2**
- PHYSIOLOGY AND PATHOPHYSIOLOGY OF CATHEPSIN C TARGETS**
- Chair: A. Ö. Yildirim [Germany]
- 10:05 **P. McDonald** [Canada]
- Cathepsin C and its substrates vs neutrophil functional responses
- 10:25 | 11:00 **Coffee break**
- 11:00 **T. Secher** [France]
- The proteolytic airway environment associated to pneumonia acts as a biological barrier for antibody treatment
- 11:20 **N. Leborgne** [Switzerland]
- Neutrophil serine proteases degrade SARS-CoV-2 spike protein and reduce virus replication and inflammation *in vivo*
- 11:40 **R. Stockley** [UK]
- Alpha-1 antitrypsin deficiency the path from antiproteases to clinical management
- Selected 5 min Talks :**
- C.H. Chen** [UK] : *A novel in vitro cellular model for assessing neutrophil proteinase biomarkers in chronic lung disease*
- E. Johnson** [UK] : *The relationship between neutrophil proteins, clinical severity and the airway microbiome in bronchiectasis*

friday April 19th

12:15 | 14:30

Lunch Buffet

14:30

R. Vanbever [Belgium]

- Assessment of neutrophil elastase protein inhibitors in muco-obstructive lung diseases

14:50

U. Specks [USA]

- PR3-ANCA interactions with its target antigen: what can they tell us about the disease

15:10

M. Zoidakis [Greece]

- Proteomics for characterization of cathepsin C target serine proteases

15:30

L. Hellman [Sweden]

- Mast cell proteases: *key players in inflammation and tissue homeostasis*

15:50 | 16:10

Coffee break

16:10

Session 3**PHARMACOLOGICAL TARGETING OF CATHEPSIN C**

Chair: M. Sięńczyk [Poland]

16:10

R. Domain [France]

- Stability and activation of neutrophil elastase-related serine protease zymogens in myelomonocytic cells

16:30

J. Cichy [Poland]

- Targeting cathepsin C in the context of neutrophil function

17:00

I. Borek [Austria]

- Inactivation of cathepsin C in pulmonary arterial hypertension

17:20

S. Korkmaz-Icöz [Germany]

- Role of neutrophil serine proteases in ischemia/reperfusion injury following heart transplantation

20:00

Gala Dinner

09:00

M. Rhimi [France]

- Inactivation of cathepsin C in inflammatory bowel diseases

09:20

K.J. Chen [USA]

- The role of a cathepsin C inhibitor in lupus nephritis

09:40

I. Couillin [France]

- Consequences of cathepsin C inactivation in cigarette smoke or ozone-induced lung inflammation in mice

10:00 | 10:20

Coffee break

10:20

M. Long [UK]

- The cathepsin C inhibitor brensocatib reduces airway azurocidin-1 levels in bronchiectasis

10:40

S. Kreideweiss & M. Schlepütz [Germany]

- Preclinical and phase I development of the novel oral cathepsin C inhibitor BI 1291583

11:00

G. Szabo [Germany]

- Design of clinical trials evaluating cathepsin C inhibition in heart transplantation

11:20

C. Taggart [UK]

- Pharmacological inhibition of cathepsin S and consequences for cathepsin C activation

11:40

D. Scherman [France]

- Foundation for Rare Diseases [FFRD]

12:00 | 14:00

Lunch Buffet



Prof. Christine Pham (M.D.)

This symposium is organized in the honor of Prof. Christine Pham who is the Guy and Ella Mae Magness Professor of Medicine, professor of Pathology and Immunology, and Chief of the Division of Rheumatology in the Department of Medicine at Washington University in Saint Louis, Missouri USA.

Dr. Pham has been part of the Washington University community for more than 30 years. After earning bachelor's and medical degrees from the University of Florida, she completed an internship and residency at Barnes (now Barnes-Jewish) Hospital, and a clinical fellowship in rheumatology at Washington University School of Medicine. Following her clinical fellowship, she joined the laboratory of Dr. Timothy Ley where she began her research career exploring the contribution of serine and cysteine proteases in immunity and autoimmunity. She joined the faculty in 1997 and is an attending physician at Barnes-Jewish Hospital and at the John Cochran Veterans Affairs Medical Center. In addition to serving as Chief of the Division of Rheumatology Dr. Pham also directs the Washington University Rheumatic Diseases Research Resource-based Center, a National Institute of Arthritis and Musculoskeletal and Skin Diseases-funded P30 program.

While in the laboratory of Dr. Ley, Dr. Pham cloned and characterized the cysteine protease cathepsin C [also known as dipeptidyl peptidase 1], an enzyme critically involved in the maturation of neutrophil serine proteases that are the targets of autoantibodies found in patients with ANCA-associated vasculitis. Through the generation of loss-of-function mutations, she further defined the contribution of cathepsin C and neutrophil serine proteases in murine models of human diseases including inflammatory arthritis, vasculitis/aortitis, viral-induced asthma phenotype, and many others.

In 1999 two separate groups of investigators, Toomes & colleagues and Hart & Hart described loss-of-function mutations in the cathepsin C gene that result in the disease known as Papillon-Lefèvre syndrome [PLS]. Dr. Pham subsequently recruited a cohort of patients with PLS and established that cathepsin C loss-of-function leads to severe reduction in the activity and stability of neutrophil serine proteases but retains granzyme activities in cytotoxic lymphocytes, providing a molecular explanation for the lack of generalized immunodeficiency in individuals with PLS.

In summary, Dr. Pham's work on cathepsin C provided proof-of-concepts for the development of inhibitors that are currently in clinical trials in patients with neutrophil-dependent lung diseases and potentially will extend to treatment of various inflammatory processes. The cathepsin C and neutrophil serine protease loss-of-function models continue to provide unique tools for the work of investigators all over the world.

The scientific community thanks Prof. Christine Pham, for her invaluable input to immunobiology of neutrophil serine proteases.

Brice Korkmaz

Clinical trials with cathepsin C inhibitors

James Chalmers

Division of Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK

Abstract

Introduction: Dipeptidyl peptidase-1/Cathepsin C inhibitors may be promising drug candidates for the treatment of inflammatory diseases associated with excessive release of neutrophil serine proteases [NSPs]. Bronchiectasis is a chronic inflammatory disease where sputum levels of NSPs are associated with increased exacerbations and worse quality of life. This presentation will discuss recent studies with Cat-C/DPPI inhibitors.

Trials to date: Three DPPI/Cat-C inhibitors are known to be in clinical development. Phase 1 data in healthy volunteers will be discussed for two compounds [Brensocatib/INS1007] and BI 1291583 which have shown reductions in NSPs in healthy volunteers. In bronchiectasis, a phase 2 trial has been completed for brensocatib [The WILLOW trial]. 256 patients were randomized to treatment with brensocatib 25mg, brensocatib 10mg or placebo with a treatment period of 6 months. The primary outcome was time to first pulmonary exacerbation. The WILLOW trial found a significant prolongation of time to first exacerbation in both treatment groups vs placebo, reductions in sputum neutrophil elastase activity, and an acceptable safety profile. A large phase 3 trial which has enrolled over 1600 participants with brensocatib is ongoing.

A phase 2 trial of BI 1291583 is also ongoing. A phase 2 study of brensocatib in cystic fibrosis patients has also been reported.

Clinical trials of DPPI/Cat-C inhibitors have also given important insights into the biology of DPPI. Reductions in activity of the associated NSPs elastase, proteinase 3 and cathepsin G were demonstrated in the phase 2 WILLOW trial. Further analysis of samples from the WILLOW trial have demonstrated downstream benefits on protease inactivation of host defence proteins such as secretory leukoprotease inhibitor and defensin-3. Proteomic analysis of neutrophils from the STOP-COVID trial in which subjects with COVID-19 were treated with 25mg brensocatib for 28 days has demonstrated potential novel effects of DPPI/Cat-C inhibition on host defence.

Conclusion: DPPI/Cat-C inhibitors may be potential drug candidates for the treatment of bronchiectasis and potentially other chronic inflammatory diseases associated with dysregulation of neutrophilic inflammation.

Exploring the impact of oligomeric state on cathepsin C activity

notes _____

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Exploring the impact of oligomeric state on cathepsin C activity

Milena Stojkovska Docevska, **Marko Novinec**

University of Ljubljana, Faculty of Chemistry and Chemical Technology, Vecna pot 113, SI-1000 Ljubljana, Slovenia

Session 1

Abstract

Cathepsin C has several structural and functional features that are unique in the family of papain-like cysteine peptidases. In contrast to its almost exclusively monomeric homologs, cathepsin C is a homotetramer organized as a dimer of dimers. This characteristic arrangement is facilitated by its unique exclusion domain, which also determines its dipeptidyl peptidase activity. Mature tetrameric cathepsin C is assembled by a two-step process that involves a dimeric zymogen form. During maturation of the zymogen, the propeptide is removed so that the exclusion domain remains non-covalently linked to the catalytic domain, which in turn is cleaved into heavy and light chains.

The exact sequence of events during the maturation of cathepsin C and the significance of the tetrameric form for its biochemical properties are not fully understood. Our objective is to solve these puzzles by studying the equilibrium between different oligomeric forms of human cathepsin C at the zymogen and mature enzyme stages in vitro using thermodynamic and kinetic

methods. These experiments are supported by site-directed mutagenesis and computational modelling aimed at modifying the interactions between pairs of subunits.

We also aim to isolate and characterize individual oligomeric forms to determine whether they have different kinetic properties, such as kinetic parameters for the hydrolysis of common substrates, or thermodynamic properties, such as thermal stability. These results will allow us to determine whether targeting the oligomerization of cathepsin C is a viable strategy to modulate its activity.

Our work on cathepsin C is part of a larger project aimed at determining whether advanced functional properties, such as cooperativity, can be engineered into oligomeric enzymes. From this point of view, we are also interested in determining whether higher oligomeric forms of cathepsin C have functional properties not found in lower oligomers, and whether such properties can be engineered into cathepsin C. These findings will then be applicable to industrial enzymes to improve their activity.



18th April 2024

Ali Aghdassi

**Intracellular vesicle transport and involvement
of cathepsin C in acute pancreatitis**

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Intracellular vesicle transport and involvement of cathepsin C in acute pancreatitis

Ali Aghdassi, Marcel Gischke, Lukas Zierke

Department of Medicine A, University Medicine Greifswald, Germany

Session 1

Abstract

Acute pancreatitis is a primarily sterile inflammatory disorder caused by a premature and intracellular activation of digestive proteases and an infiltration of inflammatory cells into the pancreas. The co-localization of the lysosomal enzyme cathepsin B [CatB] with the zymogen granule proenzyme trypsinogen is regarded as a prerequisite for disease onset but the underlying mechanisms of co-localization are still under debate.

Cathepsin C [CatC] is a lysosomal cysteine proteinase with pro-inflammatory capabilities and is expressed in several organs. The disease modulating effect has been demonstrated in various diseases, among them acute pancreatitis, where it promotes the cleavage of cell-contact molecules caused by an activation of the neutrophil serine protease elastase. The subsequent edema of the pancreatic tissue ultimately facilitates leukocyte invasion. Moreover, CatC is localized in lysosomes of acinar cells and is redistributed into the secretory vesicles in the early phase of acute

pancreatitis. However, lysosomes seem to be dispensable for initiation of acute pancreatitis as CatB is already present in zymogen granules of healthy pancreas so that the relevant components for premature protease activation are localized in the same subcellular compartment. Permeabilization of lysosomal membranes by the lysosomotropic compound glycyl-L-phenylalanine 2-naphthylamide [GPN] still allows intracellular trypsinogen activation due to the preserved activation of CatB inside the secretory compartment. GPN is hydrolysed by CatC, which leads to the selective disruption of lysosomes. Conversely, lysosomal disruption is abolished in CatC deficient mice. These observations were confirmed by L-leucyl-L-leucine methyl ester [LLOMe], another lysosomotropic detergent. Our findings underline the role of CatC in the pathogenesis of acute pancreatitis. However, the initiation of the disease seems to be independent of lysosomes.

Cathepsin C, a historical perspective

Christine Pham

MD

Guy and Ella Mae Magness Professor of Medicine

Chief, Division of Rheumatology Washington University, St Louis MO USA

Abstract

Cathepsin C, also known as dipeptidyl peptidase I or DPP1, is a ubiquitous, lysosomal enzyme in the papain-like superfamily of cysteine proteases that is highly conserved between human and mouse. Cathepsin C processes and activates serine proteases found in several effector cells including cytotoxic lymphocytes, neutrophils, and mast cells. This activation of serine proteases, especially in neutrophils, by cathepsin C is believed to modulate the severity of several chronic inflammatory conditions and has been

the focus of numerous clinical and preclinical studies for over three decades. Mutations in the cathepsin C gene also lead to a rare condition termed Papillon-Lefèvre syndrome [PLS], an autosomal recessive disorder characterized by periodontal disease and palmoplantar hyperkeratosis. Here I will review the initial development and characterization of the cathepsin C loss-of-function mutation in the mouse. The findings will be compared and contrasted to the findings in PLS patients.

Impact of cathepsin C and its substrates on neutrophil functional responses

Patrick McDonald^{1,2}, Vanessa de Carvalho Oliveira¹, Dedong Li², Jessica Basso², Mei Fong Peng², Kuan-Ju Chen², Walter Perkins², David Cipolla²

¹ Université de Sherbrooke, Canada | ² Insmad Incorporated, USA

Abstract

Neutrophils and their products play a key role in acute and chronic inflammation, and neutrophil serine proteases [NSPs] contribute to tissue damage associated with inflammatory reactions. NSPs are synthesized as inactive pro-proteins during granulocyte development, and later cleaved into active proteases during neutrophil maturation in the bone marrow by cathepsin C [CatC, also known as DPP1]. Inhibiting NSPs individually or collectively [via CatC] therefore has therapeutic potential. Accordingly, CatC knockout mice show increased resistance to disease development in several chronic inflammatory models. Likewise, brensocaticib [a novel small-molecule CatC inhibitor] is currently in Phase 3 clinical trials [ASPEN, NCT04594369] for non-cystic fibrosis bronchiectasis, following a Phase 2 trial [WILLOW, NCT03218917].

While it is widely accepted that NSP inhibition prevents disease progression by reducing inflammation-related tissue damage, NSP inhibition might also help decrease overall neutrophil activation. For instance, NSPs have been implicated in the generation of neutrophil extracellular traps [NETs], which are pro-inflammatory and a source of auto-antigens. Likewise, mature neutrophils can release catalytically active CatC, raising the possibility that CatC might feedback on the cells to amplify

neutrophil-driven inflammation. In this study, we evaluated the impact of CatC inhibition on neutrophil differentiation and functional responses.

We first determined that brensocaticib does not interfere with neutrophil differentiation in either mice or humans. Additionally, CatC inhibition [either during neutrophil differentiation or in mature neutrophils] did not affect the ability of the cells to migrate towards chemoattractants, to undergo phagocytosis, or to generate reactive oxygen species in mice or humans. Though brensocaticib similarly did not prevent NET formation in mature mouse or human neutrophils, it led to a dramatically decreased response when administered during the differentiation of mouse neutrophils. This suggested that NSPs contribute to NET formation in mice, which we confirmed using triple-NSP knockout animals. In humans however, NSP inhibition did not affect NET formation elicited by physiologically relevant stimuli. We finally explored whether CatC or NSPs that are present on NETs are catalytically active. Collectively, our findings indicate that NSPs generally do not affect neutrophil function [with the exception of NET formation in mice] and that brensocaticib acts in a highly selective manner towards these cells.

The proteolytic airway environment associated to pneumonia acts as a biological barrier for antibody treatment

Blin Timothée^{1,2,3,*}, Parent Christelle^{1,2,*}, Pichon Gabrielle^{1,2}, Guillon Antoine^{1,2,4}, Youenn Jouan^{1,2,5}, Hassan Allouchi^{1,2,6}, Aubrey Nicolas^{2,7}, Fanny Boursin^{2,7}, Domain Roxane^{1,2}, Korkmaz Brice^{1,2}, **Sécher Thomas**^{1,2,5}, Heuzé-Vourc'h Nathalie^{1,2,5}

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* equally contributed to experiment implementation | ⁵ equally contributed to the study design

Abstract

Pneumonia, like COVID-19, is characterized by a massive infiltration of innate immune cells into the airways and alveolar spaces, like polymorphonuclear leukocytes, which release proteases that may degrade therapeutic antibodies and limit their efficacy. Here, we investigated the impact of neutrophil elastase, proteinase 3 and cathepsin G, the 3 main serine proteases of neutrophils, on anti-SARS-CoV2 IgG1 and other IgG subclasses [IgG2 and IgG4], in vitro and ex-vivo, in endotracheal aspirates from patients with severe COVID-19

conditions. IgGs were sensitive to neutrophil serine proteases, and IgG2 were more resistant to proteolytic degradation. Anti-SARS CoV2 antibodies were sensitive to the lung proteolytic environment but inhibitors against neutrophil serine proteases only partly controlled their degradation. Overall, our results show that the dysregulated balance between proteases and their inhibitors in the airways, during pneumonia, contributes to anti-viral antibody degradation and is a significant mechanism of therapeutic antibody catabolism.



18th April 2024

Nathan G.F Leborgne

Neutrophil serine proteases degrade SARS-CoV-2 spike protein and reduce virus replication and inflammation in vivo

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Neutrophil serine proteases degrade SARS-CoV-2 spike protein and reduce virus replication and inflammation in vivo

Nathan G.F Leborgne^{1,2#}, Christelle Devisme^{1,2#}, Nedim Kozarac^{1,2,6#}, Inês Veiga^{1,2}, Nadine Ebert^{1,2}, Aurélie Godel^{1,2}, Llorenç Grau Roma³, Melanie Scherer^{4,6}, Philippe Plattet^{4,5}, Volker Thiel^{1,2,5}, Gert Zimmer^{1,2}, Adriano Taddeo^{1,2}, Charaf Benarafa^{1,2,5}

¹Institute of Virology and Immunology, Mittelhäusern, Switzerland | ²Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland | ³COMPATH, Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland | ⁴Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bern, Switzerland | ⁵Multidisciplinary Center for Infectious Diseases [MCID], University of Bern, Bern, Switzerland | ⁶Graduate School for Cellular and Biomedical Science, University of Bern, Bern, Switzerland

Contributed equally

Abstract

Serine proteases TMPRSS2 and furin, or cathepsins L and B are critical for SARS-CoV-2 entry by proteolytic processing of the spike protein [S]. Severe COVID-19 is associated with a massive influx of neutrophils in the lungs and these inflammatory cells are known to release potent serine proteases [neutrophil elastase [NE], cathepsin G [CatG], and proteinase-3 [PR3]] as a host defense mechanism against the virus. Little is known about the role of neutrophil serine proteases [NSPs] in SARS-CoV-2 replication and pathogenicity. Using purified human NSPs, we found that all three NSPs degrade the S protein of the original pandemic virus protein S[D614G] or the mouse-adapted S[MA10] protein. Pre-incubation of chimeric vesicular stomatitis virus [VSV] expressing S [VSV*dG-S] with each NSP significantly reduced virus entry and replication in vitro. Pretreatment of Vero/TMPRSS2 cells with CatG modestly reduced

VSV*dG-S entry, whereas NE and PR3 had no effect. NSPs had no effect on VSV*dG-S previously adsorbed to cells. Pre-incubation of SARS-CoV-2 with each NSP significantly reduced virus entry and replication in vitro. In NSPs knock-out mice infected with SARS-CoV-2 MA10, we demonstrate that deletion of CatG, but not of NE nor PR3, is associated with higher virus titers in the lung. Importantly, we show that lung cytokine and chemokine expression, and pulmonary pathology were particularly increased in NE.CatG^{-/-} double-deficient mice compared to wild-type mice. These findings suggest that NSPs contribute to the early anti-viral defenses against SARS-CoV-2 infection via proteolytic inactivation of the S protein and by limiting pulmonary inflammation. Therefore, therapeutic inhibition of NSPs or CatC in COVID-19 patients should be evaluated carefully during the acute phases of infection.

18th April 2024

R.A. Stockley

Alpha-1 antitrypsin:

The path from antiproteinases to clinical management

notes _____

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Alpha-1 antitrypsin: The path from antiproteinas to clinical management

R. A. Stockley

MD DSc FRCP

Lung Investigation Unit

Medicine - University Hospitals Birmingham NHS Foundation Trust

Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Edgbaston Birmingham, B15 2GW

Abstract

The Pro-inflammatory and destructive role of Serine proteinases has evolved from the human genetic model of Alpha-1 Antitrypsin deficiency first described in 1963. The following year in his thesis Sten Eriksson^[1] described the clinical features of affected patients including early onset emphysema bronchitis and bronchiectasis. He implicated an enzyme like trypsin as the likely mediator of the destructive process.

Over the last 60 years animal models suggested lung inflammation and connective tissue destruction by serine proteinases [predominantly neutrophil elastase] was central to the lung pathologies and, by extrapolation, other chronic neutrophilic diseases by association although the mechanisms seem largely obscure.

By the 1980s intravenous augmentation for deficient subjects with COPD and emphysema, was introduced and biochemical modulation of lung Elastase activity was shown^[2] leading to widespread [and expensive] usage although clinical evidence of efficacy has remained elusive.

This not only questions the role of serine proteinases in the pathophysiology of chronic lung disease even in those with genetic AAT deficiency, but provides challenges to the

development and validation of anti proteinase therapies for both Antitrypsin deficient and non deficient chronic lung [and other organ] diseases. There are several crucial steps in validation leading to acceptance of such therapies into clinical practice:

1. Is the disease and its severity associated with neutrophilic inflammation?
2. Do serine proteinases generate pathological changes specific to the disease?
3. Is there a supportive animal model?
4. Can a sound phase 2 study show abrogation of the proteinase and its effects
5. Can recruitment of appropriate patients be achieved to phase 3 studies for long enough to modulate validated end points

All of which is not as easy as it sounds even after 60 years in patients with established deficiency and its consequences.

¹ Eriksson S. Studies in α -antitrypsin Deficiency. Acta Med Scand :1965. Supplement to volume 177.

² Wewers MD, Casolaro MA, Sellers SE, Swayze SC, McPhaul KM, Wittes JT. Replacement therapy for alpha-1 antitrypsin deficiency associated with emphysema. N Engl J Med 1987; 316:1055-1062.

Assessment of neutrophil elastase protein inhibitors
in muco-obstructive lung diseases

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Assessment of neutrophil elastase protein inhibitors in muco-obstructive lung diseases

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Abstract

Muco-obstructive lung diseases are characterized by chronic neutrophilic inflammation and an imbalance between proteolytic enzymes and their inhibitors, thereby causing lung matrix destruction and perpetuating inflammation. Elastase is one of the major serine proteases secreted by neutrophils and we aimed to inhibit its activity by using either alpha1 antitrypsin [AAT], its natural inhibitor, or, a total neutrophil elastase [NE]-inhibiting nanobody. PEGylation was used to improve the therapeutic value of both protein inhibitors. Conjugation of AAT to a two-arms 40kDa polyethylene glycol [PEG] chain did not alter its biological activity, improved its proteolytic resistance and increased eight-fold its plasma half-life in mice. However, it only slightly increased the lung exposure to AAT following IV injection. PEGylation increased

two-fold AAT half-life in the lungs and it significantly improved its therapeutic efficacy following pulmonary delivery in a murine model of chronic obstructive pulmonary disease [COPD]. As AAT, the anti-NE nanobody did not lose its inhibitory activity after PEGylation. Both the non-PEGylated and PEGylated nanobodies were able to inhibit NE activity in sputa from patients with cystic fibrosis [CF], bronchiectasis and COPD more efficiently than AAT. PEGylation prolonged the nanobody inhibitory activity in sputa over time, probably through a protection against proteases, which is supported by a decrease in nanobody proteolysis after PEGylation in vitro. PEGylation greatly increased the lung residence time of the nanobody in healthy and β ENaC mice, a model of the CF lung disease, leading to a residence time longer than AAT.

PR3-ANCA interactions with its target antigen: what can they tell us about the disease?

Ulrich Specks

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Abstract

Antineutrophil cytoplasmic antibody [ANCA] associated vasculitis [AAV] is comprised of 3 syndromes, including granulomatosis with polyangiitis [GPA], microscopic polyangiitis [MPA] and eosinophilic granulomatosis with polyangiitis. GPA and MPA share many disease features and are often studied together in clinical trials. GPA is characterized by ANCA directed against proteinase 3 [PR3] and MPA by ANCA directed against myeloperoxidase [MPO]. Both types of ANCA are pathogenic through their capacity to induce and perpetuate vascular and tissue damage by a variety of mechanisms including full activation of primed neutrophils, promoting NETosis, and inducing cytokine synthesis and release from neutrophils and monocytes. Yet, PR3-ANCA and MPO-ANCA are associated with different genetic predisposition, relapse risk, response to treatments, such as rituximab, as well as cytokine patterns at time of active disease.

For the pathogenic mechanisms to play out, both binding of PR3-ANCA to its target antigen, PR3, expressed on the membranes of neutrophils and monocytes, constitutively and enhanced during inflammation, as well as engagement of Fcγ receptors on the surface of neutrophils and monocytes are necessary.

My laboratory has focused on characterizing the molecular interactions of PR3-ANCA with PR3 for 2 major reasons.

First, PR3-ANCA in the serum of patients target different PR3 epitopes. Clinical observations suggest that, depending on the epitopes recognized, the pathogenic potential of PR3-ANCA may vary substantially. This limits the clinical utility of serial PR3-ANCA monitoring in patients over the course of the disease. It also suggests that some PR3-ANCA may have “protective” potential and could be developed into novel therapeutic agents. Our recent efforts using human-mouse chimaeric rPR3 molecules have shown that distant mutations as well as engagement of distant epitopes by anti-PR3 monoclonal antibodies can cause activation of latent epitopes so that the binding of other PR3-ANCA is altered [Arthritis Rheumatol. 2023;75:748-59]. This observation is consequential for the design of potential therapeutic monoclonal PR3-ANCA.

Second, we have use labeled rPR3 to detect PR3-specific B-cells by flow cytometry and have shown that PR3-specific B-cells are enriched in the memory B-cell pool only in patients with PR3-AAV, but not MPO-AAV. Furthermore, the reappearance of PR3-specific plasmablasts after successful treatment of GPA with rituximab is associated with disease relapse [Arthritis Rheumatol. 2023; 75:736-47]. These results indicate that antigen-specific B-cells may be targets for novel therapy approaches targeting these cells selectively.

18th April 2024

Jérôme Zoidakis

Proteomics for characterization of cathepsin C target serine proteases

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Proteomics for characterization of cathepsin C target serine proteases

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Abstract

The development of high sensitivity proteomics methods has expanded the spectrum of identified proteins in biological samples. Moreover, chemoproteomics approaches enable the identification of protein targets for specific inhibitors.

Total cell extracts obtained from healthy human neutrophils and lymphocytes were analyzed by high resolution mass spectrometry approaches and resulted in extensive characterization of their proteome. The total number of identified proteins was over 3000 for each cell type representing a significant portion of all expressed proteins. Several proteases with important roles in immune cell biology were present among the identified proteins. Some representative proteases are cysteine cathepsins [C, L, K and S] and neutrophil serine proteases. These results

indicate that it is possible to characterize in depth the proteome of immune cells and open the way for comparative studies using samples collected from patients and healthy controls.

Chemoproteomics analysis allows the elucidation of the specificity of Cathepsin inhibitors through identification of their targets^[1]. It is possible to determine the affinity [Kd, IC50] of inhibitors at the global proteome scale and allows. Thus, a wide range of collaborative projects can be initiated along these general directions.

[1] Lechner, S., Steimbach, R.R., Wang, L. et al. Chemoproteomic target deconvolution reveals Histone Deacetylases as targets of [R]-lipoic acid. *Nat Commun* 14, 3548 [2023]. <https://doi.org/10.1038/s41467-023-39151-8>

Mast cell proteases: key players in inflammation and tissue homeostasis

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Abstract

Serine proteases constitute major granule components of several mammalian immune cells including mast cells, neutrophilic granulocytes, cytotoxic T cells and NK cells. Lower amounts are also found in basophils and in CD4 positive T cells. All of these proteases are stored in their active form in tight complex with negatively charged proteoglycans such as heparin and chondroitin sulfate and at low pH to keep the enzymatic activity low within the granules. Almost all of these serine proteases are dependent on cathepsin C for their activation. Following transfer into the endoplasmic reticulum and cleavage by the signal peptidase, the majority of these enzymes contain a two amino acid N-terminal activation peptide that has to be removed for the enzymes to become active. This cleavage is performed by cathepsin C. The two amino acids of the activation peptide differ from one protease to another but does always contain at least one charged amino acid, often two. The removal of this charged N-terminal allows the remaining N-terminal to move into the hydrophobic interior of the protease, and the structure thereby changes slightly so that the amino acids of the active site moves into position. The granule stored serine proteases

constitute the major protein component of the mast cell granules and can account for up to 35% of the total cellular protein. Human mast cells contain three major serine proteases, cathepin G, trypsin and chymase. Mast cell granules also contain a mast cell and basophil specific carboxypeptidase, the CPA3. These proteases have a relatively broad specificity allowing multiple potential targets. However, cleavage analysis has still seen a relatively restrictive cleavage of some protein families including cytokines and chemokines. Analysis of their potential in vivo targets have identified, several potential functions for these proteases including, detoxification of snake, gila monster and scorpion venoms, cleavage of angiotensin I, generating angiotensin II, thereby having a blood pressure regulating function. They have also been found to cleave a highly selective set of cytokines, including primarily TH2 cytokines, and thereby down regulating TH2 immunity. The mast cell chymase has also been shown to effectively degrade anticoagulant proteins of several ectoparasites including, ticks, mosquitos and leeches. I will here give an overview of these proteases, their production, processing and potential in vivo functions.

Stability and activation of neutrophil elastase-related serine protease zymogens in myelomonocytic cells

Roxane Domain^{1,2}, Thibault Chazeirat^{1,2§}, Quentin Guilbert^{1,2§}, Lise Vanderlynden^{1,2}, Patrick P. McDonald³, Rich Williams⁴ and Brice Korkmaz^{1,2*}

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Abstract

Elastase, proteinase 3, cathepsin G and NSP4 [Neutrophil Serine Protease 4] are pro-inflammatory serine proteases found within the granules of promyelocytes during neutrophil differentiation in the bone marrow. The vast majority of NSPs are activated by cathepsin C and, to a much lesser degree, by NSPs-AAP-1 [NSP-Alternative Activating Protease-1] from their inactive precursor forms via proteolytic cleavage of their prodipeptides. In this work, we investigated the respective roles of cathepsin C and NSPs-AAP in the activation of NSPs in pro-monocytic cell lines [U937, THP-1] and in primary monocyte-derived macrophages. In U937 cells, we observed a neutrophil-like activation of NSPs involving

cathepsin C and NSPs-AAP-1. In THP-1 cells, elastase and proteinase 3 were generated by cathepsin C and a unknown protease called NSPs-AAP-2, while cathepsin G was activated by cathepsin C. Furthermore, we showed that active forms of proteinase 3 and cathepsin G were not stable and proteolytically degraded in THP-1 cells, which explains the relatively low abundance of these NSPs in this cell line, compared to other myelomonocytic cells. In conclusion, our study shows that NSP activation in myelomonocytic cells is cell type-dependent and more importantly, that the human genome contains at least three proteases involved in NSP activation.

Targeting cathepsin C in the context of neutrophil differentiation and function

Joanna Cichy

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Abstract

Neutrophils play a dual role in the host, contributing to both health and disease, in part through their serine proteases, such as neutrophil elastase [NE]. Given that these enzymes are regulated by cathepsin C, we hypothesized that inhibiting cathepsin C would significantly impact NE-mediated processes, including neutrophil development and metabolic changes in the host. In this context, we discuss

our recent data on generation of neutropenia models based on induced pluripotent stem cells [iPSCs] in order to study the inhibition of cathepsin C activity in developing neutrophils. In addition we present our recent data on the blocking cathepsin C activity in mouse model with enforced metabolic changes associated with the massive migration of neutrophils to fat deposits.

Therapeutic targeting of cathepsin C in pulmonary arterial hypertension

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Therapeutic targeting of cathepsin C in pulmonary arterial hypertension

Izabela Borek¹, Johannes L Berg¹, Marko Navinec², Thibault Chazeirat³, Matthias Evermann⁴, Konrad Hoetzenecker⁴, Brice Korkmaz³, Grazyna Kwapiszewska^{1,5}

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Abstract

Pulmonary arterial hypertension (PAH) is a severe clinical condition marked by pronounced pulmonary vascular remodeling and persistent elevation of mean pulmonary arterial pressure. Pre-clinical models support the central pathogenic role of immune cell-derived inflammatory serine proteases in PAH development. Intriguingly, our transcriptomic screening identified elevated levels of cathepsin C [CtsC], an upstream activator of serine proteases, in the lung tissue of PAH patients. The primary objective of this study is to characterize the role of CtsC in the pathogenesis of PAH and to assess the therapeutic potential of a CtsC inhibitor.

Our single-cell RNA sequencing [scRNAseq] data reveal that, compared to healthy donor lungs, pulmonary arteries [PAs] in PAH exhibit an altered immune cell composition, marked by an expansion of CtsC-positive cells. Protein analysis further validates elevated levels of CtsC in PAH lung samples, along with the presence of proteolytically active CtsC in pulmonary arterial smooth muscle cells [PASMCS]. The increased autophagy and proliferation are characteristic features of PAH-associated

PASMCS implicated in vascular remodeling. In our in vitro experiments, we observe increased proliferation of PASMCS treated with an active recombinant human CtsC, and concurrent suppression of both autophagy and proliferation in cells exposed to a CtsC inhibitor. In vivo experiments in chronic hypoxia mouse PH model confirmed that animals treated with CtsC inhibitor exhibit reduced right ventricular systolic pressure compared to the control group. Flow cytometry immunophenotyping further discloses that the inhibition of CtsC results in a decreased number of T cells and neutrophils in bronchoalveolar lavage, along with an increased number of alveolar macrophages in lung tissue. Further analysis is ongoing to fully characterize the effects of the CtsC inhibitor on disease parameters in vivo.

Our findings suggest the involvement of CtsC in PAH pathophysiology and identify it as a potential therapeutic target. The beneficial effects of the CtsC inhibitor are not limited to the suppression of inflammatory serine protease activation but also extend to the regulation of PASMCS behavior.



18th April 2024

Sevil Korkmaz-Icöz

Inhibiting the cysteine protease cathepsin C enhances post-transplant graft function in rats

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Inhibiting the cysteine protease cathepsin C enhances post-transplant graft function in rats

Baoer Liu^{1,2}; Brice Korkmaz³; Patricia Kraft¹; Tobias Mayer¹; Alex A. Sayour⁴; Marc A. Grundl⁵; Roxane Domain³; Matthias Karck¹; Gábor Szabó^{1,2}; **Sevil Korkmaz-Icöz^{1,2}**

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Abstract

Background: Heart transplantation (HTX) is the established treatment for end-stage heart failure. Nonetheless, reperfusion following an ischemic period may contribute to myocardial damage. Neutrophil infiltration, coupled with the subsequent release of tissue-degrading neutrophil elastase [NE]-associated serine proteases and oxygen-derived radicals, is linked to unfavorable graft outcomes. Inhibiting cathepsin C [CatC] has been demonstrated to block NE-related protease activation. Our hypothesis suggests that the CatC inhibitor BI-9740 enhances post-transplant graft function.

Methods: In a rat model of HTX, recipient Lewis rats were orally administered either a placebo (n=12) or BI-9740 (n=11, 20 mg/kg) once daily for 12 days. Hearts from untreated donor Lewis rats were explanted, preserved in a cardioplegic solution, and subsequently heterotopically implanted. In vivo left-ventricular (LV) graft function was evaluated after 1 hour of reperfusion. The proteolytic activity of neutrophil serine proteases was assessed in bone marrow lysates. Additionally, morphological changes in the myocardium were examined, and heart samples underwent immunohistochemistry and western blot analysis.

Results: The rats treated with BI-9740 exhibited a significant reduction in NE-related proteolytic activity in bone marrow cell lysates compared to the placebo group. Hearts from BI-9740-treated animals showed decreased histopathological lesions, lowered levels of CatC and myeloperoxidase-positive cell infiltration, and reduced nitrotyrosine immunoreactivity, with a decreased number of poly(ADP-ribose) polymerase [PARP]-1-positive cells compared to those receiving the placebo. Regarding the functional parameters of the transplanted graft, improvements were observed in systolic function [LV systolic pressure 110±6 vs 74±6 mmHg; dP/dtmax 2782±149 vs 2076±167 mmHg/s, LV developed pressure, at an intraventricular volume of 200 µl, p<0.05] and diastolic function in the hearts of BI-9740 treated animals compared with those receiving the only placebo. Additionally, the BI-9740 administration led to a shorter graft re-beating time compared to the placebo group. However, this study did not provide evidence of DNA fragmentation, the generation of both superoxide anions and hydrogen peroxide, correlating with the absence of protein alterations related to apoptosis.

Conclusions: Our experimental finding support that pharmacological inhibition of CatC enhances graft function after HTX in rats.

19th April 2024

Moez Rhimi

Cathepsin C Inhibition: towards a new hope for IBD?

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Cathepsin C Inhibition: towards a new hope for IBD?

Vincent Mariaule¹, Amin Jablaoui¹, Soufien Rhimi¹, Meshal Almkali¹, Hela Mkaouar¹, Juan Hernandez^{1,2}, Adam Lesner³, Brice korkmaz⁴, Emmanuelle Maguin¹, **Moez Rhimi¹**

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Abstract

Inflammatory bowel diseases (IBD) constitute a main health challenge of increasing incidence worldwide and poorly understood etiology. Available treatments are not well tolerated nor generally effective. In the absence of a proven cure, the burden of the disease both financially as well as on patients' wellbeing continues to grow. It is therefore essential to set out new therapeutic approaches to treat these diseases. Several reports have demonstrated a key role for neutrophil serine proteases in IBD. Therefore, targeting the proteolytic balance seems to be crucial in IBD. Cathepsin C acts as a major coordinator for the activation of most tissue-degrading neutrophil serine proteases. It has been recognized that Cat

C is responsible for neutrophil recruitment and production of chemokines and cytokines involved in IBD. Thus, CatC seems to be a potential therapeutic target to impair protease-driven tissue degradation in the disease. To address and counterbalance unwanted effects of elastase-related proteases, we investigated the potential of CatC inhibition in mice with colitis. Interestingly, we show that CatC deficient mice exhibited significant protective effects against the development of colitis. These results not only provide insights into the importance of the proteolytic balance in IBD but also point to CatC inhibition as a potential therapeutic strategy in managing the disease.

The role of a cathepsin C inhibitor in lupus nephritis

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The role of a cathepsin C inhibitor in lupus nephritis

Kuan-Ju Chen, Jessica Basso, Daniel Lasala, Patrick McDonald, Walter Perkins, David Cipolla

Insmed Incorporated, New Jersey, USA

Abstract

Neutrophils play a key role in initiating and perpetuating systemic lupus erythematosus, contributing to the resulting kidney damage in patients with lupus nephritis [LN]. This involvement is, in part, due to the excessive release of neutrophil serine proteases [NSPs]. The activation of NSP zymogens during neutrophil maturation, facilitated by dipeptidyl peptidase 1 [DPP1], leads to the release of activated proteases by mature neutrophils in response to inflammatory stimuli. Therefore, a promising strategy to mitigate disease progression in LN involves the inhibition of DPP1.

Brensocatib, a novel small-molecule DPP1 inhibitor, is currently in Phase 3 clinical trials [ASPEN, NCT04594369] for non-cystic fibrosis bronchiectasis [NCFBE], following positive data in the NCFBE Phase 2 trial [WILLOW, NCT03218917] that demonstrated that a reduction in sputum NE was associated with decreased exacerbations.

In this work, we evaluated bremsocatib's capacity to alleviate LN progression in an interferon-alpha [IFN α]-accelerated NZB/W

F1 mouse model. Repeated oral administration of bremsocatib at 2 and 20 mg/kg/day to naïve NZB/W F1 mice for 7 days revealed a significant reduction in bone marrow NSP activities, validating bremsocatib's pharmacodynamic effect in this mouse strain.

To induce LN progression, mice were injected with an IFN α -expressing adenovirus. Subsequent administration of three bremsocatib doses over 6 weeks significantly reduced severe proteinuria, lowered the urine albumin-to-creatinine ratio, and decreased blood urea nitrogen levels. These findings indicate an improvement in kidney damage and renal function in this 8-week study.

Histopathological analysis revealed that bremsocatib treatment significantly lowered renal tubular protein and nephropathy scores, with a trend towards reduced glomerulonephritis. Bremsocatib also significantly reduced LN mouse kidney infiltration by inflammatory cells. These findings suggest that bremsocatib alters disease progression in this LN mouse model, prompting further evaluation of DPP1 inhibition in LN.

19th April 2024

Isabelle Couillin

Consequences of cathepsin C inactivation in cigarette smoke or ozone-induced lung inflammation in mice

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Consequences of cathepsin C inactivation in cigarette smoke or ozone-induced lung inflammation in mice

S. Huot-Marchand¹, R. Domain², S. Carignon¹, N. Riteau¹, A. Gombault¹, B. Korkmaz² and **I. Couillin¹**

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Abstract

Introduction. Cathepsin C [CatC], a lysosomal amino peptidase belonging to the papain family of cysteine, activates neutrophil serine proteinases [NSPs]. Neutrophil-associated local proteolysis is a common pathogenic even in chronic inflammatory lung diseases. NSPs elastase, proteinase 3, cathepsin G, and NSP4 are fully processed and stored as active enzymes in granules of the regulated secretory pathway. To counterbalance unwanted effects of granule-associated serine proteinases amplified by CatC, we evaluated the consequence of pharmacological or genetic depletion of CatC in two mouse models of lung diseases, cigarette smoke- or ozone-induced pulmonary inflammation.

Methods. Wild type [WT] C57/Bl6 mice were exposed to 3R4F cigarette smoke 3 times a day for 4 days. Mice were treated or not with mouse IcatCXPZ-01 SC injections [4.8mg/kg 2x/day, 8 hours apart, from D1 to D11. In addition, WT or cathepsin C deficient [CatC^{-/-}] mice were acutely exposed to ozone [1ppm for 1hrs]. Inflammation was analyzed 24 hours after the last exposure.

Results. Using the acute CS model, we observed degradation [around 80%] of elastase zymogen in bone marrow neutrophils of mice treated with SC injections of the CatC inhibitor IcatCXPZ-01

indicating that CatC activity was efficiently reduced in neutrophil precursors. Analyzing the inflammatory response, we observed that CatC inhibitor treatment decreased neutrophil, but not macrophages influx into the BAL. Treatment decreased also MPO, CXCL1, CXCL5, MMP-9 and TIMP-1 levels in BALF and lung, and IL-1 β in lung. Using the acute ozone model, we observed reduced inflammation in CatC^{-/-} mice with decreased neutrophil and eosinophil influx, MPO, MMP9 and TIMP-1 levels in BALF. Analysis of mRNA expression in lung tissue indicated reduced Cxcl1, Asc and Zbp1 levels in CatC^{-/-} mice.

Conclusion. These results demonstrate that IcatCXPZ-01 efficiently reduced CatC activity in neutrophil precursors, leading to decreased pulmonary inflammation induced by acute CS exposure in mice. In addition CatC deficiency dampens ozone-induced airway inflammation in mice.

Perspectives. We project to decipher the mechanisms by which CatC regulates inflammation in particular, analyzing the effects of CatC inhibition or deletion on neutrophil activity, IL-1 β secretion, pyroptosis cell death, NET formation and ROS generation.

The Dipeptidyl peptidase-1/cathepsin C inhibitor brensocatib reduces airway azurocidin-1 levels in bronchiectasis

Merete B Long, Emma Johnson, and James D Chalmers, on behalf of the WILLOW and STOP-COVID19 Authors

Division of Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK

Abstract

Introduction:

DPPI inhibition with brensocatib reduced neutrophil elastase activity in bronchiectasis and in COVID19, prolonging time to first exacerbation in bronchiectasis. DPPI knockout in animal models has broad anti-inflammatory effects; utilising two clinical trials of brensocatib, we explored the effect of DPPI inhibition across the neutrophil proteome.

Methods:

The WILLOW [NCT03218917; 10mg and 25mg] and STOP-COVID19 [ISRCTN30564012; 25mg] phase-2 double-blind placebo-controlled trials of once-daily oral brensocatib in bronchiectasis and COVID19, respectively, were utilised. In the STOP-COVID19 sub-study, blood samples were obtained at baseline and days 8, 15 and 29 post-randomisation [end-of-treatment [EoT]], isolated peripheral blood neutrophil proteomes were profiled using LC-MS, and serum azurocidin-1 [Azul] measured by ELISA. In a post-hoc analysis of WILLOW, sputum supernatant at baseline and 169 days post-randomization [EoT] was analysed by Azul ELISA [25mg and placebo arms only].

Results:

In STOP-COVID19 [brensocatib: n=75, placebo: n=77], neutrophil proteomics identified significant changes in several proteins including the pseudoenzyme Azul [FDR<0.01] by day 29 [EoT] in the treatment arm, accompanied by significantly lower levels of serum Azul [p<0.0001].

In bronchiectasis patients in the WILLOW study [brensocatib: 55, placebo n=55], sputum Azul levels were also significantly reduced with brensocatib treatment [p<0.0001; mean Azul at baseline vs day 169, brensocatib: 104µg/ml vs 4µg/ml, Placebo: 66µg/ml vs. 48µg/ml]. No differences were identified at baseline between the treatment and placebo group.

Conclusion:

In acute and chronic lung disease, brensocatib treatment significantly reduced azurocidin-1, an underrecognized potential DPPI target associated with bronchiectasis severity and impaired mucociliary clearance.

The Willow study was funded by Insmed Incorporated. The STOP-COVID trial was an investigator initiated study funded by Insmed Incorporated and Sponsored by the University of Dundee.

19th April 2024

Stefan Kreideweiss & Marco Schlepütz

**Preclinical and phase 1 characterization of
the novel cathepsin C inhibitor BI 1291583**

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Preclinical and phase I characterization of the novel cathepsin C inhibitor BI 1291583

Stefan Kreideweiss, Wiebke Sauter, Marco Schlepütz

Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riß, Germany

Abstract

Introduction: An imbalance between neutrophil serine proteases [NSPs], including neutrophil elastase [NE], cathepsin G [CatG], and proteinase 3 [PR3], and their inhibitors has been implicated in bronchiectasis [BE]. Cathepsin C [CatC], which activates NSPs, is a viable target for reducing disease progression in BE. Here, we detail preclinical and Phase [Ph] I data of the novel CatC inhibitor BI 1291583.

Methods: Preclinical studies of BI 1291583 examined in vitro CatC binding kinetics and inhibition, cathepsin selectivity, and inhibition of active NSP production in progenitor cells and lipopolysaccharide-challenged mice. Ph I studies in healthy Caucasians assessed single- and multiple-dose safety, tolerability, pharmacokinetics and pharmacodynamics, relative bioavailability under fasted/fed conditions, and effects of coadministration of itraconazole. A Ph I study in healthy Japanese volunteers was also conducted.

Results: In preclinical studies, BI 1291583 bound human CatC with a KD of 0.43nM. BI 1291583 inhibited human CatC with an IC50 of 0.9nM, displayed a >6000-fold selectivity for CatC vs related cathepsins, inhibited active NE production in neutrophil progenitor cells with an IC50 of 0.7nM, and displayed up to 99%, 94%, and 99% inhibition of active NE, PR3 and CatG production in vivo. In the Caucasian volunteers, BI 1291583 was readily absorbed and had a long half-life [33.6–60.2h]. Exposure increased in a supra-proportional manner.

Inhibition of CatC was dose-dependent with up to 86% blood NE activity inhibition and 65% inhibition of PR3. No food effect was apparent. Co-administration of itraconazole increased exposure by up to twofold. A higher exposure in Japanese vs. Caucasian volunteers, was likely due to bodyweight differences. Exposures were within defined safety limits. BI 1291583 was safe, well tolerated, and the incidence of treatment-related skin exfoliation was similar in BI 1291583 vs placebo groups in all study. No drug-related treatment-emergent adverse events [AEs] or AEs of special interest [skin or oropharynx AEs] were reported.

Conclusions: BI 1291583 is safe, well tolerated and reduces NSP activity. Phase 2 trials investigating BI 1291583 are underway for treatment of non-cystic fibrosis-related BE and cystic fibrosis-related BE.

DISCLOSURE STATEMENT

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19th April 2024
Gábor Szabó

Design of clinical trials evaluating cathepsin C inhibition in heart transplantation

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Design of clinical trials evaluating cathepsin C inhibition in heart transplantation

Gábor Szabó^{1,2}, Baoer Liu^{1,2}, Brice Korkmaz³, Patricia Kraft¹, Sevil Korkmaz-Icöz^{1,2}

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Abstract

Heart failure impacts a global population exceeding 64 million individuals, with approximately 5% of heart failure patients reaching the end-stage. Heart transplantation (HTX) is recognized as an optimal therapy for patients with end-stage heart failure. Nevertheless, the inevitable occurrence of ischemia/reperfusion (IR) injury during HTX significantly results in adverse outcomes for recipients. It is crucial to devise solutions to mitigate this effect and enhance post-transplanted graft function and patient outcomes. Cathepsin C [CatC], also known as dipeptidyl peptidase 1 [DPP1], plays a crucial role in the activation of pro-inflammatory neutrophil serine

proteases [NSPs], which are associated with chronic inflammatory processes and autoimmune diseases under pathological conditions. Two ongoing clinical studies, Brensocatib [INS1007] in phase 3 by Insmed and BII291583 in phase 2 by Boehringer Ingelheim, are investigating the potential benefits of CatC inhibition for patients with bronchiectasis, a NSPs-driven disease. An orally bioavailable CatC inhibitor, has been demonstrated in our recent study to improve post-transplanted graft function in a rat model of HTX after 12 days of oral administration. The presentation will give insight in designing clinical trials in the fields of heart transplantation with particular reference of CatC inhibition.



19th April 2024
Cliff Taggart

Pharmacological inhibition of cathepsin S and consequences for cathepsin C activation

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Pharmacological inhibition of cathepsin S and consequences for cathepsin C activation

Cliff Taggart

Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast

Abstract

Cathepsin S [CTSS] is a potent elastolytic protease that plays a role in inflammation, tissue destruction and antigen presentation. We have previously demonstrated upregulated CTSS activity and protein in the lungs of patients with Cystic Fibrosis [CF], Chronic Obstructive Pulmonary Disease [COPD] and Acute Respiratory Distress Syndrome [ARDS]. Utilising murine models of acute [direct instillation of lipopolysaccharide] and chronic [ENaC-Tg and smoking] lung disease, we have demonstrated a role for CTSS in lung inflammation, mucus production and lung damage. Notably, in all murine models of lung disease we have observed a significant decrease in lung neutrophil recruitment which is due, in part, to activation of the protease activated receptors, PAR-1 and PAR-2. In recent data, we have shown that neutrophils can also produce CTSS which suggests a role for CTSS in neutrophil function. We have focused on the involvement of CTSS in the processing of Cathepsin C [CTSC], which itself is an integral protease for the activation of

the Neutrophil Serine Proteases [NSPs], which are integral and critical to the normal function of neutrophilic cells. Initial investigations were carried out in the neutrophil precursor-like cell line PLB-985, as the processing of CTSC and NSPs occurs early in the neutrophil maturation process. In these cells we have shown that inhibition of CTSS activity leads to an arrest in CTSC processing, as confirmed by the appearance of a processing intermediate via Western blot and a reduction in CTSC activity via fluorometric activity assay. These findings were further confirmed in the HL-60 neutrophil precursor cell line. The effect of these inhibitors on NSP activity was then also investigated via fluorometric activity assay and reductions in the activity of NSP activity were noticeable with CTSS inhibitor treatment. Future investigations will involve analysing the effect of CTSS inhibition on neutrophil function and NSP activity in vivo. The findings of this study could implicate neutrophil-derived CTSS in the pathogenesis of acute and chronic lung disease.

The research actions of the Rare Disease Foundation

Daniel Scherman

Director of "Fondation for Rare Diseases"

Abstract

By definition, a disease is designated "rare" when it affects less than one person in 2,000 in the population. People living with a rare disease and those around them face numerous social, psychological and financial obstacles every day. They are mainly children, because 80% of rare diseases are of genetic origin and these diseases appear from childhood.

These illnesses are chronic, often very disabling physically and/or mentally retarded. They are sometimes lethal. They are little known because each affects very few patients. Because of this, several years pass before the family of a sick child knows the name of the illness: this is what we call "diagnostic wandering".

There are more than 8,000 different rare diseases which, taken together, affect around 5% of the population, or 3 million people in France. Less than 5% of these 8,000 diseases benefit from a treatment.

Scientific research has enabled spectacular advances in identifying the cause of rare diseases, thus speeding up diagnosis, advances to which the Foundation has made a notable contribution. In addition, spectacular progress has been made over the last 5 years with, among other examples, medicine drugs against cystic fibrosis and transthyretin amyloidosis, and gene therapies to treat infantile spinal muscular atrophy, beta-thalassemia, sickle cell anaemia, severe immune deficiencies, or certain forms of blindness. These examples will be presented

Born from a joint desire of the actors involved in research and representatives of patient associations [AFM-Téléthon and the Rare Diseases Alliance], the Rare Diseases Foundation is a Scientific Cooperation non-profit Foundation, which aims at accelerating academic research and public/private partnership through 3 objectives: identify the cause of illnesses to help with diagnosis and identify therapeutic targets, help develop new treatments, and improve the life course of sick people and their families through social sciences and humanities research.



notes



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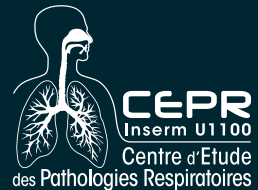
17 > 19 April 2024 | Tours, France

4TH INTERNATIONAL SYMPOSIUM ON CATHEPSIN C

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Scientific Committee: Prof. Gilles Lalmanach, *Inserm/Université de Tours-France*,
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