Role and effect of amyloid proteins on NET formation in the CNS

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Ethical review	Approved WMO
Status	Pending
Health condition type	Autoimmune disorders
Study type	Observational invasive

Summary

ID

NL-OMON53562

Source
ToetsingOnline

Brief title
NETs

Condition

- Autoimmune disorders
- Central nervous system infections and inflammations

Synonym
Neurodegeneration, neuronal toxicity

Research involving
Human

Sponsors and support

Primary sponsor: Radboud Universiteit Nijmegen
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: Amyloid proteins, Immune system, NETs, Neutrophils

Outcome measures

Primary outcome

The data obtained from in vitro experiments will be the outcome of the study.

Secondary outcome

Not applicable.

Study description

Background summary

Neutrophils are actively recruited first responders to sites of infections and protect our bodies from infiltrating harmful pathogens. Upon stimulation, neutrophils undergo a specific cell death mechanism called NETosis, which is a critical innate immune response characterized by ROS production and the release of chromatin decorated with antimicrobial peptides, and serine proteases, accompanied by various pro-inflammatory and immunostimulatory molecules. While up to 60% of our circulating blood consists of neutrophils, they are virtually absent from the central nervous system in healthy individuals. It was recently found, however, that neutrophils can pass the otherwise protective blood-brain barrier and enter our brain under specific neuropathological conditions such as Alzheimer's disease (AD), Multiple Sclerosis (MS), amyotrophic lateral sclerosis (ALS), and Parkinson*s disease (PD).

In line with the presence of neutrophils in the diseased brain, it is becoming increasingly more evident that bacterial infections may contribute to the developmental onset of several neurological pathologies. For example, chronic periodontal infection was linked as a possible cause of Alzheimer*s disease, and Porphyromonas gingivalis was recently identified as a keystone pathogen. Here, a positive correlation was found between bacterial infection and the formation of Aβ plaques, a characteristic feature of AD and recently assigned to have antimicrobial properties. In line with their role in innate immunity, neutrophils were also found in the surroundings of Aβ deposits and the reduced cortical blood flow commonly observed in AD patients was assigned to neutrophil adhesion in brain capillaries. On a similar note, neutrophils and neutrophil extracellular traps (NETs) were also found in the cerebrospinal fluid of patients with other neurodegenerative diseases such as MS.

Besides the currently accepted mechanism of microbial-induced neutrophil activation and NET formation, it was recently shown that amyloid fibrils themselves from three different protein sources (e.g. α-synuclein, Sup35, and transthyretin) also induced NET formation in vitro. The accompanying release of NET-associated proteases further degraded the amyloid fibrils into shorter peptide products that showed increased toxicity on liver and kidney cells. Because of the cell-damaging processes involved, it is tempting to speculate that amyloid-induced NETosis contribute to neurotoxicity. The in vivo pathogenic relevance of the presence of neutrophils in brain tissue and the effect of the accompanying NETs on neuronal cells and disease progression, however, has not yet been systematically investigated.

Study objective

In this research proposal, we aim to develop chemical reporter molecules to study neutrophil activity and NET formation in real-time. We will systematically study the effect of brain-associated amyloid fibrils on NET formation and investigate the effect of the accompanying secretion molecules on neuronal activation and synapse growth in vitro and in vivo. The specific objectives are as follows:

1. Development of molecular reporters and activity-based probes to study and identify NETs in vitro and in vivo.
2. Evaluate neutrophil infiltration and activation in neurodegenerative disorders using immunostaining and activity-based probes.
3. Evaluate the effect of amyloids and aggregates on NET formation on isolated neutrophils in vitro.
4. Evaluate the effect and toxicity of NETs on neuron cells.

Study design

In order to study neutrophils and NET formation in vitro and in vivo, we will develop activity-based probes (ABP) for a set of proteins that are specifically secreted by neutrophils during NETosis namely Neutrophil Elastase (NE), Cathepsin G (CatG), Peptidyl Arginine Deiminase (PAD) and Myeloperoxidase (MPO). The prepared probes will first be evaluated for their suitability to detect enzyme activity during NETosis on neutrophils.

The process of NETosis can be initiated by several triggers and proceed via a NADPH oxidase-dependent as well as an NADPH oxidase-independent pathway. Both pathways result in NETs with unique molecular compositions and it is therefore interesting to investigate the effect of amyloid-induced NETosis in more detail. Here, we aim to examine whether amyloid proteins of different structure and size activates neutrophils and induces NETosis. We will generate different conformations of amyloid ranging from monomers to oligomers and fibrils using established protocols, and incubate them with neutrophils in vitro. Activation

will be quantified using the developed activity-based probes and by NETosis.

Neutrophils are very short-lived (roughly 1 day) and thus, will need to be isolated from fresh blood samples. Blood samples (10-40 mL) will be collected via venapuction at the RadboudUMC in EDTA anti-coagulation tubes after signed informed consent. The exact amount depends on the nature of the planned experiment and the number of neutrophils that is needed to perform it. Donors will not be followed over time or subjected to other procedures than donating blood. This research will not yield findings that may be stressful or clinically relevant to the donors, since the only clinical parameter that will be checked are cell counts.

Neutrophils will be isolated from the donated blood according to standard operating procedures (SOP) and subsequent in vitro experiments will be performed on the day of donation. After the experiments are finished, all materials will be discarded; biomaterials involved in this study will not be stored.

Study burden and risks

Risk and burdens: risk is negligible and the burden is minimal. The most common risks related to blood donation from the subject's arm are brief pain and/or bruising. Infection, excess bleeding, clotting, or fainting are also possible but unlikely.

Benefits: there will be no direct benefit for the donor other than a 15¤ Bol.com voucher. However, participation of subject will help the researchers to gain knowledge.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)

Inclusion criteria

Healthy volunteers between 18 and 64 years old. Specific characteristics such as sex and ethnic background are irrelevant.

Exclusion criteria

Subjects who are not healthy, not feeling well, older than 64 or younger than 18.

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Pending
Start date (anticipated):	01-06-2023
Enrollment:	40

Type:	Anticipated

Ethics review

Approved WMO Date:	17-07-2023
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL82453.091.23