Effects of a preconditioning oral nutritional supplement (pONS) on pig livers after warm ischemia

hepatic ischemia-reperfusion injury

Jaeschke H; Am J Physiol 2003
Pharmacologic preconditioning

A. FREE RADICAL GENERATION

- Reactive oxygen species: $O_2^-$, $H_2O_2$, $OH^-$

B. CELL INJURY BY FREE RADICALS

- Membrane lipid peroxidation
- DNA fragmentation
- Protein cross-linking and fragmentation

C. NEUTRALIZATION OF FREE RADICALS – NO CELL INJURY

- Peroxisomes
- Cytosol
- Mitochondria
- Glutathione peroxidase
- SOD
- Glutathione reductase
- Catalase
- Vitamin C
- Fisetin

Fenton reaction:

- $O_2^-$ + $H_2O_2$ → $OH^-$ + $OH^-$
Warm ischemia of liver tissue
application of pONS (Fresenius Kabi)

• 70 g pONS in 250 ml tap water
  glutamine (15 g), GTE (1 g; 99.3 % polyphenols),
  Vit. C (0.75 g), E (0.25 g), beta carotene (0.05 g),
  selenium (150 μg), zinc (0.1 g), carbohydrates (50 g)

• 24, 12, and 2 hrs before and 3 hrs after ischemia

• pre OP: feeding; post OP: jejunostomy
**Protocol -24 hrs -12 hrs -2 hrs 0.5 hrs 3 hrs 6 hrs 8 hrs**

- pONS / control 70 g in 250 ml water (feeding)
- 40 min hepatic ischemia (total portal venous and arterial vascular occlusion)
- PR, MAP, CVP, PVF, HAF, ALT, AST, bile volume
- histology (H&E), immunohistochemistry (TNF-α, myeloperoxidase, caspase 3)
- pONS / control 70 g in 250 ml water (jejunostomy)

**Abbreviations:**
- PR: pulse rate
- MAP: mean arterial pressure
- CVP: central venous pressure
- PVF: portal venous flow
- HAF: hepatic artery flow
Hemodynamic parameters (1)

**pulse rate**

![Graph showing pulse rate over time after reperfusion](image)

**mean arterial pressure**

![Graph showing mean arterial pressure over time after reperfusion](image)

**central venous pressure**

![Graph showing central venous pressure over time after reperfusion](image)

values are Mean ± SEM; * : p< 0.05
Hemodynamic parameters (2)

Hepatic arterial flow

Portal venous flow

values are Mean ± SEM; * : p< 0.05
Transaminases

**ALT (GPT)**

![Graph showing ALT (GPT) levels over time after reperfusion for control and pONS groups.](image)

**AST (GOT)**

![Graph showing AST (GOT) levels over time after reperfusion for control and pONS groups.](image)

Values are Mean ± SEM; *: p < 0.05
Bile production

values are Mean ± SEM; * : p< 0.05

control, n=6
pONS, n=5
### Histology, H&E staining (×100)

<table>
<thead>
<tr>
<th>histopathology</th>
<th>Control</th>
<th>pONS</th>
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<tr>
<td></td>
<td>n</td>
<td>Median</td>
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<tr>
<td>necrosis</td>
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<tr>
<td>leukocyte infiltration</td>
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</table>

Histomorphological changes:
- grade 0, minimal or no evidence of injury;
- grade 1, mild injury including cytoplasmic vacuolation and focal nuclear pyknosis;
- grade 2, moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular borders; and
- grade 3, severe necrosis (a) with disintegration of hepatic cords (b), hemorrhage (c), and neutrophil infiltration (d).

To investigate leukocyte infiltration into the hepatic tissue, a scale from 1 to 4 was used:
- grade 1, <10 leukocytes/field (focal infiltration);
- grade 2, 10-20 leukocytes/field (mild infiltration);
- grade 3, 21-50 leukocytes/field;
- grade 4, >50 leukocytes/field.

n; number of microscopic fields assessed
Immunohistochemistry was evaluated by the semiquantitative technique, relating the score of 0 to 4 points to the fraction of stained cells:

0, 0% cells; 1, <5% cells; 2, 5%-20% cells; 3, 20%-40% cells; 4, >40% positive cells.

<table>
<thead>
<tr>
<th>expression</th>
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<th>pONS</th>
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<tr>
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<td>25%</td>
<td>75%</td>
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<td>100</td>
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<td>1</td>
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<td>&lt;0.001</td>
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</table>
Conclusion

• Oral pharmacologic preconditioning is feasible and beneficial.

• pONS protects liver from warm ischemia / reperfusion injury by decreasing:
  - oxidative stress,
  - lipid peroxidation,
  - apoptosis, and necrosis.
Dankeschön!