Pathophysiology of *E. coli*- mediated Hemolytic Uremic Syndrome

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May 7, 2015
Financial Disclosures

Research funding or other support from Thallion Pharmaceuticals, Teijin Pharma, Ltd., Asahi Pharmaceuticals, Inc., Kaketsuken. This support is not associated with the content of this lecture.

The speaker and her spouse does not have a relevant financial interest in any amount which has occurred within the past 12 months or other relationship with the manufacturer(s) of any products or provider of services that may be described in this lecture.
At the conclusion of this activity, participants will be able to:

1. Distinguish Shiga toxin-mediated HUS from other thrombotic microangiopathies
2. Describe the basic pathophysiology of the nonhuman primate model of hemolytic uremic syndrome
3. Compare pathophysiology differences induced by Shiga toxin-1 and -2
4. Identify use of clinical parameters and physiological biomarkers to monitor efficacy of therapeutic intervention

LOs are provided for compliance with ACCME requirements
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Case Study: December 2014
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Day 0: A 20 year old college student presents to Student Health with bloody diarrhea and crampy abdominal pain with no obvious food precipitant. He is otherwise healthy. His roommates are well. Stool studies are sent. He is hydrated intravenously with 1L normal saline and sent home.

Day 2: He returns 2 days later with worsening abdominal pain, continued bloody diarrhea, anorexia, and generalized malaise. Stool sample is positive for Campylobacter antigen, and Shiga toxin. He is hydrated again, and given a prescription for levofloxacin, of which he took one or two doses only, limited by nausea and anorexia.

Day 4: He presents to the emergency department two days after the second visit to Student Health with persistent gastrointestinal symptoms, generalized weakness and dizziness. He has a low-grade fever to 100F, BP 95/70, pulse 120 regular, and looks fatigued but nontoxic. The State Lab calls: E coli 0157:H7.

<table>
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<tr>
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<th>Second Student Health visit</th>
<th>Emergency Dept 2 days later</th>
<th>Hospital Day 2-3</th>
<th>Hospital day 6*</th>
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<td>WBC</td>
<td>19,500</td>
<td>15,200</td>
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<td>Hemoglobin</td>
<td>17</td>
<td>13.6</td>
<td>6.5</td>
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<tr>
<td>Hematocrit</td>
<td>50</td>
<td>38.5</td>
<td>19</td>
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<tr>
<td>PLT</td>
<td>161,000</td>
<td>30,000</td>
<td>23,000</td>
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<td>Total bilirubin</td>
<td>1.0</td>
<td>3.6</td>
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<td>Creatinine</td>
<td>0.9</td>
<td>2.4</td>
<td>2.6</td>
<td></td>
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<tr>
<td>LDH</td>
<td>not done</td>
<td>1598</td>
<td>1999</td>
<td></td>
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<tr>
<td>ADAMTS13</td>
<td></td>
<td></td>
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<td>89% (day4)</td>
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Renal Insufficiency: nonoligouric

*transfused. Discharged day 10 feeling better: normal plts, creatinine, LDH 1074; total 2 units blood transfused, no plasma exchange
E. coli O157:H7 -> Shiga toxin producing E. coli

Enterohemorrhagic E. coli (EHEC) / Shiga toxin producing E. coli (STEC) / Diarrhea associated-HUS

Figure 3 | Attaching and effacing histopathology caused by EPEC and EHEC. The attaching and effacing histopathology results in pedestal-like structures, which rise up from the epithelial cell on which the bacteria perch. Image courtesy of J. Girón.
E. coli O157:H7

Very low infectious dose ~100 – 1000 organisms for humans
Transmission oral-fecal, person-to-person

Hamburger
Filet mignon
Spinach
Lettuce
Bean sprouts
Alfalfa sprouts
Cake
Cheese curds
Chicken
Coleslaw
Pork
Cookie dough
Onions
Raw cow milk
Raw apple cider
Raw apple juice
Radish sprouts
Raw goat milk

Bean dip
Egg salad
Fruit salad
Lettuce, iceberg
Lettuce, romaine
Lasagna
Lamb
Venison jerky
Macaroni salad
Meatballs
Cantaloupe
Pea salad
Pears
Salad bars
Potato salad
Mashed potatoes
Tacos (Taco Bell)
Pizza

Day care centers
Cheerleading squads
Petting farms
Zoos
Dairy farms
Summer camp
Family members

Drinking water
Water reservoirs
Swimming ponds
Swimming lakes

http://www.cspinet.org/foodsafety/outbreak
STEC: Shiga toxin producing *E. coli*
Shiga toxins are the primary virulence factors

**Ribotoxic**

**Stx1, Stx2**

Antigenically distinct

AB$_5$ toxins similar to Ricin from castor beans & Shiga toxin from *Shigella dysenteriae serotype 1*

Bind to Gb3, retrograde trafficking to ER

“stop” protein synthesis

ER stress

Endothelial dysfunction

Pro-thrombotic
Clinical Course and Consequences of STEC Infection

Adapted from Tarr, P., et al. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. The Lancet 365 (9464): 1073-1086, 2005

Ingestion

Diarrhea
Cramping
Abdominal Pain
Vomiting
~Fever

Diarrhea improves

Blood Diarrhea ~90%

Resolution ~85%

5-15% HUS

Hemolytic Uremic Syndrome
Thrombocytopenia
Hemolytic anemia
Thrombotic microangiopathy

Acute Renal Injury
Kidney Failure
Neurologic Abnormalities
High risk of death
Long-term morbidity
Shiga toxin, *E. coli* O157:H7, bloody diarrhea, thrombocytopenia, anemia/MAHA, acute kidney injury, HUS

**E. coli**-mediated hemolytic uremic syndrome (HUS)

Thrombotic Microangiopathies

- EHEC / STEC / Diarrhea-HUS
- Atypical HUS
- Thrombotic thrombocytopenic purpura

Toxigenic bacteria

- Complement, genetic, Eculizumab
- ADAMTS13, autoimmune, genetic

Distinct from Disseminated Intravascular Coagulation (DIC), a consumptive coagulopathy
Animal model of HUS?

Pathophysiology, biomarker development, therapeutic intervention

Thrombotic microangiopathy
Thrombocytopenia
Renal glomerular injury (sporadic)

No coagulopathy
Platelets normal
Renal tubular epithelial injury
Animal Models

Mouse

↑ Inexpensive, genetics, familiar
↓ Hematology, renal injury

Gnotobiotic piglet

↑ Good model of neuro injury
↓ Hematology, renal injury, special care

Dutch belted rabbit

↑ Hematology, familiar
↓ Renal injury

Nonhuman primate
Papio baboon

↑ HUS, renal injury, neuro
Genetically close to humans
↓ Expensive, special care
Everybody knows that

All Shiga toxins work pretty much the same way

Thrombocytopenia? Must be HUS

Passive immunity will never work. Too late.
Q: Do the toxins elicit similar or distinct pathophysiology?

**STUDY DESIGN**

Dose-response: I.V. injection of toxin, periodic blood and urine collection

- **Toxin Challenge intravenous**
  - Stx1
    - 10 ng/kg
    - 50 ng/kg
    - 100 ng/kg
  - Stx2
    - 10 ng/kg
    - 50 ng/kg

[Image: Diagram showing a timeline from 0 to 7 days with arrows indicating the progression of toxin challenge and response over time.]
Survival dose differences, dose-dependent thrombocytopenia, anemia

All Shiga toxins work pretty much the same way (not really)
Dose-dependent loss of kidney function

Stx1 and Stx2

Fibrin
Plt
RBC

Stx2—50ng/kg  Bar = 1 μm

Stx1—10
Stx1—100

Infect Immun, 2010
PMID: 20308301
Am J Pathol, 2013
(PMID: 23402998).
Biomarkers of HUS? DAMPs and D-Dimers

Mitochondrial DNA vs bacterial DNA: Distinguish bacterial sepsis from sterile inflammation

Mitochondrial DNA increases in plasma after Stx1

Increased D-dimer is early, but not specific for HUS

Biomarkers of HUS? (cyto/chemokines)

A

Stx1

Urine Biomarker (pg/ml)

Time (hrs)

0 24 48 72

0
2000
4000
6000

Stx2

Urine Biomarker (pg/ml)

Time (hrs)

0 24 48 72 96 120

0
2000
4000
6000

B

IL-8

MCP-1

MIP-1α

VEGF

IL-6

0 5 10 15 20 25

0
500
1000
1500
2000

Why work so hard? Can’t we just add a little LPS?

Thrombocytopenia? Must be HUS (not really)

STEC/Stx

Stx2 + LPS

Frequently Diarrhea+

Underlying Diseases, such as sepsis

Platelets ↓

Platelets ↓

HUS and DIC are clinically distinct entities

Stx

HUS

LPS

DIC

Frequently Diarrhea+

Underlying Diseases, such as sepsis

Platelets ↓

Platelets ↓

HUS

DIC

Fibrinogen

↓ ~ ↑

Coagulation factors

↓ ~ ↑

FDP/D-dimer

↑ ~ ↓

MAHA

+++ +/- ~ +

↑ increase

↓ decrease

↑↑↑ marked increase

↓↓↓ marked decrease

→ little or no change


J Intensive Care, 2:65, 2014
HUS and DIC are not the same thing

- Gm(-) bacteremia with DIC

Stx2 → no coagulation problems
Stx2 + LPS → thrombocytopenia and kidney injury = HUS

Thrombotic Microangiopathies

EHEC or STEC or Diarrhea-HUS

Atypical HUS

Complement, genetic, Eculizumab

Toxigenic bacteria

Thrombotic thrombocytopenic purpura

ADAMTS13, autoimmune, genetic

Shiga toxin-induced HUS: Is complement a major player?

Complement inhibition for everyone?
Shiga toxin-induced HUS: Is complement a major player?

*Blood,* 122(5):803-806, 2013 (PMID:23733336)

Need more clinical data for data-driven decisions
How do we fix it?

1. Target the bacteria (crossed out)

2. Target the toxins

3. Target the host response
Target the Toxins

(The FASEB Journal. 2006;20:2597-2599.)
© 2006 FASEB

A multivalent peptide library approach identifies a novel Shiga toxin inhibitor that induces aberrant cellular transport of the toxin

Kiyotaka Nishikawa*,†,1, Miho Watanabe*, Eiji Kita§, Katsura Igai*,†, Kazumi Omata‡, Michael B. Yaffe|| and Yasuhiro Natori*

* Department of Clinical Pharmacology, Research Institute, International Medical Center of Japan, Tokyo, Japan;† PRESTO, Japan Science and Technology Agency, Saitama, Japan;§ Department of Bacteriology, Nara Medical University, Kashihara, Japan;† Department of Medical Ecology and Informatics, Research Institute, International Medical Center of Japan, Tokyo, Japan; and|| Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

1 Correspondence: Department of Clinical Pharmacology, Research Institute, International Medical Center of Japan, 1–21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. E-mail: knishika@ri.imcj.go.jp
TVP peptide binds only to Stx2 B subunit - does not bind Stx1.

Vero cells stained for Stx2 and inhibitory b-TVP peptide

Image by Caitlin L. Parello, Pathology Department

Q: Will the tetravalent peptide work in our baboon i.v. Stx2 toxemia model?

STUDY DESIGN

Lethal Dose 50ng/kg

Stx2 only

Prevention TVP(0)

Rescue at 6 h TVP(6)

Rescue at 24 h TVP(24)

28 days
TVP prevents and rescues baboons from lethal Stx2 challenge

TVP preserves kidney function

**Urinalysis**

**A**
- Hemoglobinuria (0-4)

**B**
- Proteinuria (mg/dl)

**C**
- Urine output (ml/kg/hr)

**Stx2**

Target the Toxin

Passive immunity will never work. Too late. (not really)

TMA-15 rescued all 4 animals and ameliorated thrombocytopenia even at 48 hours after challenge. All were survivors.

Red (n=6): No treatment, Stx2 at 50 ng/kg (all lethal)
Brown (n=2): 1mg/kg, 24 hours after Stx
Green (n=2): 1mg/kg, 48 hours after Stx

Passive Immunity Works. Its not too late
How do we fix it?

1. Target the bacteria

2. Target the toxins

3. Target the host response
Target the Host Response

Recombinant Activated Protein C: anti-coagulant, anti-inflammatory

Rescue protocol at 24 or 48 hours after lethal dose Stx2

Platelet (E+3/cmm)

Time (hours)

50 ng/kg Stx2
x2stx24-A
x2stx24-B
x2stx48-A
x2stx48-B

24hrs
48hrs
†
Moving Forward

How late can we administer passive immunity to rescue from HUS and still preserve kidney function? (looking good for 72hrs). Best biomarkers?

Have an NHP HUS model, but drawbacks. Can we make a better mouse model? Increase Gb3 toxin receptor expression on murine endothelial cells (not Fabry’s)

Other small animal models?
Hamsters respond to coagulation pressures similarly to humans
◆ Shiga toxin-mediated HUS is distinct from other thrombotic microangiopathies and disseminated intravascular coagulation [thrombocytopenia ≠ HUS]

◆ Stx1 and Stx2 induce HUS in nonhuman primates and the path to HUS is different between the 2 toxins

◆ Therapeutic approaches should be based on evidence-driven decisions
  E.g., clinical parameters, biomarkers, complement, passive immunity
  Not as much progress on HUS biomarkers as we’d like....
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