Oxidative Stress in Chronic Pancreatitis

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CP: Oxidative Stress

• Outline:
  – Insight into development of Chronic Pancreatitis
  – Oxidative Eustress and Oxidative Distress
  – Experimental evidence of oxidative stress
  – Evidence of oxidative stress in Human CP
How does CP develop?
Pancreatitis

Acute pancreatitis

Recurrent acute pancreatitis

Chronic Pancreatitis

20%-30%

50%-90%
• 75 patients with RAP
• 47% developed CP during follow-up

(Garg et al. Clin Gastro Hepatol 2006)
Progression of disease

Ductal changes
Progression of Disease

Parenchymal changes
Progressive model of disease

Paradigm of disease progression

Injury $\rightarrow$ Inflammation $\rightarrow$ Fibrosis
Recurrent $\rightarrow$ Recurrent $\rightarrow$ CP
Oxidative stress in CP

- Oxidative stress could be a link between cellular injury and inflammation
- Not a primary event but a step in the cascade
CP: Etiopathogenesis

Environmental factors

Oxidative stress

Mutations (SPINK1, CFTR, PRSS1, Cathepsin B, CTRC)
CP: Oxidative Stress

- Outline:
  - Oxidative Eustress and Oxidative Distress
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  - Evidence of oxidative stress in Human CP
Terms

• Redox signaling
• Redox control (Enzymatic and non-enzymatic)
• Redox Medicine
• Oxidative Eustress (physiological)
• Oxidative Distress (pathological)
Redox Mechanisms

- Reduction and Oxidation: essential for cellular functions
- Reactive species:
  - Free radicals: $O_2^-$, HO$^-$, NO
  - Nonradical: $H_2O_2$
- Perform important functions
- Oxidative Eustress (physiological)
Redox processes

Oxidative stress

- DNA and RNA oxidation
- Mutations
- Metal ion interactions
- Protein processing, folding, interactions, and trafficking
- Lipid oxidation and signaling
- Glycan modifications
- Redox reactions
- Electrophile reactions
- Sulfane reactions
- Nitration

Exposome

- Nutrition
- Lifestyle and exercise
- Chemical and drugs
- Air pollution
- UV light
- Ionizing radiation
- Microbiome

Genome

Epigenome

Transcriptome

Redox proteome

Redox metabolome

Redox networks, structure, and function

Biological processes
Oxidative Eustress

ROS (Oxidants)

Intracellular signaling

Scavengers

Oxidative Eustress

Antioxidants

Oxidative Distress
CP: Oxidative Stress

- Oxidative Distress:
  - Lipid peroxidation
  - Protein modification
  - DNA/RNA modification
  - Disturbed cellular function
CP: Oxidative Stress

- Regulatory mechanisms to control OS:
- Nrf 2-Keap 1: constitute Genetic antioxidant response element (ARE)
- Exert:
  - Cytoprotection
  - Antioxidant
  - Anti-inflammatory
CP: Oxidative Stress

- Cellular responses to OS:
  - ER stress response: UPR
  - Autophagy, apoptosis
  - NFκβ: inflammation
CP: Oxidative Stress

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Oxidative Stress in Pancreatitis

- Endogenous production of ROS:
  - ER stress
  - ER stress → ROS
  - Evidence?
Caerulein induced Pancreatitis
- ER Stress: Up-regulation of ATF6 and PERK pathways
Endogenous ROS

Acinar cells (AR42J cell lines)

Tunicamycin

Caerulein
ROS production: by tunicamycin
ROS production: ↑ by Caerulein
ROS and Antioxidant Response

Nrf2 up-regulation

anti NRF2

Antioxidant response suggestive of OS
CP: Oxidative Stress

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Environmental factors

Oxidative stress

Mutations (SPINK1, CFTR, PRSS1, Cathepsin B, CTRC)
Oxidative stress in CP

- **Sources:**
  - **Exogenous (Environmental):**
    - Alcohol
    - Smoking
    - Diet
    - Xenobiotics
  - **Endogenous**
    - Intracellular: physiological
    - Pathological: ?
Inadequate antioxidant defenses

↑ xenobiotic load

↑ production of free radicals

↑ toxic metabolites overwhelm Phase II

Increased OS

Inflammation

CHRONIC PANCREATITIS

Induction

Xenobiotics

Phase I pathway CYP enzymes

Phase II pathway Glutathione etc.

Attach OH/O: ↑ solubility; compound may become more bioactive

Bio-conjugation/reduction
Genetic mutations in CP

• How genetic mutations may lead to oxidative stress?
Genetic mutations in CP

- *PRSS1, CTRC, CFTR* gene mutations
- Mechanism of injury not well understood
- How mutations lead to inflammation?
Mutations and Oxidative stress

PRSS1, CTRC gene mutations

Unfolded protein response (UPR)

ER stress*

ROS

CP: Etiopathogenesis

Environmental factors

- Oxidative stress
- ER stress
- Mutations (PRSS1, CTRC)
Oxidative stress in CP

• Enough evidence from experimental and human studies:

• ↑ Oxidative stress in CP
Case-control study

- Patients: 127 patients with CP
- Controls: 104 healthy controls
Lipid peroxidation (TBARS, nmoles/ml)

Baseline | One month | Six months
---|---|---
Placebo | Antioxidant

P=0.001
FRAP ($\mu$molesFe$^{+2}$ lib): pre, post and change

P=0.038
Progression of Disease

Q: does oxidative stress play role at this stage?
Case-control study

- 50 Patients with RAP, 50 controls
- Markers of OS:
  - 4-hydroxynonenol (4-HNE),
  - Malondialdehyde (MDA)
- Antioxidant status:
  - Ferric reducing the ability of plasma (FRAP)
  - Glutathione peroxidase (GPX)
  - Vitamin C
Oxidative stress in RAP

• 4-HNE significantly increased in patients with RAP compared with controls (3.03 ± 2.35 vs. 2.12 ± 1.29 ng/ml; p=0.03)

• Antioxidant levels were reduced in RAP compared with healthy controls
  – FRAP (707.0 ± 144.9 vs. 528.8 ± 120.0 µmol/Fe2+ liberated; p=0.001)

(Bopanna et al. Pancreatology 2017)
Q: Does AO supplementation help?
AIIMS study: RCT

CP (n=127)

Intervention Group*  Placebo Group

*0.54 g vitamin C, 9000 IU β-carotene, 270 IU vitamin E, 600 µg organic selenium, and 2g methionine in 3 divided doses

• 6 months intervention

(Bhardwaj, Garg et al, Gastroenterology 2009)
Results

Number of painful days per month

P = 0.012

Placebo

Antioxidant
Number of oral analgesics used/month

![Bar chart showing the number of oral analgesics used per month for Placebo and Antioxidant groups.](chart)

- **Placebo**:
  - Previous: 10
  - Post: 5

- **Antioxidant**:
  - Previous: 15
  - Post: 10

**P = 0.06**
Percentage of Patients who became Pain free

Placebo  P<0.001  Antioxidants

Pain free
Not pain free
Long term outcome of Patients

Long-term pain relief with optimized medical treatment including antioxidants and step-up interventional therapy in patients with chronic pancreatitis

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Response to Step-up therapy

Medical therapy (n=288)
- Response: 52.1%
- No Response: 47.9%

Endoscopic therapy (n=67)
- Response: 16.7%

Surgery (n=26)
- Response: 6.7%

No response at 1 year: 15.4%
Proportion pain free with increasing duration of disease

Proportion of patients pain free

Follow up (years)

N= 208  N= 193  N= 151  N= 80  N= 38
ANTICIPATE Trial: No benefit

- 70 patients, 33 patients on AO
- Characteristics:
  - 70% were alcoholic with a mean alcohol intake of 222 g/d (~ 20 drinks/day)
  - Smoking 21 cigarettes/day
ANTICIPATE Trial

• >85 mg/d of opiates/day addiction and neuropathic pain
• 54% prior intervention, unresponsive disease and probably neuropathic pain
ANTICIPATE Trial

• Results: No benefit with AO
• Why?
Mechanism of Pain

- Late stage, poor functional reserve

- Ductal obstruction, atrophy
  - Little inflammation
  - Oxidative stress +
  - Neuropathic pain
Neuropathic pain
- NK-1, NGF, BDNF
- Substance P, CGRP
CP: Antioxidants

- RCTs
- Meta-analysis

GRADE 1B evidence
Oxidative Stress in CP: Summary

- Oxidative Eustress: physiological
- Oxidative Distress: pathological
- AP ➔ RAP ➔ CP: oxidative stress one of the mechanism of injury/inflammation
- Experimental & clinical evidence of OS
- Antioxidants: Therapeutic benefit +
- Not effective in neuropathic pain