Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

NX210c

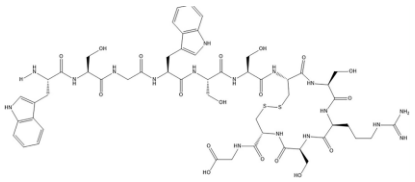
Evidence Summary

Studies in rodents and cell culture have reported neuroprotective effects in neurodegenerative models. No studies have tested NX210c in humans to date, but a phase I study has tested NX210 (linear form).

Neuroprotective Benefit: In mouse models, NX210c improves cognitive function, reduces pathological markers, inhibits apoptosis, and increases synaptic proteins/transmission. No studies have tested NX210c in humans yet.

Aging and related health concerns: No studies have tested NX210c for age-related diseases other than in models of neurodegeneration.

Safety: Intervention with NX210c has not been studied in humans. In a phase I study, NX210, which cyclizes to NX210c, was safe and well-tolerated with mild adverse events including dizziness, headache, and somnolence.

Availability: in clinical development	**Dose**: not established for NX210c. In a phase I study of NX210, single intravenous doses of 0.4, 1.25, 3.5, 5, or 10 mg/kg have been tested.	**Chemical formula:** H-WSGWSS[CSRSC]GOH (brackets represent the disulfide bond) **Structure**:  Source: Deletage et al., 2021
Half-life: NX210c has a plasma half-life of 6-20 minutes after a single infusion of NX210.	**BBB**: likely penetrant based on a rodent study	
Clinical trials: none available	**Observational studies**: none available	

What is it?

NX210c is a cyclic conformation of NX210, a 12-amino-acid-long peptide derived from the large glycoprotein, subcommissural organ-spondin (SCO-spondin)(Deletage et al., 2021). SCO-spondin is synthesized by the SCO ependymal cells during embryogenesis and plays an important role in neuronal development, differentiation, migration, survival, neurite outgrowth, and axon fasciculation (Gobron et al., 2000; Vera et al., 2013). In humans, the SCO becomes atrophic shortly after birth, possibly contributing to the failure of neuronal repair in neurodegenerative diseases and injuries in adulthood (Deletage et al., 2021). NX210 has two cysteines that create a disulfide bond under oxidative conditions, forming a cyclic form, NX210c. NX210c exerts neuroprotective effects via integrin receptors and γ-secretase substrates, subsequent activation of the PI3K/Akt/mTOR pro-survival pathway, and disruption of the apoptotic cascade.

NX210c is under clinical development by Axoltis Pharma for the treatment of CNS damage.

Neuroprotective Benefit: In mouse models, NX210c improves cognitive function, reduces pathological markers, inhibits apoptosis, and increases synaptic proteins/transmission. No studies have tested NX210c in humans yet.

Types of evidence:

- A phase I study of NX210
- Several laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

No studies have tested an NX210c treatment for the prevention of dementia or cognitive decline. There has been a phase 1 study that tested NX210, which cyclizes to NX210c. In this phase I double-blind randomized controlled single-ascending dose study of 39 healthy volunteers, a single intravenous dose of NX210 (0.4, 1.25, 3.5, 5, or 10 mg/kg) resulted in a dose-related decrease in homocysteine levels (-19.6% at the 10 mg/kg dose) (Bourdes et al., 2022). Homocysteine is a sulfur-containing amino acid that is derived from the metabolism of the amino acid methionine. B vitamins (B12, B6, and B9/folate) are required for homocysteine metabolism. Higher homocysteine levels are associated with many age-related diseases including dementia risk (Zuin et al., 2021). Theoretically, the reduction in homocysteine levels seen with NX210 infusion could be neuroprotective. Although the mechanism by which NX210 (or NX210c) reduces homocysteine levels is unclear. Authors of the phase 1 study speculated that NX210 could bind with homocysteine and promote its elimination, which was supported by in silico modeling and docking simulations (unpublished data discussed in Bourdes et al., 2022).

Human research to suggest benefits to patients with dementia:

None available.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

In a central nervous system biodistribution study, a single intravenous administration of [3H]NX210c in male rats suggested that NX210c passes through the blood-cerebrospinal fluid (CSF) barrier (Bourdes et al., 2022).

In a mouse model of Alzheimer's disease (mice with intracerebroventricular injection of Aβ25-35 oligomers), intraperitoneal injections of NX210c (and NX210) significantly improved cognitive function, decreased markers of Alzheimer's disease (Aβ42, p-tau, inflammation, astrogliosis, and lipid peroxidation), and increased synaptic markers (synaptophysin and PSD95)(Le Douce et al., 2021).

NX210c treatment (1, 2, and 3.75 mg/kg, i.p.) fully prevented Aβ oligomer-induced deficits in working memory (measured by spontaneous alternation on the Y-Maze) such that performance was comparable with mice not injected with Aβ25-35 oligomers (Le Douce et al., 2021). This protection was more pronounced with NX210c compared to NX210 (when the 1 mg/kg dose failed to significantly prevent working memory deficits). No benefits were observed at the lowest (0.1 mg/kg) or highest (30 mg/kg) doses with NX210 or NX210c. The most effective doses of NX210c were as beneficial as donepezil in restoring working memory in Aβ-injected mice. For contextual long-term memory (measured by step-through latency passive avoidance), NX210c treatment (1, 2, and 3.75 mg/kg, i.p.) fully prevented Aβ-induced deficits and the effects were comparable to those of donepezil treatment, or control mice (not injected with Aβ25-35 oligomers). For spatial learning and memory (measured with Morris water maze), NX210c treatment (2 mg/kg) demonstrated the same level of learning as that of control mice (not injected with Aβ25-35 oligomers) and a trend for better performance compared to mice treated with donepezil ($p=0.059$).

In Aβ25-35-injected mice, NX210c treatment (2 mg/kg, i.p.) started 1 hour after Aβ25-35 injection and continued for 11 days restored levels of Aβ42, p-tau, astrogliosis (GFAP), inflammation (TNF-α), oxidative stress (lipid peroxidation), endoplasmic reticulum stress (caspase-12), and synaptic markers (synaptophysin and PSD-95) in the hippocampus and prefrontal cortex (Le Douce et al., 2021). NX210c was more effective than NX210 in restoring p-tau and caspase-12 levels in Aβ25-35-injected mice. Levels of these markers after treatment with NX210c were comparable to control mice (not injected with Aβ25-35 oligomers) and mice treated with donepezil.

In Aβ25-35-injected mice, subtherapeutic doses of donepezil and NX210c (0.25 and 0.1 mg/kg, respectively), started 1 hour after Aβ25-35 injection, significantly reduced cognitive deficits, as measured by spontaneous alternations and step-through latency (Le Douce et al., 2021).

When Aβ25-35-injected mice were treated with NX210c (2 mg/kg/day) after the disease was already established (starting 11 days after Aβ25-35 injection), spatial working memory and spatial learning and memory were rescued, measured by the Y-maze spontaneous alternation and Morris water maze, respectively (Le Douce et al., 2021). Aβ25-35-injected mice treated with NX210c showed the same learning capacity as that of control mice not injected with Aβ25-35. Delayed daily treatment with NX210c also prevented the Aβ25-35-induced decrease in step-through latency.

In Aβ25-35-injected mice, early NX210c treatment (2 mg/kg), started 1 hour after Aβ25-35 injection and continued for 120 days, significantly reduced working memory deficits, as measured by spontaneous alternations on the Y-maze (Le Douce et al., 2021). Performance was comparable to control mice not injected with Aβ25-35. In Aβ25-35-injected mice that received delayed NX210c treatment (2 mg/kg), started 11 days after Aβ25-35 injection and continued for 4 weeks, working memory deficits were fully restored 18 days after treatment. Intriguingly, the restoration of working memory was sustained up to 4 months, despite the cessation of treatment on Day 38.

In Aβ25-35-injected mice, a combination therapy of subtherapeutic doses of NX210c (0.1 mg/kg) and donepezil (0.25 mg/kg), started 1 hour after Aβ25-35 injection and continued for 120 days, led to a sustained recovery of working memory for the 120-day duration (Le Douce et al., 2021). Daily treatment with donepezil (1 mg/kg, active dose) showed decreased efficacy over time, at which point NX210c treatment was initiated (on Day 44, scaled up from 2, 4, to 8 mg/kg over time), and working memory deficits were temporarily rescued at the 2 mg/kg dose, followed by full recue at the highest dose (8 mg/kg), such that performance was comparable to control mice not receiving Aβ25-35 oligomers.

In a mouse model of synaptic dysfunction (induced by blockade of the glutamate NMDA receptor with phencyclidine), a single systemic injection of NX210c (5 mg/kg, i.p.) increased the glutamate AMPA- and GluN2A-containing NMDA receptor-mediated excitatory postsynaptic currents in the brain (Lemarchant et al., 2022). This was accompanied by restoration of spatial working memory, measured by spontaneous alternations on the T-maze. In the same mouse model, NX210c treatment for 3 days (5 mg/kg/day, i.p.) increased protein levels of the glutamate GluN2A-NMDA receptor by two-fold and fully restored its downstream signaling mediator, p-CREB, while also restoring spatial working memory.

In rat cortical and hippocampal neurons exposed to glutamate (excitotoxic), simultaneous treatment with NX210c promoted survival of the neurons and prevented neurite network retraction (Deletage et al., 2021). NX210 treatment promoted neuronal survival in cortical (but not hippocampal) neurons and protected neurite networks in hippocampal (but not cortical) neurons. Overall, NX210c showed more consistent neuroprotective effects than NX210. These neuroprotective effects appeared to occur via integrin receptors and γ-secretase substrates, subsequent activation of the PI3K/Akt/mTOR pro-survival pathway, and disruption of the apoptotic cascade (by decreasing apoptotic mediators AIF1, cytochrome c, and active caspases 3 and 7 and promoting anti-apoptotic Bcl2-mediated mechanisms). Integrins and γ-secretase substrates are putative receptors or mediators of the neuroprotective actions of NX210/210c.

NX210c also showed neuroprotective benefits in human fetal primary cortical neuron cultures exposed to glutamate (Deletage et al., 2021). NX210c treatment promoted neuronal survival and neurite growth, while decreasing release of lactate dehydrogenase, a marker of neuronal damage/death.

Although NX210c was not tested, in two rat models of spinal cord injury (dorsal funiculi aspiration and spinal cord contusion), treatment with NX210 (the linear form; 100 µg/kg) stimulated axonal regrowth and improved functional recovery (Sakka et al., 2014). An *in vitro* study in B104 neuroblastoma cell culture showed that NX210 treatment prevented oxidative damage and cell death induced by hydrogen peroxide.

APOE4 interactions: Unknown.

Aging and related health concerns: No studies have tested NX210c for age-related diseases other than in models of neurodegeneration.

Types of evidence:

- None

No studies have evaluated the effects of NX210c for prevention or treatment of age-related conditions beyond neurodegenerative conditions discussed above.

Safety: Intervention with NX210c has not been studied in humans. In a phase I study, NX210, which cyclizes to NX210c, was safe and well-tolerated with mild adverse events including dizziness, headache, and somnolence.

Types of evidence:

- A phase I study of NX210
- A few laboratory studies

No studies have tested NX210c directly in humans.

In a phase I double-blind randomized controlled single-ascending dose study of 39 healthy volunteers, an intravenous dose of NX210 (0.4, 1.25, 3.5, 5, or 10 mg/kg) was safe and well-tolerated (Bourdes et al.,

2022). A total of 17 treatment-emergent adverse events were recorded in 13 participants and all of them were of mild severity. Twelve adverse events (70.6%) were deemed drug-related (6 out of 12 events occurred with the 1.25 mg/kg dose); 7 of those (58.3%) involved the nervous system, such as dizziness (n=3), headache (n=1), and somnolence (n=2). Other treatment-emergent adverse events were gastrointestinal (n=1), cardiac disorders (n=1), eye disorders (n=1), renal disorders (n=1), and general disorders (n=1). All adverse events resolved spontaneously, except for one, which was worsening of leukocyturia, with unknown outcome. NX210 had a short half-life in plasma (6-20 minutes) and rapid clearance, while pharmacodynamic effects were sustained for several hours based on plasma, or for 48 hours based on urine and EEG findings (Bourdes et al., 2022). Based on *in vitro* studies, NX210 is not cardiotoxic, mutagenic, or cytotoxic (unpublished data discussed in Bourdes et al., 2022).

Drug interactions: Drug interactions have not been studied.

Sources and dosing: NX210c is under clinical development by Axoltis Pharma for the treatment of CNS damage. Dosage of NX210c in humans has not been established. In a phase I study of NX210, single intravenous doses of 0.4, 1.25, 3.5, 5, or 10 mg/kg were tested (Bourdes et al., 2022). In mouse models, NX210c doses ranging from 0.1 mg/kg to 30 mg/kg (i.p.) have been tested (Le Douce et al., 2021).

Research underway: There are currently no ongoing clinical trials testing NX210c, based on ClinicalTrials.gov. NX210c is under clinical development by Axoltis Pharma for the treatment of CNS damage.

Search terms:
Pubmed, Google: NX210c

Websites visited for NX210c:

- Clinicaltrials.gov (0)
- NIH RePORTER (0)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem
- DrugBank.ca (0)
- Cafepharma (0)

- Pharmapro.com (0)

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