Oxidative Stress in End-Stage Kidney Disease

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Oxidative Stress in End-Stage Kidney Disease

• **What is oxidative stress?**
• Why is oxidative stress important in end-stage renal disease patients?
• Why is there oxidative stress in end-stage renal disease patients?
• Why is oxidative stress harmful?
• oxPTH- oxidative stress and PTH
**Oxidative stress** represents an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.
**Reactive Oxygen Species (ROS)**

<table>
<thead>
<tr>
<th>Radicals:</th>
<th>Non-Radicals:</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{O}_2^-\cdot$ Superoxide</td>
<td>$\text{H}_2\text{O}_2$ Hydrogen peroxide</td>
</tr>
<tr>
<td>$\cdot\text{OH}$ Hydroxyl</td>
<td>$\text{HOCl}^-$ Hypochlorous acid</td>
</tr>
<tr>
<td>$\text{RO}_2\cdot$ Peroxyl</td>
<td>$\text{O}_3$ Ozone</td>
</tr>
<tr>
<td>$\text{RO}\cdot$ Alkoxy</td>
<td>$^{1}\text{O}_2$ Singlet oxygen</td>
</tr>
<tr>
<td>$\text{HO}_2\cdot$ Hydroperoxyl</td>
<td>$\text{ONOOO}^-$ Peroxynitrite</td>
</tr>
</tbody>
</table>
Major cellular sources of Reactive Oxygen Species in living cells

a) Mitochondria

Stimuli inducing increased mitochondrial generation of ROS:
- serum deprivation
- integrin signalling
- apoptosis
- TNFα
- hypoxia
- ceramide
- p53
- oncogenic Ras

b) NADPH oxidase

Phagocytic Cells

Non-Phagocytic Cells

Stimuli for activation of NADPH oxidase and 5-lipoxygenase

- integrin signalling
- growth factors
- cytokines/hormones
- immunological stimuli
- hypoxia
- oncogenic Ras

c) 5-lipoxygenase
Endogenous sources of ROS and RNS

- Mitochondria
- Lysosomes
- Peroxisomes
- Endoplasmic Reticulum
- Cytoplasm
- Plasma Membrane
- Myeloperoxidase (phagocytes)
- Transition metals
- Fe
- Cu
- Electron transport

- Microsomal Oxidation, Flavoproteins, CYP enzymes
- Xanthine Oxidase, NOS isoforms
- Oxidases, Flavoproteins
- Lipoxygenases, Prostaglandin synthase, NADPH oxidase
- Transition metals
- Electron transport
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The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia

The malnutrition inflammation syndrome tips the redox balance toward oxidative stress and cardiovascular disease.

Clinical trials using a range of interventions aimed at improving CVD outcomes in HD patients have been unsuccessful. These include:


The only two intervention trials to show a positive effect in this population were those using antioxidant therapy.

<table>
<thead>
<tr>
<th>Total CVD endpoints</th>
<th>Vitamin E (n=97)</th>
<th>Placebo (n=99)</th>
<th>Relative risk (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Including sudden death</td>
<td>18 (18-8%)</td>
<td>34 (34-3%)</td>
<td>0.54 (0.33-0.89)</td>
<td>0.016</td>
</tr>
<tr>
<td>Excluding sudden death</td>
<td>15 (15-5%)</td>
<td>33 (33-3%)</td>
<td>0.46 (0.27-0.78)</td>
<td>0.014</td>
</tr>
<tr>
<td>Myocardial infarctions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Including sudden death</td>
<td>8 (8-2%)</td>
<td>18 (18-2%)</td>
<td>0.45 (0.20-0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>Excluding sudden death</td>
<td>5 (5-2%)</td>
<td>17 (17-2%)</td>
<td>0.30 (0.10-0.80)</td>
<td>0.016</td>
</tr>
<tr>
<td>Fatal, including sudden death</td>
<td>5 (5-2%)</td>
<td>9 (9-1%)</td>
<td>0.57 (0.20-1.60)</td>
<td>0.3</td>
</tr>
<tr>
<td>Fatal, excluding sudden death</td>
<td>2 (2-1%)</td>
<td>8 (8-1%)</td>
<td>0.26 (0.06-1.17)</td>
<td>0.1</td>
</tr>
<tr>
<td>Non-fatal</td>
<td>3 (3-1%)</td>
<td>9 (9-1%)</td>
<td>0.35 (0.10-1.24)</td>
<td>0.08</td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any cause</td>
<td>31 (31-2%)</td>
<td>29 (29-3%)</td>
<td>1.09 (0.70-1.70)</td>
<td>0.7</td>
</tr>
<tr>
<td>Cardiovascular disease*</td>
<td>9 (9-3%)</td>
<td>15 (15-2%)</td>
<td>0.61 (0.28-1.30)</td>
<td>0.25</td>
</tr>
<tr>
<td>Sudden death</td>
<td>3 (3-1%)</td>
<td>1 (1-1%)</td>
<td>3.06 (0.30-29-00)</td>
<td>0.3</td>
</tr>
<tr>
<td>Secondary outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>5 (5-2%)</td>
<td>6 (6-1%)</td>
<td>0.85 (0.30-2.70)</td>
<td>0.8</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>3 (3-1%)</td>
<td>8 (8-1%)</td>
<td>0.39 (0.11-1.43)</td>
<td>0.2</td>
</tr>
<tr>
<td>Unstable angina pectoris</td>
<td>2 (2-1%)</td>
<td>4 (4-1%)</td>
<td>0.51 (0.09-2.70)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

CVD=cardiovascular disease and includes fatal and non-fatal myocardial infarction, fatal and non-fatal ischaemic stroke, unstable angina, peripheral vascular disease (not including the arterio-venous fistula) in a limb not previously affected. *Deaths from cardiovascular disease include fatal myocardial infarction, fatal stroke, and sudden death.

Table 2: Effect of vitamin E treatment on cardiovascular outcomes in the SPACE cohort

Figure 2: Kaplan-Meier survival curves from primary cardiovascular disease endpoints
CVD=cardiovascular disease.
The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial.

Kaplan-Meier survival curves from primary end points. Hemodialysis patients were randomly assigned either to receive acetylcysteine (ACC, 600 mg BID) or placebo (control group). The primary end point was a composite variable consisting of cardiac events including fatal and nonfatal myocardial infarction, cardiovascular disease death, need for coronary angioplasty or coronary bypass surgery, ischemic stroke, peripheral vascular disease with amputation, or need for angioplasty. Relative risk, 0.60 (95% CI, 0.38 to 0.95), \( P=0.03 \).

<table>
<thead>
<tr>
<th>Number at risk</th>
<th>ACC</th>
<th>64</th>
<th>40</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>70</td>
<td>33</td>
<td>13</td>
<td></td>
</tr>
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</table>

DPP4 inhibition Offers a New Therapeutic Approach for ARB-resistant Diabetic Nephropathy - data from diabetic eNOS -/- mice

ANOVA mit Bonferroni
p>0.001
*, ** p<0.05; p<0.01 vs. diabet Placebo
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Mechanisms resulting in elevated oxidative stress in hemodialysis patients

- Chronic kidney disease
- Malnutrition
- Loss of antioxidants during dialysis
- Interactions between blood and membrane
- Bacterial products in dialysate

Antioxidant therapy in hemodialysis patients: a systematic review
Jeff S. Coombe and Robert G. Fassett
Fig. 6. Plasma myeloperoxidase levels during hemodialysis with a cuprophan membrane. $N = 5$, $^*p \leq .05$ vs. predialysis.

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Relation between oxidant stress and various pathways that have been implicated in diabetic nephropathy

Hyperglycemia

- Oxidative stress
  - Mitochondrial dysfunction
  - AGE / RAGE
  - NAD(P)H oxidase
  - PKC
  - Angiotensin II

Apoptosis

- Loss of podocytes
- Loss of mesangial cells

Activation of transcriptional pathways such as NF-κβ, API

- Proteinuria
- Increased ECM
- Nodular glomerulosclerosis
- Loss of kidney function

Oxidants in Chronic Kidney Disease
Sudhir V. Shah et al.
Low-density lipoprotein

- 1 apolipoprotein B-100
- free cholesterol
- phospholipids
- triacylglycerol (fat)
- cholesterol esters
Oxidized LDL

Hypertension  Hyper-/dyslipidemia

Diabetes  Smoking

Reactive oxygen / nitrogen species 
ROS / RNS

↓

LDL oxidation

Malondialdehyde-LDL  Copper-oxidized LDL
MDA-LDL  Cu-LDL

Ox-LDL is highly immunogenic → auto-antibody induction

Ox-LDL plays a major role in initiating arteriosclerosis
Ox-LDL and arteriosclerosis

Binding to endothelial cells (EC) via LOX-1:
- endothelial dysfunction and inflammation
- ROS generation
- attraction of monocytes (Mo)

Uptake by macrophages (Mac) via scavenger receptors (CD36, SR-A1, SR-A2, LOX-1):
- bypass of the regulated LDL receptor path
- foam cell induction → … plaque
- growth factors
- inflammatory cytokines
- chemokines
Ox-LDL in clinical studies

Cumulative risk of cardio-vascular disease in 765 initially healthy individuals from a general community followed from 1995 to 2005

Tertiles of ox-LDL and the Framingham Risk Score groups: added value to conventional risk factors!

BRUNNECK Study: Kiechl, Arterioscler Thromb Vasc Biol 2007; EPIC-Norfolk Study: Tsimikas, JACC 2010
DNA: Hot Spots for Free Radical Attack

[Diagram of DNA molecule with highlighted hot spots for free radical attack]
Oxidative Damage to the Bases

HO\(^{-}\) attack on pyrimidines

Oxidative Damage to the Bases

HO\(^*\) attack on purines

Adapted from: von Sonntag C. (1987)
*The Chemical Basis of Radiation Biology.*
Taylor & Francis London, NY.
Examples of Oxidized DNA Bases

Oxidation of DNA can lead to Mutations

8-hydroxyguanine

8 OH-Gua (enol) ⇌ 8 oxo-Gua (keto)

Basepairs
A-T
G-C

8 OHdG→A

Misreading of 8-OHdGua can lead mutation (GC→AT transversion).
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PTH has two methionine residues at position 8 and 18, and indeed studies by independent groups have repeatedly shown that PTH is subject to oxidation at the methionine residues.

Fig. 1. Structures of methionine and its oxidation products.
hPTH (1-84)
Oxidized PTH is biologically inactive

FIG. 2. Calcemic response in vitamin D-deficient (A) and 1,25(OH)\textsubscript{2}D\textsubscript{3} (500 ng)-repleted (B) TPTX rats after infusion of hPTH 1–34 (—○—) and oxidized hPTH 1–34 (—●—). Graded amounts (0.1–0.8 mmole/hr) of the hormones were infused continuously during the last 18 hr of experimental period into the TPTX rats. Values represent the mean ± SEM for each group. Statistically different values are indicated as *p < 0.01 for hormone groups compared to control group (no hormone infused).

NOBORU HORIUCHI

Effects of Oxidation of Human Parathyroid Hormone on Its Biological Activity in Continuously Infused, Thyroparathyroidectomized Rats

Oxidized PTH is biologically inactive

FIG. 4. Effects of infusion of hPTH 1–34 and oxidized hPTH 1–34 on urinary excretion of P (A), cAMP (B), and Ca (C) in vitamin D-deficient TPTX rats. The hormones were infused at a rate of 0.8 nmol/hr during the last 4 hr of the experimental period. Values at zero time point represent basal levels of urinary P, cAMP, and Ca. Solid lines (—O—) show hPTH 1–34 groups and broken lines (—●—) indicate oxidized hPTH 1–34 groups. Values represent the mean ± SEM for each group.

NOBORU HORIUCHI
Effects of Oxidation of Human Parathyroid Hormone on Its Biological Activity in Continuously Infused, Thyroparathyroidectomized Rats
Current PTH assays do not distinguish between oxidized PTH and real intact, biologically active PTH.

An assay that is able to distinguish between oxidized PTH and real intact, biologically active PTH is urgently needed.
Parathyroid Hormone (PTH) is subject to oxidation. Oxidized PTH is biologically inactive.

**Step 1:** Affinity chromatography

Patient sample:

- PTH native (bioactive)
- PTH oxidized (bioinactive)

**Step 2:** Immunoassay for bioactive PTH

Antibody-coated chromatography beads binding oxPTH:

- PTH native (bioactive)

Microtiter plate:

- TMB
- Peroxidase
- Peroxidase

Colored substrate product:

- H$_2$O$_2$
The intact hPTH(1-84)ox sample shows TIC-peaks at 18 - 20 min and several mass peaks due to +16, +32, +48 and +64 Da mass shifts caused by the oxidations (methionine sulfoxide, +16 Da and sulfone, +32 Da for each methionine residue, and combinations thereof).
No significant mass peaks were observed that can be assigned to any of the hPTH(1-84)ox species by nanoLC-ESI-FT-MS analysis of the flow-through and wash fractions (equilibrating buffer and water) of the column,
Summary/Conclusions

• Oxidative stress plays a key role in end-stage kidney disease and damages lipids, proteins and DNA
• Treatment of oxidative stress (directly or indirectly) may improve CV mortality
• Oxidation of hormones like PTH may result in loss of function of these hormones – this is not reflected by the currently available PTH assays
• Thank you!