Complement activation after gut ischemia-reperfusion injury (GI I/R) in man

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To examine cellular damage, intestinal barrier function loss, activation of the Complement system and inflammatory alterations induced by ischemia and reperfusion of the human small and large intestine.Second, we aim to study the consequences of...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Gastrointestinal inflammatory conditions
Study type	Observational invasive

Summary

ID

NL-OMON38154

Source
ToetsingOnline

Brief title
Complement activation after GI I/R

Condition

- Gastrointestinal inflammatory conditions

Synonym
Gut ischemia/reperfusion; 'gut infarction'

Research involving
Human

Sponsors and support

Primary sponsor: Academisch Medisch Centrum
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: Complement, Gut, Human, Ischemia/reperfusion

Outcome measures

Primary outcome

Firstly, we will compare cellular damage (I-FABP, ILBP, ZO-1) in the tissue, which was subjected to ischemia-reperfusion with the tissue, which was not subjected to ischemia-reperfusion.

Secondly, we will compare complement activation (eg MBL, TCC) in the tissue, which was subjected to ischemia-reperfusion with the tissue, which was not subjected to ischemia-reperfusion.

Thirdly, we will compare inflammation (eg Calprotectin, MPO, NGAL) in the tissue, which was subjected to ischemia-reperfusion with the tissue, which was not subjected to ischemia-reperfusion.

Fourth, we will study the consequences of intestinal I/R on intestinal barrier function loss, by comparing the translocation of sugars from the gut lumen to the circulation, before ischemia, after ischemia and after 30 and 120 minutes reperfusion.

Fifth, we will study macroscopic mucosal changes during intestinal IR using a endo-videocapsule. Data will be analyzed in collaboration with the department of Gastroenterology.

At last we want to study the gene expression patterns in tissue samples of IR exposed intestine, in order to identify possible targets for preventive and therapeutic interventions.

Secondary outcome

Not applicable.

Study description

Background summary

Gastro-intestinal ischemia-reperfusion (GI I/R) injury occurs when the gut is temporarily deprived of blood supply and where restoration of the blood supply triggers an intense inflammatory response. GI I/R remains a common major clinical problem (partly as a result of the lack of diagnostic tools and poor treatment options) with a reported mortality of 60-80% for patients with acute mesenterial thrombosis. Furthermore, small and large intestinal ischemia is considered a crucial factor associated with complications after abdominal and (cardio-) vascular surgery, trauma and sepsis. It is seen as a major contributor to the development of post-operative complications and multiple organ failure.
Recent animal studies using complement-knockout mice showed an important role of complement activation in the onset of inflammation and tissue injury after GI I/R. To delineate the role of (three pathways of) complement activation in human GI I/R injury, we will study complement activation peroperatively after GI I/R.
Local tissue inflammation, as complement activation, is considered important to resolve I/R induced damage, but derailment of the inflammatory response can result in further complications.
Derailment of the inflammatory response can be triggered by the translocation of highly pro-inflammatory intestinal intraluminal components, like endotoxin, as a result of a compromised intestinal barrier function. 20 Therefore, the next goal is to study the effect of intestinal ischemia and reperfusion on the intestinal barrier function.

Apart from increasing knowledge on the molecular mechanisms involved in the pathophysiology of intestinal IR, it is also of importance to get insight into the macroscopic mucosal changes during intestinal IR. In this way, we can improve early recognition of intestinal ischemic lesions using endoscopy.

Study objective

To examine cellular damage, intestinal barrier function loss, activation of the Complement system and inflammatory alterations induced by ischemia and reperfusion of the human small and large intestine.
Second, we aim to study the consequences of human intestinal ischemia reperfusion induced barrier function loss, i.e. the translocation of intestinal

intraluminal content into the circulation.
Third, we aim to study macroscopic mucosal changes during intestinal IR.
At last, we aim to study the consequences of human intestinal ischemia reperfusion on gene expression patterns for identification of targets for preventive and therapeutic strategies.

Study design

Small Intestinal I/R
In this study the effects of ischemia followed by reperfusion (I/R) of the human small intestine will be investigated. To this end we will collect blood and tissue samples during major upper abdominal surgery or major colorectal surgery (model for large intestinal I/R). In some surgical procedures the duodenum and duodenal-jejunal transition are resected and continuity of the gastro-intestinal tract is restored by a gastro-jejunal or entero-enteric anastomosis.
For research purposes a 3-6 centimeter small and isolated part of the jejunum will be submitted to I/R during surgery. Because of the limited size of the jejunal segment subjected to I/R, we suspect that the I/R induced changes have no systemic implications, but will enable us to investigate the important processes following GI I/R. The supplying small arterial branches (aa. jejunales) and draining venous structures (vv. jejunales) of the specific jejunal element are identified in the mesentery and subsequently clamped to halt circulation. Following 15, 30, 45 or 60 minutes of ischemia the clamp, occluding the vascular branches, is removed and the jejunum is inspected for reperfusion. Reperfusion is continued for a subsequent two hours, after which the tissue is removed along with the total resection specimen. The previously identified venous mesenterial branches enable us to specifically draw blood from the outflow of the isolated tissue segment. To analyze segment inflow arterial samples can be taken from the arterial line, introduced for surgery.

To study the consequences on intestinal barrier function loss during ischemia and reperfusion the differential sugar test is used. This test is in common use in every day clinical practice and consists of the oral administration of two sugars, lactulose and l-rhamnose, and their consequent measurement in the plasma. This method is based on the fact that lactulose, a disaccharide, is too large to cross a healthy intestinal barrier, but in case of barrier function loss, lactulose is able to cross the barrier to the circulation. High plasma levels of lactulose will therefore reflect increased intestinal permeability. L-rhamnose is a monosaccharide which can cross the intestinal barrier freely, and is added to the test to serve as an internal control for confounders as gastric dilution and intestinal perfusion. Using high performance liquid chromatography combined with mass spectrometry, both saccharides can be detected in plasma.

To study macroscopic changes during small intestinal IR, a endo-videocapsule will be inserted in the isolated gut-segment in a subgroup of 6 patients. This

small camera (size of a pill), will be inserted through an incision proximal of the isolated segement. Directly after insertion, the part of intestine with the incision-cut will be removed, to prevent leakage of intraluminal content through the incision. To study IR-induced changes, the isolated gut-segment will be exposed to 60 minutes of ischemia with 120 minutes of reperfusion, which is in line with current IR-protocols.

The blood samples will be analyzed at the general surgery laboratories for markers of cellular damage ILBP, I-FABP and L-FABP, inflammatory markers like TNF-α, IL-6 and IL-10, neutrophil activation products (Calprotectin, MPO and NGAL) and complement proteins like MBL and the terminal complement complex (TCC), as well as for markers for intestinal permeability (lactulose and L-rhamnose).
The tissue samples will be analyzed with various standard laboratory procedures like western blot and immunohistochemistry, qPCR, electron microscopy, gene expression analysis and other relevant techniques.

Another 3 cm of tissue is harvested from the already resected specimen and will function as an internal healthy control. Essential in this procedure is the fact that no extra tissue will be taken from the patient and all studies can be performed on tissue already resected during the elective surgical procedure.

Large Intestinal I/R
Next to small intestinal ischemia reperfusion (IR), also large intestinal IR will be studied. To this end we will collect blood and tissue samples during major colorectal surgery, (i.e. Low Anterior resection or Hartmann*s procedures). In this surgical procedure a part of the healthy colon is removed for surgical/medical reasons. For research purposes a 3-6 centimeter isolated colon segment will be submitted to IR during surgery in a similar manner as already was performed in patients undergoing pancreaticoduodenectomies (for studying small intestinal IR). This model is harmless for the patient, since we take advantage of the fact that a part of healthy colon is resected for surgical reasons. No extra tissue will be resected for research purposes. Conform the study of the small intestine, the consequences of different ischemic periods (15, 30, 45 and 60 minutes ischemia) and extent of the ischemia (hypoperfusion) will be studied in the same manner as described for small intestinal IR. Following ischemia, the clamp, occluding the vascular branches, is removed and the colonic segment is inspected for reperfusion. Reperfusion is continued for a subsequent two hours, after which the tissue is removed along with the total resection specimen. Blood samples will be collected at four different time points during surgery. The previously identified venous mesenterial branches enable us to specifically draw blood from the outflow of the isolated tissue segment. To analyze segment inflow arterial samples can be taken from the arterial line, introduced for surgery.

Study burden and risks

The patients enrolled in this study will all undergo major abdominal surgery with duration of at least 2 hours. Because I/R is applied on intestinal tissue which will be resected anyway during the surgical procedure, this will not interfere with standard surgical care. The only actions undertaken by the surgeon solely related to this research proposal, is the isolation of 3-6 cm of gut and apply 15, 30, 45 or 60 minutes ischemia to it prior to its removal along with the rest of the gut which is to be resected for medical reasons.
During the operation, two sugars, lactulose and l-rhamnose, will be injected into the intestine using a syringe with needle. The sugars will be administered just after the part of intestine is isolated for the study protocol (see above).Directly after injection, the part of isolated intestine with the injection site will be removed using a linear cutting stapler to prevent leakage of intraluminal content through the injection site (see also the endo-videocapsule protocol). All materials are sterile. Lactulose and L-rhamnose are both harmless saccharides which have been used since the 70s to investigate intestinal permeability. Both saccharides have been extensively used and can be administered without risk of adverse effects. These saccharides are registered for oral use. In addition, it has to be taken into account that these saccharides will only be introduced into the part of intestine, that will be removed for surgical reasons anyway. Our research and clinical department have experience with peri-operative administration of test substances into the gut (see also MEC 03-032.5).
To study macroscopic changes during small intestinal IR, a endo-videocapsule will be inserted in the isolated gut-segment in a subgroup of 6 patients. This small camera (size of a pill), will be inserted through an incision proximal of the isolated segement. Directly after insertion, the part of intestine with the incision-cut will be removed, to prevent leakage of intraluminal content through the incision. No extra part of intestine will be removed for this procedure. The videocapsule is sterile.
During the operation the surgeon will sample blood from the vein of the isolated gut segment. These actions will not add substantially to the total duration of the operation. The additional risks for the patients are minimal and will not increase the total risk of the operation. The arterial line used for blood sampling is part of standard anesthetic care.

The technical protocol for large intestinal ischemia-reperfusion is the same as the protocol for small intestinal ischemia reperfusion. There*s no interference with the standard surgical procedure, and there are no additional risks. The only actions undertaken by the surgeon solely related to this research proposal, is the isolation of 3-6 cm of colon and apply ischemia to it prior to its removal along with the rest of the gut which has to be resected for surgical reasons.

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

Adult patients
Major upper abdominal surgery
Major colorectal surgery
right hemicolectomy

Exclusion criteria

We will not include patients known with Inflammatory Bowel Disease (IBD), because of pre-existing effects of the disease on the inflammatory status of the gut. Acute major abdominal procedures will not be included, because of the possible pre-existing effects of trauma or hemorrhage on the gut. We will exclude those patients who have not given informed consent.

Study design

Design

Study type: Observational invasive

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

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	01-11-2006
Enrollment:	139
Type:	Actual

Ethics review

Approved WMO Date:	26-06-2006
Application type:	First submission
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	02-07-2007
Application type:	Amendment
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	14-05-2008
Application type:	Amendment
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	11-08-2008
Application type:	Amendment

Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	08-04-2009
Application type:	Amendment
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	14-04-2010
Application type:	Amendment
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	08-10-2012
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

No registrations found.

In other registers

Register	ID
CCMO	NL12407.068.06