Glycocalyx assessment in temporal lobe epilepsy

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Primary objective of this study: To establish glycocalyx properties of temporal lobe epilepsy (TLE) patients. Secondary objectives of this study:- To establish differences in glycocalyx properties of glycocalyx between TLE patients and controls.- To...

Ethical review	Approved WMO
Status	Completed
Health condition type	Structural brain disorders
Study type	Observational non invasive

Summary

ID

NL-OMON47782

Source
ToetsingOnline

Brief title
Glycocalyx in epilepsy

Condition

- Structural brain disorders

Synonym
temporal lobe epilepsy

Research involving
Human

Sponsors and support

Primary sponsor: Medisch Universitair Ziekenhuis Maastricht
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: Epilepsy, Glyocalyx, Temporal lobe epilepsy

Outcome measures

Primary outcome

Primary outcome is cortical glycocalyxthickness.

Glycocalyxthickness is expressed in the two dimensions, PBR and Dperf (both continuous variables in expressed micrometers).

PBR and Dperf are presented as mean (±standard deviation) if normally distributed, and as median (range) if non-normally distributed. Differences between the two groups will be calculated using t-test or Mann-Whitney U-test. Missing data will be replaced by a mean or median value. p-value <0.05 is considered statistically significant.

Secondary outcome

Sublingual, cortical and hippocampal comparison

Sublingual and cortical glycocalyx dimensions in TLE and control patients will be compared between groups using the two-tailed student*s T-test. Also, differences between sublingual, cortical (and hippocampal in TLE patients) intra-individually will be compared using the two-tailed student*s T-test.

Epilepsy type and frequency, age of epilepsy onset (years of epilepsy)

Epilepsy-specific factors such as type of seizures and seizure frequency of the included TLE patients will be correlated to glycocalyx dimensions.

Statistics used: uni- and multivariate regression analysis.

Hippocampal sclerosis

In TLE patients the differences in glycocalyx dimensions will be compared between patients with hippocampal sclerosis and no hippocampal sclerosis using the two-tailed student*s T-test. In the hippocampal sclerosis group we will try to correlate the glycocalyx dimensions to the degree of hippocampal sclerosis (using Wyler and Blümcke classicification) using uni- and multivariate regression analysis.

History of FS or TBI

Differences in glycocalyx dimensions will be compared between TLE patients with and without a history FS or TBI using the two-tailed student*s T-test.

Viability of microvascular structures in an in-vitro setting

Small arteries will be isolated from resected cortical tissue and positioned in an arteriograph. Next, using vasoconstrictive and vasodilatative agents viability will be examined. This will be illustrated in dose-response curves.

Examination of the neurovascular unit in an in-vitro setting

Small arteries will be isolated from resected cortical tissue and positioned in an arteriograph. If viable, arteries will be exposed to electrical field stimulation for examination of the neurovascular unit. This will be illustrated using a voltage-response curve.

Examination of the glycocalyx in an in-vitro setting

Small arteries will be isolated from resected cortical tissue and positioned in an arteriograph. In-vitro measurement of the glycocalyx in small cortical arteries by intraluminal dextran-proteins might be possible using 2-photon microscopy. If possible, the results will be correlated to the results of the in vivo measurements using chi²-test.

Study description

Background summary

Epilepsy is the most common neurological problem after stroke. Most patients can be treated with antiepileptic drugs, but 30% is pharmacoresistant. Since so many patients are affected, this is a major problem, both medically and socioeconomically.

The most frequent type of epilepsy is temporal lobe epilepsy (TLE). Despite more than 50 years of extensive research, the pathophysiology of epilepsy has not been elucidated yet. Over the years, we have learned several things, e.g. that febrile seizures in childhood and traumatic brain injury (TBI) are the most important risk factors for developing temporal lobe epilepsy, that the sclerotic hippocampus (present in 60% of TLE patients) is site for mossy fiber sprouting and granular cell dispersion, resulting in disturbed neuronal networks in which deep nuclei such as the thalamus play a role, and that after a seizure, the blood brain barrier opens and inflammation occurs. However, we still do not know if and how inflammation leads to epilepsy, nor why some develop TLE after having suffered from FS or TBI earlier in life, while others do not. We hypothesize that the cerebral microcirculation, and in particular the glycocalyx, may play a role.

The glycocalyx is a thin gellayer lining the endothelium on its luminal side. It consists of a skeleton that is bound to the endothelium and is made up of proteoglycans and glycoproteins, and of several soluble molecules from the endothelium and plasma, such as free proteoglycans, antithrombin III and cytokines. This endothelial glycocalyx appears to play a central role in vascular homeostasis, and protects the endothelium from circulating blood. In the brain, it is the first barrier between the blood and the brain, and can be considered part of the BBB. The BBB has been shown to be affected in some way in epilepsy, but in what way exactly is unclear. No data on glycocalyx in epilepsy are available. Experimental studies have shown that loss of glycocalyx increases vascular permeability, and that inflammation can disrupt the

glycocalyx. Furthermore, experimental TBI affects the glycocalyx.

No studies on glycocalyx in (temporal lobe) epilepsy patients have been published so far. Maastricht offers the unique combination of an enormous experience with glycocalyx measurements and the opportunity to perform these measurements in brain tissue in an in vivo situation.

Study objective

Primary objective of this study: To establish glycocalyx properties of temporal lobe epilepsy (TLE) patients.

Secondary objectives of this study:
- To establish differences in glycocalyx properties of glycocalyx between TLE patients and controls.
- To establish the correlation between sublingual glycocalyx measurements and cortical glycocalyx measurements in TLE patients and controls.
- To establish differences in glycocalyx properties between TLE patients with hippocampal sclerosis and those without hippocampal sclerosis.
- To establish differences in glycocalyx between TLE patients with a history of febrile seizures and/or traumatic brain injury and those without a history of febrile seizures and/or traumatic brain injury.
- To establish the correlation of glycocalyx properties in TLE patients to epilepsy-specific factors such as type of seizures and seizure frequency.
- To establish the examination of viability of small cortical arteries in an in-vitro setting using an arteriography.
- To establish the examination of the neurovascular unit of small cortical arteries in an in-vitro setting using an arteriograph and electrical field stimulation.
- To establish the examination of the glycocalyx of small cortical arteries in an in-vitro setting using an arteriograph and 2-photon microscopy and to correlate this to the in-vivo results.

Study design

In our department, we treat 30-40 patients per year for medically refractory localisation-related epilepsy by resective brain surgery. These patients constitute a group of epilepsy patients that suffer from localisation-related epilepsy, and in whom it is expected that they will be seizure free or have a major seizure reduction after resection of the seizure focus in the brain. The prior extensive diagnostic examinations and possible surgery indication is appointed by the Academisch Centrum voor Epilepsie.
In most cases, the seizure focus is located in the mesiotemporal region, in casu amygdala and hippocampus. The hippocampus is located on the mesial side of the temporal lobe. After performing a temporal lobe resection plus resection of the (sclerotic) hippocampus and amygdala, around 70% of the patients will be

rendered seizure-free. During surgery, the (anterior part) temporal lobe is fully exposed. This gives us the unique opportunity to use the SDF camera directly on the cortical and hippocampal surface, in order to directly measure glycocalyx in cortical and hippocampal vessels, respectively. This may increase surgery time (normally three to four hours) with maximally five minutes.
The control population consists of patients that require a (small) craniotomy or burr hole surgery for tumor resection or biopsy, and patiens between 18 and 60 years that require a craniotomy because of neurovascular surgery, like arteriovenous malformations of aneurysms. In these cases it is also possible to use the same probe on the cortical surface prior to performing the corticotomy.

The camera has been used successfully on human tissue in vivo before, mainly on kidney and sublingual.

Method
The study is an observational case-control study. In order to include sufficient patients, the study will take four years.

Measurements
The glycocalyx measurement will take place by means of a small video-microscoop (SDF camera, MicroVision Medical, Amsterdam, CE-certified, Maastricht University equipment registration number H.08IHVI06265). Using GlycoCheck ICU analysing software, red blood cell (RBC) column width will be measured automatically in approximately 3000 vessel segments with a diameter of 5-30 micrometer. Subsequently, the perfused boundary region (PBR) will be calculated for all 3000 vessel segments.

In all patients (TLE and control) a standard sublingual glycocalyx measurement will be performed as measurement 1:
Measurement 1: Directly following anesthesia induction the measurement will take place in the operation room. The patient is not aware of this measurement.

Continuation of method in TLE patients:
Measurement 2: When the cortex is fully exposed, measurement two can take place. Location: operation room.
Measurement 3: Usually the hippocampus will be exposed thirty minutes following cortical resection. When fully exposed, the hippcampal measurement is performed. Location: operation room.

Continuation of method in control patients:
Measurement 2: When the cortex is fully exposed, measurement two can take place . Location: operation room.

All glycocalyx measurement procedures that are performed will lead to a delay of surgery time of maximally 10 minutes. Following the final measurement (measurement 3 in TLE patients and measurement 2 in control patients) the patient has reached the end point of this study.

A significant volume of brain tissue is resected during TLE surgery. Part of the cortex and hippocampus that were used for glycocalyx measurements will be transferred to the microcirculation lab where other (in vitro) experiments on vascular permeability and glycocalyx quality will be performed. Other parts of the cortex, and part of the hippocampus, are stored in our biobank, while the rest of the hippocampus and cortex are sent to the department of pathology, for standard histopathological analysis (e.g. to determine the degree of hippocampal sclerosis). Using this minimally invasive device with minimal time investment on epilepsy patients and others undergoing brain surgery, gives us the unique opportunity to gather direct information on brain microcirculatory function.

Additional information on the in vitro experiments
Correlation to in vitro experiments: epilepsy patients
During a standard temporal lobectomy including amygdalohippocampectomie resected cortical, amygdala and hippocampus are partly resected en bloc. Once resected this tissue is divided in several pieces for clinical and research matters. The tissue is considered as remnant tissue.

For regular clinical purposes one part of the cortical tissue, one part of the hippocampus and the whole of the amygdala (too small to be further divided) is forwarded to the department of pathology. Important pathological parameters like focal cortical dysplasia and (degree of) hippocampal sclerosis are determined by the pathologist. Since these parameters are important for especially research considerations, they will be included in the compiled clinical data and are included as secondary study parameters/end points.

Another part of the cortical and hippocampal tissue is freshly frozen on dry ice. This part will be stored in our bio-bank (-80C refrigerator storage). The tissue in our bio-bank might be used for future study ideas, by example for genetic analysis. When studies on this tissue will be elaborated, additional METC approval will be requested.

A third part of the cortical and hippocampal tissue is fixated using Formaldehyde. After two days of fixation the tissue is embedded in Paraffine. This tissue is stored in the histological biobank.
The histological biobank for this tissue is located at the MHeNS lab. All tissue is stored in a locked closet located on the locked room of GH. Only OS, GH and RH have a key for opening this closet. When the tissue for neuropathological review was not conclusive or too little to review, the histological biobank tissue can be used for re-review. Tissue stored in the histological biobank can be used for future (histological) experiments. When studies on this tissue will be elaborated, additional METC approval will be requested.

A fourth part of the cortical tissue (hippocampal vascular structures are currently too small for handling) of the tissue is stored in a HEPES-buffer

which is important for conservation of vascular features. Direct vascular dynamics related experiments can then be performed on this tissue. This tissue is only stored for a maximum of 48 hours. After this time period vascular dynamical experiments can not be considered viable.
Interesting microvascular features that we want to include is neurovascular unit viability of vascular segments in the resected cortical tissue. Using microdissection arterial segments are isolated. Next, an arterial segment is mounted in an organ chamber between two glass cannulas and exposed to a continuous distending pressure of 70mmHg. Strong vasoconstrictive agents will then be applied, followed by strong vasodilative agents. In this way, viability of vascular wall dynamics is being observed. Once viability is confirmed, several receptor agonists and antagonists will be applied to further determine the necessary vasoconstrictive and vasodilatative agents/cascade. Also, vascular permeability can be determined more thoroughly by comparing responses to intraluminal and extraluminal application of vasoconstrictive agents.

If viable, supplementary extraluminal electrical field stimulation can be applied. Vasoconstriction as a function of the neurovascular unit can be mimicked in this way. Again several receptor agonists and antagonists will be applied additionally to further determine the necessary vasoconstrictive and vasodilatative agents/cascade. Since glycocalyx properties are very easily disrupted, viability of the included vessels is of importance for further glycocalyx analysis.

Glycocalyx an

Study burden and risks

Sublingual glycocalyx measurement is a non-invasive, short measurement using a SDF camera. All measurements are performed when the patient is under general anesthesia, which makes the burden low while the risk is minimal. The second and third glycocalyx measurement is performed on cerebral vessel segments during surgery. This is a non-invasive technique, comparable with intraoperative cerebral ultrasound, carrying minimal risk and zero burden for the patient.

Possible risk includes local contusion of the cortex or hippocampus. This has no consequences for the patient since the tissue possibly damaged will be resected anyway, with the exception of the neurovascular indications in which no tissue is resected regularly.

Surgery time will be extended by a maximum of 10 minutes as a consequence of the measurements, a time-investment we deem justified when taking into account the huge amount of information we are provided with.

Given the burden of disease for epilepsy patients, that is the consequence of our lack of pathophysiological knowledge, and given the fact that our proposed

study, which is non-invasive and extremely low risk/low burden for the patient, will give us unique information on the pathophysiology of epilepsy, we think it is justified to conduct the study

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Epilepsy patients
- adults between 18 and 60 years
- patients diagnosed with pharmacoresistant epilepsy, temporal lobe epilepsy, focus in non-eloquent area. , Control patients
- adults between 18 and 60 years
- patients undergoing an elective craniotomy for non-eloquent intracranial tumor resection or neurovascular indications, like aneurysm clipping and

arteriovenous malformation resection, or undergoing a burr hole for tumor biopsy.

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study: child (<18y) or elderly (>60y), pregnancy, diabetes mellitus, familiar (combined) hyperlipidemia, history of stroke or other cardiovascular diseases, use of cardiovascular medication, silent signs of cerebral small vessel disease on brain MRI.
Moreover, control patients in which no *normal*, *non-compressed* and/or non-edematous* cerebral cortex can be assessed during surgery or in whom a history of seizures is reported, will be excluded.

Study design

Design

Study type:	Observational non invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Completed
Start date (anticipated):	14-07-2016
Enrollment:	30
Type:	Actual

Ethics review

Approved WMO

Date:	20-05-2015

Application type:	First submission
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	22-06-2016
Application type:	Amendment
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

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

ID: 26910
Source: Nationaal Trial Register
Title:

In other registers

Register	ID
CCMO	NL51594.068.14
OMON	NL-OMON26910