Human intestinal ischemia and reperfusion

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This study aims at (further) revealing the pathophysiology of intestinal IR in man, with a specific interest for the role of proteases and protease-activated receptor-2 (PAR-2), cellular and inflammatory changes, barrier function and intestinal...

Ethical review	Approved WMO
Status	Completed
Health condition type	Gastrointestinal vascular conditions
Study type	Interventional

Summary

ID

NL-OMON43970

Source
ToetsingOnline

Brief title
Human intestinal IR

Condition

- Gastrointestinal vascular conditions
- Vascular disorders NEC

Synonym
Intestinal ischemia, oxigen deficiency of the bowel

Research involving
Human

Sponsors and support

Primary sponsor: Universiteit Maastricht
Source(s) of monetary or material Support: Career Development Grant van MLDS,Maag Lever Darm Stichting

Intervention

Keyword: Doxycycline, Intestinal ischemia reperfusion, Protease inhibition, Tranexamic acid

Outcome measures

Primary outcome

The primary endpoint in this study is inflammation (neutrophil influx, complement activation, interleukins, TNF-α, COX 1-2) and protease activity in tissue as well as in blood plasma. An important element of our study is the alteration in these variables after administration of a protease-inhibitor and/or MMP-inhibitor compared to a placebo.

Secondary outcome

The secondary study parameter is intestinal cell damage, which will be evaluated by assessment of plasma levels of I-FABP, ILBP as well as tissue stainings for morphology, tight junctions, apoptosis, goblet cells, mucines, cell proliferation, I-FABP, L-FABP and SM22. An important element of our study is the alteration in these variables after administration of a protease-inhibitor and/or MMP-inhibitor compared to a placebo.

Study description

Background summary

The intestinal mucosa is responsible for absorption of nutrients from the lumen and for separation of luminal content (external environment) from the host (internal environment). Disruption of this delicate balance at the mucosal-luminal interface occurs during intestinal ischemia-reperfusion (IR), a frequently observed phenomenon of normal physiology. Furthermore, intestinal IR is considered as common pathway in several pathologies, where temporary flow reduction results from vascular disease (thrombosis, embolism), surgical states (aortic surgery, cardiopulmonary bypass) and other pathologies, including

necrotizing enterocolitis, pancreatitis and shock. Moreover, splanchnic hypoperfusion is seen as a phenomenon involved in the etiology or perpetuation of inflammatory bowel disease (IBD). Intestinal IR results in local tissue damage with barrier function loss facilitating bacterial translocation, triggering systemic inflammation and multiple organ failure (MOF). Despite high morbidity and mortality rates associated with intestinal IR, no effective preventive or therapeutic strategies exist.
In search for new interventional targets, it is crucial to comprehend the underlying mechanisms associated with human intestinal IR.
For a long time only animal studies were reported on intestinal IR. This is probably one of the main reasons that no effective clinical treatment for intestinal IR was developed and the associated high morbidity and mortality rates are unchanged the last 70 years. Therefore, a new human model enabling the study of sequelae of jejunal IR was developed by our workgroup. This allowed a better understanding of several physical (mucus-layer, epithelial lining) and immunological (Paneth cells) barriers, preventing invasion of foreign or endogenous threats from intestinal lumen to sterile interior environment after 30 minutes ischemia. Prolonged (>45 minutes) ischemia followed by reperfusion leads to irreversible damage and increasingly evident endoplasmatic reticulum stress in Paneth cells, accompanied by apoptosis, resulting in bacterial translocation and systemic inflammation. Furthermore, using a newly developed human experimental colon-IR model, colonic epithelium was shown more resistant to IR-induced damage than jejunum, although the high colonic microbiota density is potentially more toxic than small intestine intraluminal content. This may be explained by differences in mucus organisation. Another explanation is the detrimental activity of intestinal wall penetrating digestive enzymes present in the proximal small intestine. Recent rat studies undergoing intestinal IR indicate that pancreatic enzymes play a major role in acute inflammatory processes resulting from intestinal ischemia, hemorrhagic and endotoxic shock. Serine proteases (e.g. trypsin, chymotrypsin, elastase) are important pancreatic enzymes, which are stored in secretory membrane-bound vesicles as zymogens. The trypsin zymogen is activated by duodenal brushborder enterokinases, while activated trypsin activates more trypsinogen and chymotrypsinogen. To avoid uncontrolled proteases activity, enzymes-inhibitors are present in the circulation. Under normal physiological conditions, proteases are compartmentalized in the intestinal lumen by the mucosal barrier (mucus-layer and epithelial lining). The pathophysiological role of proteases was discovered by adding protease-inhibitors intraluminally in rats, showing abrogation of the usual sequelae of intestinal IR, inflammation and shock. These results gave rise to the *autodigestion* hypothesis suggesting that powerful proteases leak across the intestinal mucosal barrier, initiating self-digestion of the intestinal wall and leading to systemic inflammatory response (SIRS) with MOF. Protease activity in the intestinal wall was accompanied by mucus disruption, tight-junction loss and epithelial cell disruption after intestinal IR in animals. Recently, a report showed that intraluminally administered protease-inhibitors decreased shock and sepsis in a patient with Fournier*s gangrene.

Proteases, particularly serine proteases, also act as molecules that are able to send specific signals to cells involved in intestinal inflammatory responses through the activation of G protein-coupled protease-activated receptor-2 (PAR-2). Proteases cleave PAR at specific sites, unmasking a new N-terminal sequence, acting as tethered ligand, which binds to the receptor to initiate multiple signalling cascades. PAR-2 is present on epithelial and endothelial cells and various types of immune cells with high expression in the gastrointestinal and respiratory tracts. PAR-2 is activated by trypsin, mast cell tryptase and coagulation factors (VIIa, Xa) and modulates several gastrointestinal functions, including motility and secretion. Recent studies using PAR-2 knockout-mice and specific PAR-2 agonists showed PAR mediated neutrophil recruitment in mesenteric vessels, increased expression of endothelial adhesion molecules, delayed gastrointestinal transit, and increased histological damage. PAR-2 is reported to interact at signalling level with Toll Like Receptor-4, resulting in enhanced NF-kappa B-mediated inflammatory response, establishing a novel paradigm of receptor cooperativity. Furthermore, two distinct pools of PAR-2 are present in intestinal epithelial cells: an apical pool accessible from the lumen, activated by trypsin, and a basolateral pool accessible from the interstitium and blood, activated by mast cell tryptase or proteases released by recruited leukocytes. A recent study using cell lines and mouse ileum suggests that the outcome of PAR-2 activation is dependent on the specific receptor pool that is activated, because separate signalling pathways are triggered. Epithelial PAR-2 activation directly affects cytoskeleton contraction by triggering myosin light chain with subsequent tight-junction permeability changes, probably via the basolateral PAR-2. Rodent studies suggest an important role for PAR-2 in inflammation following intestinal IR. PAR-2 mRNA and protein expression in intestinal mucosa is upregulated after IR and inhibition of PAR-activating proteases is beneficial, diminishing post-ischemic intestinal inflammation, such as myeloperoxidase activity and chemokine and adhesion molecule expression. In line, IBD patients have increased levels of PAR-2 activating proteases in lumen and colonic tissue. A human PAR-2 antagonist is not available. However, soy extracts, characterized by presence of serine protease-inhibitors, have recently attracted attention because of their anti-inflammatory, PAR-2-mediated properties in IBD animal models.

Study objective

This study aims at (further) revealing the pathophysiology of intestinal IR in man, with a specific interest for the role of proteases and protease-activated receptor-2 (PAR-2), cellular and inflammatory changes, barrier function and intestinal permeability, microscopic mucosal changes, gene expression patterns and identification of targets for diagnostic, preventive and therapeutic strategies.

Study design

Interventional study

Intervention

During the procedure, a small intestinal segment will be isolated and selectively exposed to 30, 45 or 60 minutes of ischemia, followed by up till 120 minutes of reperfusion. In a subgroup a protease inhibitor and/or MMP inhibitor (or vehicle) will be administered intraluminally. At given time points, tissue and blood is collected for further analysis.

Study burden and risks

The patients enrolled in this study will all undergo major upper abdominal surgery. Because IR is applied to intestinal tissue which will be resected anyway during the surgical procedure, this will not interfere with standard surgical care. There are no specific benefits for the participating patients, however in the future the results of our study will likely be useful for patients suffering from intestinal IR. The additional risks for the patients in this study are marginal and they will not increase the total operation risk. We already performed similar studies in the human small intestine and these have shown no negative effects or increased risk for the participating patients compared to patients who undergo a PPPD (or whipple procedure) without participation in the IR study (Hundscheid et al; article in preparation). We refer to METC approval of our previous project *Complement activation after gut ischemia-reperfusion injury in man* (MEC 06-3-044).
However, administration of a protease and MMP inhibitor are new components in our ischemia-reperfusion studies. Nonetheless, these are substances that are already administered very regularly in humans for other indications and it is not to be expected that these substances cause any harmful effects.

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 (18 years of age and older) undergoing major upper abdominal surgery
o Whipple-procedure or pylorus preserving pancreatico duodenectomy (PPPD)
o Ileo-Jejunal bypass surgery
o Roux-en-Y gastric bypass
o Total gastrectomy
o Hepatico jejunostomy
o Pancreaticojejunostomy (Frey*s procedure)
*
Patients who have given an informed consent

Exclusion criteria

<18 years of age or older but no proper understanding of the research proposal
Inflammatory bowel disease
Celiac disease
Acute major abdominal procedures
Patients who have refused informed consent;For the population who will receive tranexamic acid additional exclusion criteria have been formulated: ;Active or history of thrombo-embolic disorders such as deep venous thrombosis, pulmonary embolism or cerebral embolism
History of blood coagulation disorder (hypercoagulation state)
Subarachnoid hemorrhage
Disseminated intravascular coagulation (DIS)
Severe renal insufficiency: i.e. serum kreatinine >150 µmol/L
History of convulsions
Pregnancy
Known hypersensitivity of allergy for tranexamic acid
Simultaneous use of thrombolytics (e.g. alteplase, streptokinase)

Simultaneous use of hormonal anticonceptives or other substances that induce hemostasis.
;For the population who will receive doxycycline additional exclusion criteria have been formulated: ;Known hypersensitivity of allergy for tetracyclines.
Severe liver function disorder i.e. ASAT or ALAT or AF or γ-GT >150 U/L whether or not combined with severe renal insufficiency: i.e. serum kreatinine >150 µmol/L.
Severe renal insufficiency: i.e. serum kreatinine >150 µmol/L.
Porphyria
Myasthenia gravis
Simultaneous usage (or just before or after administration of doxycycline) of oral retinoids or substances containing metalions (such as antagel or ironpreparations)
Simultaneous use of methoxyflurane (anesthetic) or oral contraceptives
Bodyweight beneath 50 kg
History of blood coagulation disorder (inert hypocoagulation state)
Pregnancy or lactating

Study design

Design

Study phase:	2
Study type:	Interventional
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Placebo
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Completed
Start date (anticipated):	26-10-2016
Enrollment:	69
Type:	Actual

Medical products/devices used

Product type:	Medicine
Brand name:	Cyklokapron

Generic name:	Tranexamic acid
Registration:	Yes - NL outside intended use
Product type:	Medicine
Brand name:	Vibramycin
Generic name:	Doxycycline
Registration:	Yes - NL outside intended use

Ethics review

Approved WMO Date:	13-01-2016
Application type:	First submission
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	06-06-2016
Application type:	First submission
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
EudraCT	EUCTR2014-002970-36-NL
CCMO	NL54508.068.15

Study results

Date completed: 01-01-2019

Summary results
Trial ended prematurely