GLP-2 acutely ameliorates LPS related increased intestinal permeability in rats

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The “leaky gut”
Bacterial Endotoxin (LPS)

- Bacterial endotoxin (lipopolysaccharide; LPS) is an essential component of the outer cell membrane of most Gram negative bacteria
- LPS is secreted into the surrounding medium at high concentrations
- LPS is present in the intestinal lumen
- Minute amounts of the LPS in the systemic circulation can produce fever, shock, and intestinal inflammation
- Chronically increased circulating LPS is associated with the metabolic syndrome, including fatty liver, hypertension, and hyperlipidemia
- LPS is detoxified by dephosphorylation
LPS

Metabolic endotoxemia

SIRS and MOF

• The systemic inflammatory response (SIRS) can lead to multiorgan failure (MOF) complicating severe inflammatory states such as pancreatitis.

• MOF is characterized by failure of the pulmonary, renal, cardiac, hepatic, circulatory, and coagulation systems.

• MOF is associated with a high mortality.

• MOF is associated with acute upregulation of inflammatory cytokines.

• Excess circulating LPS due to a leaky gut barrier has been implicated in the pathogenesis of MOF.

Permeability and LPS in pancreatitis

Permeability

LPS vs. permeability

LPS acutely increases permeability in mice

LPS 1 mg/kg

Guo, S. J immunol 2015
### Concentration of LPS in stool

#### Table 3. Distribution of Stool Endotoxin

<table>
<thead>
<tr>
<th>Endotoxin (EU/g)</th>
<th>Low*</th>
<th>5.1–22026</th>
<th>&gt;22026</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ln EU/g</td>
<td>Low*</td>
<td>1.62–10.0</td>
<td>&gt;10.0</td>
<td></td>
</tr>
<tr>
<td>NEC-negative</td>
<td>53 (35.1%)</td>
<td>27 (17.9%)</td>
<td>71 (47.0%)</td>
<td>151 (100%)</td>
</tr>
<tr>
<td>Stage I NEC</td>
<td>12 (26.1%)</td>
<td>6 (13.0%)</td>
<td>28 (60.9%)</td>
<td>46 (100%)</td>
</tr>
<tr>
<td>Stage II, III NEC</td>
<td>0 (0.0%)</td>
<td>4 (33.3%)</td>
<td>8 (66.7%)</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>65 (31.1%)</td>
<td>37 (17.7%)</td>
<td>107 (51.2%)</td>
<td>209 (100%)</td>
</tr>
</tbody>
</table>

1 EU/g = 10 ng/g  
22026 EU/g = > 2 mg/g

The LAL test

- Most accepted test for the measurement of LPS in biological fluids
- Is dependent on factor C derived from horseshoe crabs which is converted into an active protease in the presence of LPS
- LAL results are in EU (endotoxin units). Conversion to ng is:
  
  EU = 10 ng
The intestinal barrier

LPS > 2 mg/ml

LPS < 2 pg/ml

<table>
<thead>
<tr>
<th>Antigens Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actomyosin Proteins</td>
</tr>
<tr>
<td>Occludin/Zonulin Proteins</td>
</tr>
<tr>
<td>Lipopolysaccharides</td>
</tr>
<tr>
<td>Endotoxins from Gram-Negative Bacteria</td>
</tr>
</tbody>
</table>
Systemic and Metabolic endotoxemia

Intestinal Permeability Increase

High fat diet  Dysbiosis  Systemic inflammation  EtOH

Intestinal Permeability Increase

GLP-2

Metabolic Endotoxemia  Low grade inflammation

Diabetes  Obesity

Sepsis  MOF

Plasma LPS > 10 pg/ml (0.1 EU/ml)
Glucagon-like peptides: GLP-1 & GLP-2

Insulinotropic

Intestinotrophic

Intestinal permeability

Sinclair EM & Drucker DJ. *Physiology* 2005
Teduglutide is a stable GLP-2 analog

- DPP-IV degradation resistant recombinant human GLP-2 analog
- Differs from GLP-2 only by an N-terminus substitution of glycine for alanine in position 2
- Extends half life from 2 minutes to 2 hours
- Once-daily subcutaneous injection for short bowel syndrome

Source: PubChem
GLP-2 reduces intestinal permeability


5 μg h-Gly GLP-2 4 hrs before stress
GLP-2 decreases metabolic endotoxemia

Cani PD, Gut. 2009 Aug;58(8):1091-103
Background

• Circulating LPS increases the permeability of the gut mucosal barrier, increasing LPS translocation into the circulation, augmenting endotoxemia with consequent systemic inflammation.

• Acute treatment with glucagon-like peptide-2 (GLP-2) prevents the observed LPS-related intestinal permeability increase.
Hypothesis

Acute administration of GLP-2 reduces LPS-related increased intestinal permeability
Aims

• To measure the effects of exogenous GLP-2 administration on intestinal permeability in vivo
• To examine the effect of endogenous GLP-2 and the stable GLP-2 analog teduglutide (TDG) on LPS-induced permeability increase
• To determine the therapeutic window for GLP-2 effect after LPS exposure
LPS and TDG treatment protocol

1, 3, 6 hr model
LPS ip 5 mg/kg
± TDG ip 50 µg/kg
-6 hr

± TDG ip
-3 hr

± TDG ip
-1 hr

Inh iv
-20 -15 min

LPS ip
LPS ip

Experiment
Intraduodenal FD4 infusion

24 hr model
LPS ip
TDG ip
-24 hr
-18 hr

TDG ip
TDG ip

Experiment
Intraduodenal FD4 infusion

TDG iv
-15 min

t = 0
In vivo measurement of intestinal barrier function

Male SD rats (200 – 250 g) under isoflurane anesthesia (2%) Pretreated with LPS (5 mg/kg, ip) 6 or 24 hrs before the experiments

Intraduodenal (id) bolus infusion

FITC-Dextran 4kDa (FD4, 0.1 mM, 10 ml)

Thoracic duct

Celiac artery

SMA

Portal vein
LPS treatment increased FD4 permeability increase after 6 hrs

FD4 id infusion (0.1 mM, 10 ml)

- Control
- LPS (5 mg/kg, ip) 1 hr
- LPS 3 hr
- LPS 6 hr

PV FD4 (nM)

Time (min)

*p < 0.05 vs. Control group
LPS treatment increased FD4 transport into portal vein (PV), further enhanced by a GLP-2 receptor antagonist at 6 hr

*p < 0.05 vs. Control group
†p < 0.05 vs. LPS group
LPS treatment increased GLP-2 levels in PV plasma at 6 or 24 hrs before FD4 perfusion

*p < 0.05 vs. Control
LPS treatment increased FD4 transport into PV at 24 hr, reduced by exogenous GLP-2 at 18 hr

*p < 0.05 vs. Control group
†p < 0.05 vs. LPS group
TDG treatment at -3 hr or 0 hr prevented LPS-induced FD4 permeability increase

* p < 0.05 vs. Control group
† p < 0.05 vs. LPS group
TDG treatment at -18 hr reduced LPS-induced FD4 permeability increase.

* \( p < 0.05 \) vs. Control group, † \( p < 0.05 \) vs. LPS group
Therapeutic windows of acute teduglutide treatment for LPS-induced intestinal permeability increase

1, 3, 6 hr model
- LPS ip
- ± TDG ip
- t = 0
- Inh iv
- ± TDG iv
- LPS ip
- t = 0
- Experiment
- Intraduodenal FD4 infusion

24 hr model
- LPS ip
- TDG ip
- TGD ip
- t = 0
- Experiment
- Intraduodenal FD4 infusion
GLP-2 strengthens the mucosal barrier

FD4

FD4  LPS

Permeability increase

TNFα, IL-6

LPS

GLP-2

VIP

TDG

MΦ

TLR4
Conclusions

- LPS is a potent pro-inflammatory molecule
- The intestinal mucosa forms a barrier between high LPS concentrations in the lumen and low in the circulation
- Excess LPS transport into the circulation is the likely basis for chronic diseases such as the metabolic syndrome and acute diseases such as SIRS/MOF
- Acute inflammations increases GLP-2 release into the PV
- Impairment of LPS transport by endogenous and exogenous GLP-2 may decrease morbidity and mortality of SIRS/MOF complicating critical illness
In memoriam

A Tribute to Paul H. Guth, MD (1927–2017)

Jonathan D. Knutti¹,²,³ and Yasuto Akita¹

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This article serves as a commemoration of the career of Paul H. Guth, MD, an accomplished gastrointestinal physiologist and clinician who passed away on July 4, 2017, at the age of 89 after a brief illness. As his niece and collaborator, it is my hope that this article accurately highlights and commemorates the many accomplishments of his career, as well as to remember the warm, caring, and helpful person that he was to his colleagues, friends, and family.

Paul was an inquisitive scientist, deeply devoted and passionate about his research and supportive of his colleagues. He left a strong legacy to his family, his professional colleagues, students, mentors, fellow scientists, and clinicians. My aim is to briefly summarize his career and to emphasize his personal qualities that endeared him to so many. Please note that Paul has already published extensively about his mentor and teacher Mort Grossman that provide additional insight into his long professional career [1,7].

Paul is regarded as one of the global leaders in the field of gastric cytoprotection, which outlines the study of how the gastric mucosa is defended against the highly acidic luminal content, which, with a pH as low as 1, is capable of causing mucosal injury. How the gastric mucosa remained healthy in such a caustic environment was a major interest in the field of gastroenterology ever since the 1960s, when the twin discoveries of proton pump inhibitors and the microorganism Helicobacter pylori substantially informed the clinical impact of (and research interest into) peptic ulcer disease (PUD). Nevertheless, PUD remains clinically, in particular from injecting nonsteroidal anti-inflammatory drugs (NSAIDs) and importantly, the mechanisms that protect against acid-related injury remain vital for the study of tissue injury in general. Paul’s pioneering efforts, in particular his many studies of the gastric mucosal barrier and the control of oxygen-derived radicals toward mucosal injury and protection, stand among the most significant early contributions toward the understanding of fundamental injury mechanisms that have current broad application, specifically toward the mechanism of tissue injury due to dentition or irritants. His highest impact publication, which has been cited in almost 400 research publications since its publication in 1985, addresses the contributions of...
Acknowledgements

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Thanks for the memories...
캘리포니아에서 인사드립니다
Questions?