Cathepsin C Inhibitor Proved Effective in the Treatment of Vasculitis

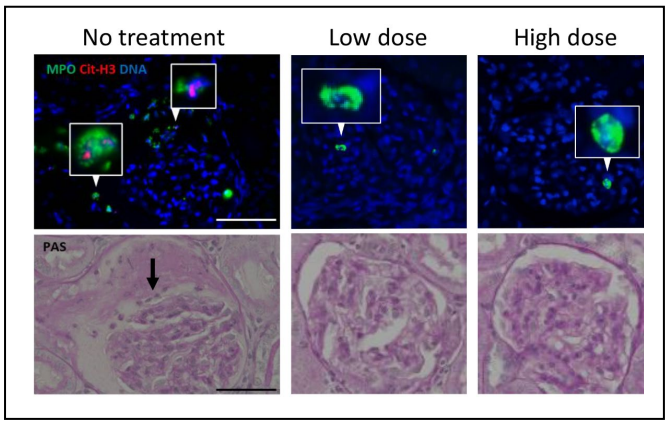
～Expectations for a Novel Therapeutic Agent for ANCA[1*]-Associated Vasculitis～

Alivexis, Inc. and Hokkaido University today announced the publication of a joint research article entitled “Cathepsin C inhibition reduces neutrophil serine protease activity and improves activated neutrophil-mediated disorders,” published in Nature Communications on August 22, 2024.

The research, led by Assistant Professor Yuka Nishibata, Dr. Sakiko Masuda, and Professor Akihiro Ishizu of the Graduate School of Health Sciences, Hokkaido University in collaboration with Alivexis, has shown for the first time in an animal model that inhibiting cathepsin C (CatC) improves ANCA-associated vasculitis (AAV). AAV is an autoimmune disease mainly observed in the elderly, which causes rapid progression of kidney and lung damage. The published study targeted CatC, an enzyme which controls the maturation of neutrophil serine protease (NSPs[2*]), since mature NSPs are essential for the formation of neutrophile extracellular traps (NETs[3*]), the underlying cause of AAV. The team hypothesized that CatC inhibition would improve AAV symptoms, and to test this theory the Hokkaido University research group developed a rat model of AAV to which they orally administered MOD06051, a novel and highly specific CatC inhibitor developed by Alivexis. In animals treated with MOD06051, both NET-forming neutrophils detected in peripheral blood and NETs deposited in the kidneys were drastically reduced, and the clinical manifestations of kidney and lung damage were also reduced. The results of this study suggest that CatC inhibition is an effective therapeutic strategy for the treatment of various diseases caused by NETs, including AAV.

Key Scientific Findings

- CatC inhibition improves kidney lesions and pulmonary hemorrhage in a rat model of AAV.
- CatC inhibition suppressed NET formation, one of the major underlying mechanisms of AAV.
- Expectations are high for the development of novel therapeutics for multiple diseases caused by NETs.



Renal glomerular lesions in an animal model of AAV and ameliorative effect of CatC inhibitor Neutrophils (green) positive for the NET marker Cit-H3[4*] (red) are observed in the glomeruli of the disease group, with ruptured glomerular capillaries (↓) resulting in blood components leaching into the Bowman's sac. Cit-H3 is not observed in the neutrophils infiltrating the glomeruli of the low-dose and high-dose treatment groups, and the glomeruli maintain normal morphology.
Bar, 50 μm

【Background】

AAV is a systemic small vessel vasculitis that develops in the presence of the autoantibody against neutrophil cytoplasmic antigen (ANCA). AAV is a disease mainly observed in the elderly and is characterized by rapidly progressing renal dysfunction and pulmonary bleeding. The Japan Ministry of Health, Labour and Welfare designates AAV as an intractable disease, with approximately 20,000 Japanese patients in 2020—a number which is increasing every year. Current treatment options for AAV include immunosuppressive drugs such as corticosteroids and cyclophosphamide, as well as molecular targeted drugs such as rituximab and avacopan. However, due to the number of non-responders to these therapies and the cases of relapse after treatment, there is a strong need for new treatment options. Furthermore, the existing therapeutics have side effects including decreased immunity, with treatment-related infections being a major issue that needs to be resolved.

NETs are DNA complexes coated in bactericidal enzymes originally expressed in the cytoplasm of neutrophils which are released extracellularly by neutrophils in response to pathogenic microorganisms. NETs released from neutrophils entangle the pathogens with their DNA and bombard them with enzymes to kill them. Although NETs play a significant role as a host defense mechanism, in the absence of pathogens the enzymes released from NETs damage the wall of blood vessels, leading to vasculitis (**Fig. 1**).

NSPs are a group of enzymes found in neutrophils, and include neutrophil elastase, proteinase 3, and cathepsin G. A common activation mechanism for all types of NSPs is the cleavage of two peptides on the N-terminal side of immature (inactive) NSPs during neutrophil differentiation in the bone marrow, resulting in their conversion to the mature (active) form. The mature form of neutrophil elastase plays an important role in the formation of NETs (**Fig. 1**).

CatC is an enzyme that acts during neutrophil differentiation in the bone marrow, cleaving the two N-terminal peptides of immature (inactive) NSPs and converting them to the mature (active) form (**Fig. 1**).

【Methods】

Animal model of AAV: Twenty-four 4-week-old male Wistar Kyoto rats were immunized with human-derived MPO, a potential antigen for autoantibodies against neutrophils.

Administration of CatC inhibitor (MOD06051): The above rats were divided into 3 groups of 8 rats each (disease group, low-dose treatment group, and high-dose treatment group). 0.3 mg/kg of MOD06051 was orally administered twice daily in the low-dose treatment group and 3 mg/kg in the high-dose treatment group. The disease group received the same dose of solvent orally twice daily. The administration period was 42 days from the day of model creation, and sampling was performed from all individuals on the 42nd day.

Evaluation items: ANCA antibody titer in serum (enzyme-linked immunosorbent assay), NET-forming neutrophils in blood (flow cytometry), NETs deposition in renal tissue (immunofluorescence), renal tissue injury (PAS staining), and lung hemorrhage (HE staining) were evaluated.

【Results】

CatC inhibitory activity of MOD06051: MOD06051 selectively inhibited only CatC across nine cathepsin family enzymes in vitro and inhibited CatC enzyme activity in peripheral blood after administration to rats. It also inhibited the activity of NSPs in neutrophils differentiated from human hematopoietic stem cells and in neutrophils extracted from rat bone marrow after repeated dosing of the compound, as well as inhibiting NET formation induced by various stimuli.

ANCA antibody titers in serum: Compared to normal rats (n=8) in the negative control group, the disease control group showed significantly higher ANCA antibody titers than the negative controls; in the MOD06051-treated group, ANCA antibody titers were as high as in the disease control group, regardless of compound dosage (**Fig 2a**).

NET-forming neutrophils in blood: Compared to the negative control group, the percentage of peripheral neutrophils forming NETs was significantly higher in the disease control group than in the negative controls, and the percentage was significantly and dose-dependently reduced to normal levels in the MOD06051 treatment groups (**Fig 2b**).

NET deposition in renal tissue: Approximately 10% of kidney glomeruli-infiltrating neutrophils formed NETs in the disease control group, while NET deposition in renal tissue was significantly and dose-dependently reduced in the MOD06051 treatment groups (**Fig 2c**).

Renal tissue injury: Approximately 10% of glomeruli in the disease control group had lesions, while the percentage of injured glomeruli was significantly reduced in the MOD06051 treatment groups (**Fig 2d**).

Pulmonary hemorrhage: MOD06051 dose-dependently reduced pulmonary hemorrhage, with statistically significant improvement in the high-dose group compared to the disease control group (**Fig 2e**).

【Future ExpectationsProspects】

This study clearly showed that CatC inhibitors have the potential to be novel therapeutic candidates for AAV. The fact that no effect on ANCA antibody titer was observed in this study means that the ability to produce antibody, i.e., liquid immunity, was not affected by CatC inhibition. This finding

indicates that, unlike existing therapeutic agents that suppress a wide range of immune functions, CatC inhibitors potentially may avoid causing immunosuppressive adverse effects. In addition, since it has been reported that mice genetically deficient in CatC have no loss of host defense-related neutrophil functions, such as ROS release, adhesion, migration, or phagocytosis of neutrophils, MOD06051 may improve disease symptoms while not effecting the neutrophil's host defense function. Further investigation, including clinical trial evaluation, is expected to confirm the safety and efficacy of the compound in human diseases. In addition to AAV, NETs are also known to contribute to the etiology of various other diseases such as sepsis, gout, diabetes, systemic lupus erythematosus, and rheumatoid arthritis. Therefore, CatC inhibition is expected to become an effective therapeutic strategy for the treatment of various NET-related diseases.

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Publication Information

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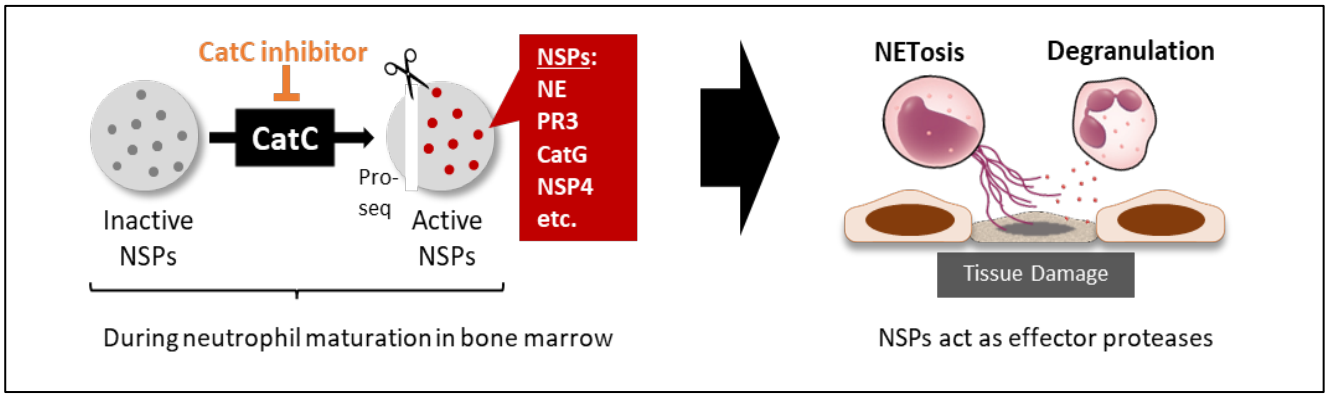
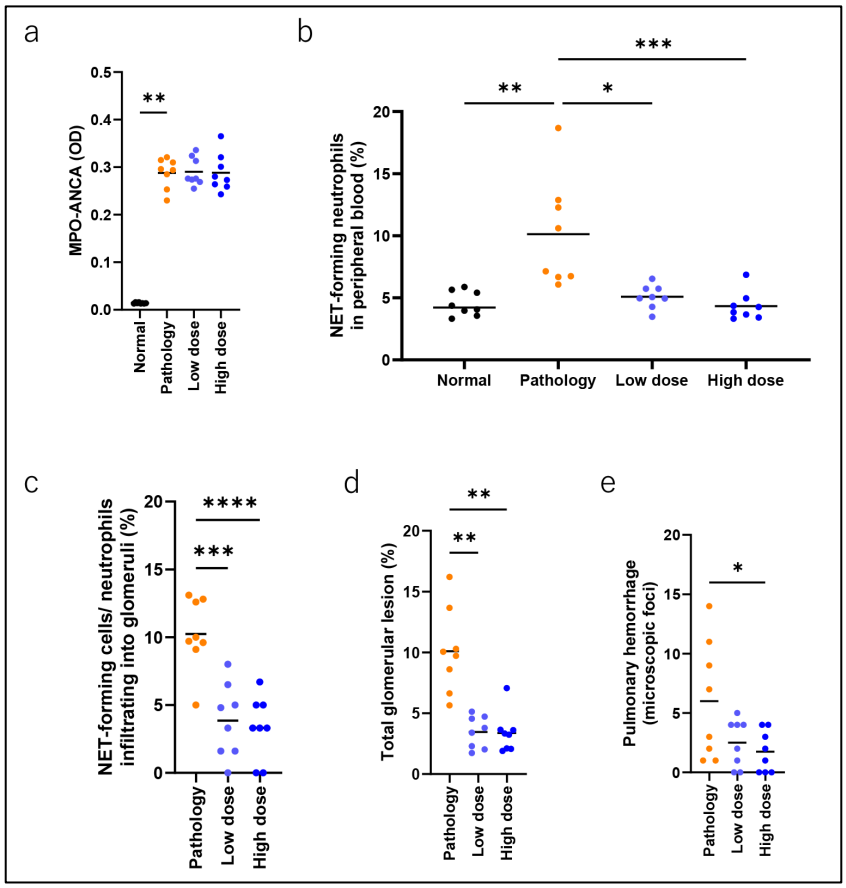


Fig 1. Mechanism of action of cathepsin C inhibitors on tissue injury by neutrophils



(**a**) ANCA antibody titer in serum. (**b**) Percentage of NET-forming neutrophils in peripheral blood. (**c**) Percentage of NET-forming neutrophils in kidney glomeruli-infiltrating neutrophils. (**d**) Renal lesions shown as the percentage of injured glomeruli. (**e**) Pulmonary hemorrhage shown as the number of foci of pulmonary hemorrhage observed under the microscope.
*p<0.05, **p<0.01, ***p<0.001, **** p<0.0001.

Fig 2. Efficacy of CatC Inhibitors in the Treatment of AAV

【Glossary】

＊1 ANCA …Anti-Neutrophil Cytoplasmic Antibody is a pathogenic autoantibody that binds to myeloperoxidase and proteinase 3 in the cytoplasm of neutrophils.

＊2 NSPs … Neutrophil Serine Proteases are a group of enzymes found in neutrophils, including neutrophil elastase, proteinase 3, and cathepsin G, among others. Neurophil elastase in its mature form plays a critical role in the formation of NETs.

＊3 NETs … Neutrophil Extracellular Traps are released from neutrophils as a defensive response to pathogenic microorganisms in the body. In AAV, however, ANCA binding causes the release of NETs from neutrophils, which in the absence of pathogenic microorganisms

cause damage to the vascular endothelium, resulting in vascular inflammation.

＊4 Cit-H3 … Citrullinated Histone H3 is produced when histone H3, a nuclear protein around which DNA coils, undergoes citrullination during NET formation, inducing DNA unwinding. The presence of Cit-H3 is an indicator of NET formation.

Link to the Press Release from Hokkaido University: https://www.global.hokudai.ac.jp/blog/new-compound-shows-great-potential-for-patients-with-neutrophil-associated-inflammation/