NX210c PEPTIDE: A DRUG CANDIDATE TO REPAIR THE BBB IN NEUROLOGICAL DISORDERS

Sighild Lemarchant[1], Chris Greene[2], Nicolas Rebergue[1], Gwen Fewell[3], Supriya Mahajan[4], Damir Janigro[4], Daniël Dumas[5], Philip Kremer[5], Juliette Le Douce[1], Sébastien Marie[1], Yann Godfrin[1,6], Annette Janus[1], Matthew Campbell[2]

[1]Axoltis Pharma, Lyon, France [2]Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland [3]SynVivo, Huntsville, USA [4]Flocel, Cleveland, USA [5]CHDR, Neurology, Leiden, The Netherlands [6]Godfrin Life-Sciences, Caluire-et-Cuire, France

INTRODUCTION

Blood-brain barrier (BBB) integrity is altered in various animal models of neurodegenerative diseases and in patients. Correlations of these alterations with functional deficits suggest that repairing barriers may represent a disease-modifying approach to prevent neuroinflammation and neurodegeneration induced by the extravasation of blood components into the parenchyma.

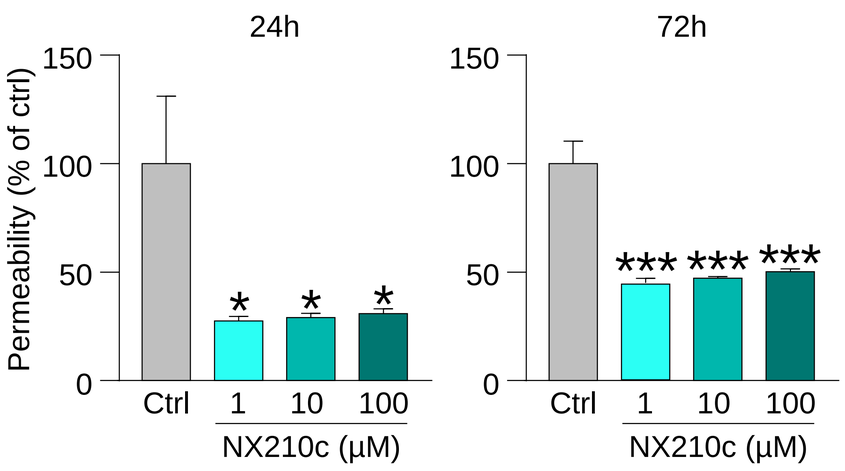
NX210c is a 12-amino acid peptide derived from the most conserved sequence of the type 1 thrombospondin repeats of the subcommissural organ-spondin; it exerts beneficial effects on cognitive and motor functions in animal models of amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease and spinal cord injury. The aim of this study was to evaluate *in vitro* and *in vivo* if NX210c could reinforce BBB integrity.

METHODS

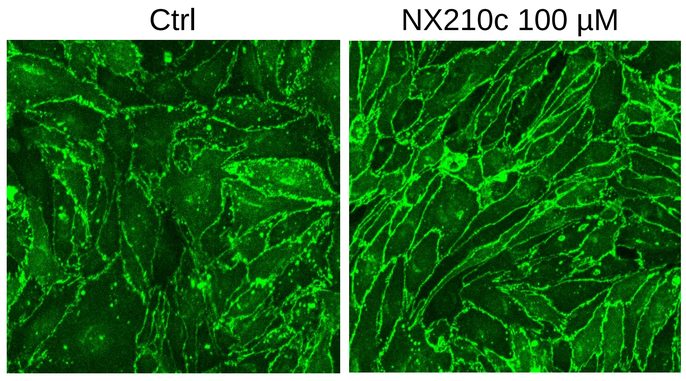
In vitro: Mouse bEnd.3 endothelial cell (EC) monolayers were treated with NX210c (1-100 µM) or its vehicle (cell culture water) for 24h and 72h. Claudin-5 and occludin protein expression were evaluated respectively by immunofluorescence (N=7-10/group) and western-blot (N=8/group). We also measured permeability of EC monolayers grown on transwell inserts to a 40-kDa FITC-dextran was measured (N=3/group) and transendothelial electrical resistance (TEER; N=15-18/group). Human BBB-on-chips (primary human brain microvascular endothelial cells (HBMVECs), pericytes and astrocytes) under shear stress were treated similarly for 4h, and the permeability was assessed using 4-kDa FITC-dextran (N=4-5/group), as well as the TEER measured (N=3/group). *In vivo:* 3- and 21-month-old C57BL/6J mice were treated intraperitoneally with NX210c at 10 mg/kg or its vehicle (water for injection) for 5 days once a day. Brains were collected at day 6 and the immunoreactive areas of tight junction proteins in the cortex and hippocampus were measured (N=5-8/group). ***p<0.001, **p<0.01, *p<0.05.

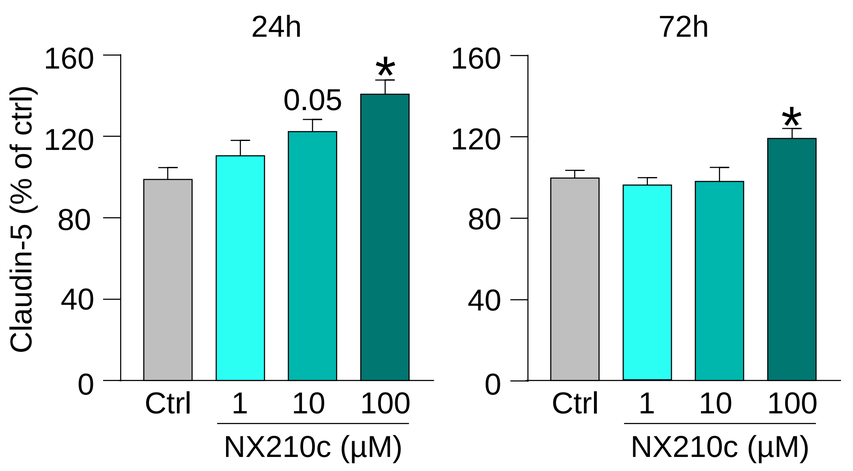
RESULTS

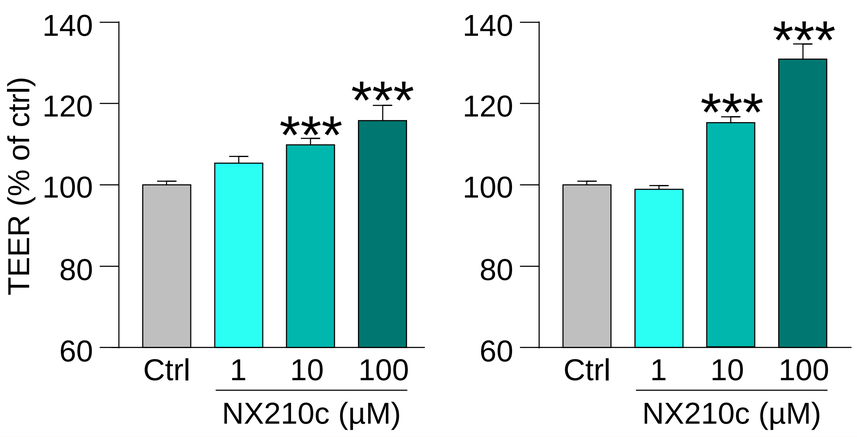
bEnd3 endothelial cell monolayers:



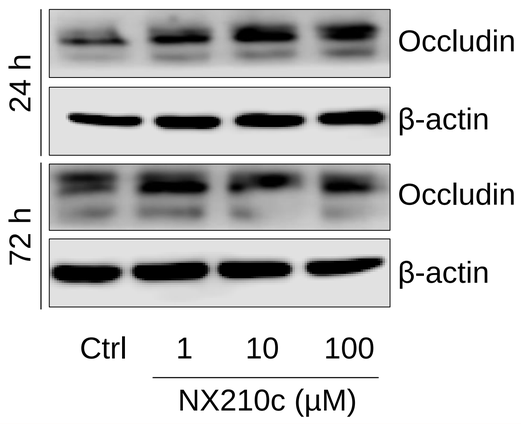
Claudin-5:

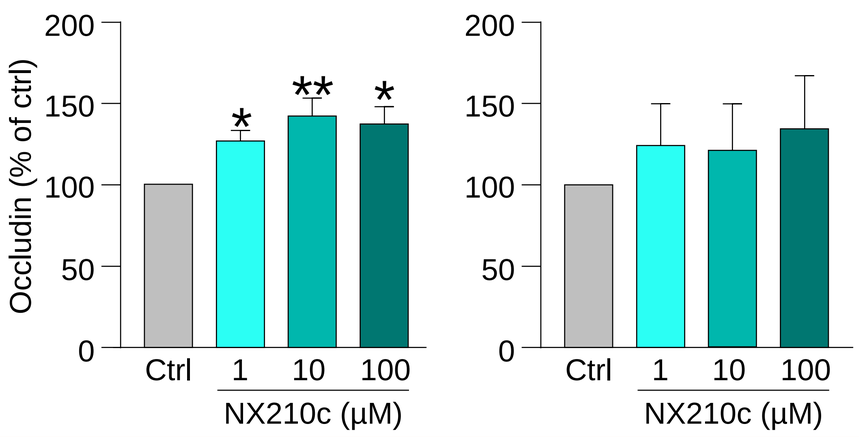




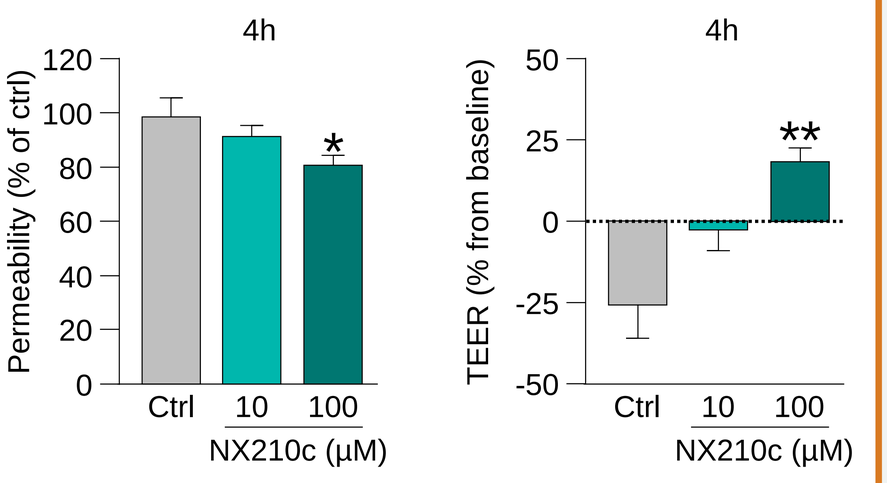


Occludin:

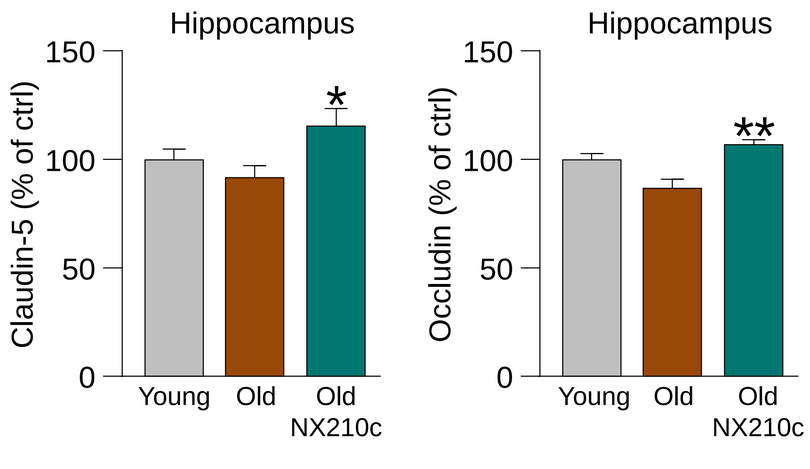


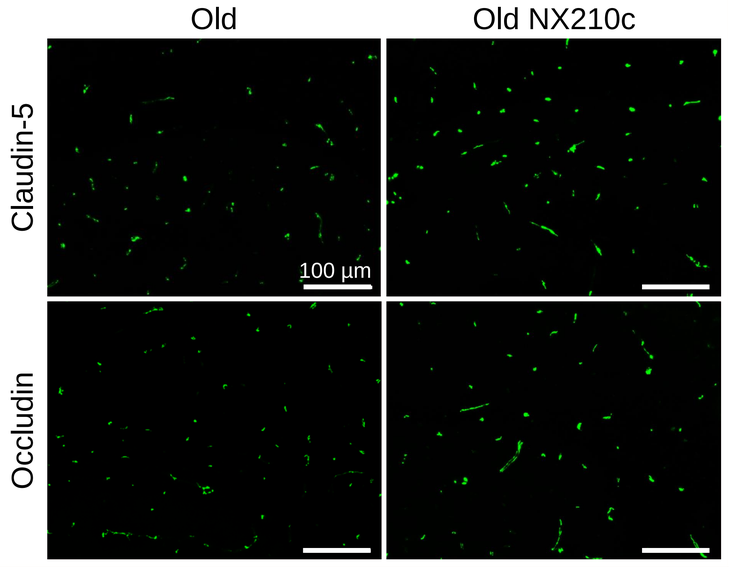


3D dynamic human BBB-on-chips:



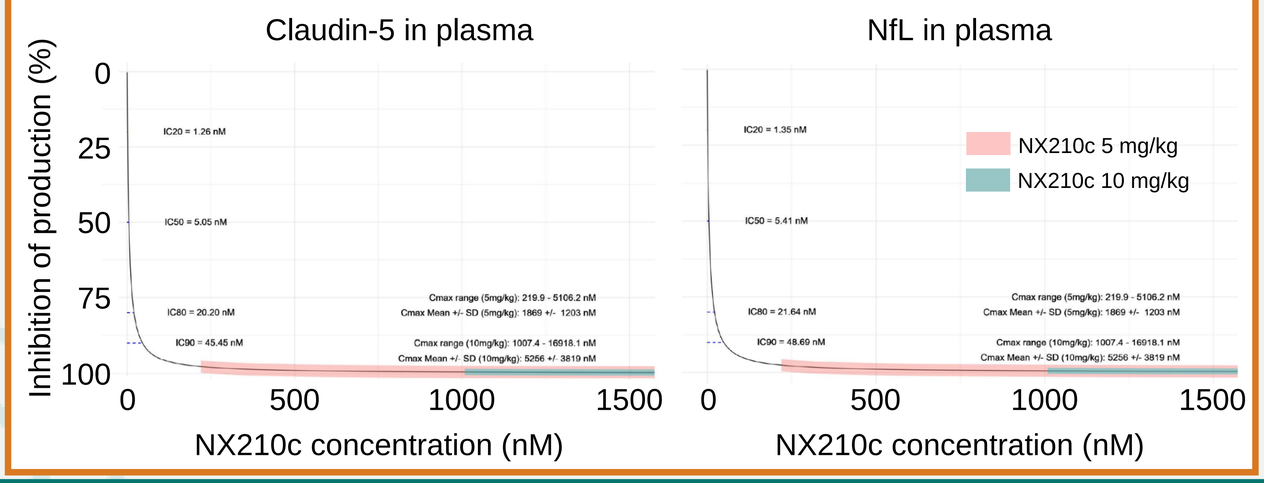
Mouse model of aging:





same in the cortex for occludin (data not shown)

Healthy elderly volunteers (Phase 1b MAD study):



CONCLUSIONS

NX210c is a promising drug candidate for the treatment of neurological diseases with BBB dysfunctions such as ALS. Indeed, we have shown that NX210c promoted BBB integrity *in vitro* by reducing BBB permeability, increasing TEER and tight junction protein expression. The increase in tight junction protein expression was confirmed *in vivo* in a mouse model of aging. A multiple ascending dose (MAD) phase 1b clinical trial (NCT05827653) in healthy elderly volunteers treated 3×/week for 4 weeks confirmed the good safety profile of NX210c and showed pharmacological effects on BBB integrity and neuroprotection. Indeed, a sustained decrease in claudin-5 plasmatic levels was observed with a PK/PD relationship. This was associated with a decrease in NfL. Axoltis Pharma got the authorization to launch a phase 2 clinical trial in ALS patients (NCT06365216).

Disclosures: SL, NR, JLD, SM and AJ are employed by Axoltis Pharma. YG is the CEO and a shareholder of Axoltis Pharma.