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• Prof. Zdenko Machala (Comenius U; Slovakia)
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Matt Pavlovich
Toshi Ono
Brandon Curtis
Carly Anderson
H Obo
Connor Galleher
Sharmin Karim
Zilan Xiong
Introduction and Overview

• Plasma-generated gas phase reactive oxygen and nitrogen species are relatively short-lived and probably react quickly after entering cells/fluids.

• Major questions: how does plasma affect tissue deeper than the surface layer of cells? How can it be therapeutic if effects are localized to surface?

• We can get some guidance from the literature: recent work on oxidative stress, aging, and radiation biology/oncology appear relevant to plasma medicine.
What is our **Conceptual Model** for Plasma-Therapy?

**Plasma device**

- **Gas Phase RONS**
  - O
  - OH
  - O3
  - NO
  - NO2

- **Liquid Phase RONS products**

**Surface Cells**

- Cell-cell communication: ‘bystander effect’?
- Immune system involvement?
- Plasma-induced increased blood flow/O2?

**Bulk Tissue/Tumor**

**RONS gas species**: transfer into liquid and **surface cells**

(Liquid: water, salt, proteins, lipids; RONS react to form products)

**Postulate**: RONS-macromolecule products must induce RONS/redox stress in surface cells, followed by cell-cell communication.
But what is known of RONS?

RONS are key species in normal physiology and homeostasis, with central roles in:

- immunity and inflammation
- cell cycle
- metabolism
- cell differentiation/development/death
- vascular tone/blood pressure/neurotransmission/platelet activation
- Protein post-translational modification

They act as *cell signaling* agents and eventually trigger gene expression by altering or stimulating transcription factors.
The ‘Bad’ Side of RONS

RONS are correlated with many important diseases (and aging), including:

- Cancer
- Cardiac diseases
- Vascular diseases
- Inflammatory diseases
- Stroke
- Neurodegenerative diseases
- Diabetes and metabolic syndrome

Most major human diseases/health conditions!
Traditional View of RONS: Oxidative Stress Model

Reactive oxygen and nitrogen are mostly damaging and must be kept in check by antioxidants.

Therefore, consumption of antioxidants should be therapeutic, and possibly life-extending, by reducing excess levels of RONS in the body.

But this traditional view *has been shown to be essentially incorrect*: e.g. antioxidants do NOT increase life span or reduce cancer risk but they CAN reduce valuable effects of exercise.
‘Hormetic’ response: favorable at low dose, unfavorable at high dose.

Transient ROS increases are ‘vaccination-like:’ inactive microorganisms exert immune response via vaccines.
Naviaux (2012) also suggests the traditional view of reactive oxygen (and nitrogen) in the context of ‘oxidative stress’ as a cause of disease/aging may be completely wrong.

He suggests ‘oxidative shielding’ is an evolutionarily conserved way that cells protect themselves.
**Oxidative Shielding Rather than Oxidative Stress**

Naviaux (2012) suggests:

“ROS and oxidative changes in chronic disease are the symptoms of disease and not the cause.”

“Oxidative shielding...ultimately increases membrane rigidity, decreases permeability and inhibits cell division.”

“The machinery of oxidative shielding evolved from pathways of innate immunity designed to protect the cell from attack and limit the spread of infection.”

*Does plasma act as an exogenous source of therapeutic oxidative shielding?*
Ionizing Radiation Shares Some Features with Plasma-Cell Exposures

Generation of RONS

DNA damage (SSB and DSB)

Apoptosis of tumor cells

Known that direct radiation effects are only part of anti-tumor effects: bystander effects affect unexposed cells

What is currently thought about the role of RONS in radiation bystander effects?
Ionizing radiation (IR) induces ROS in ‘bystander’ cells via modulation of ROS/redox. Might plasma do something similar?
IR Leads to Either **Apoptosis** or **Adaptation and Survival**

(Klammer et al., Cancer Lett., 2014)
Why Do Organisms Naturally Modulate Cellular ROS as a Generalized Stress Response?

Klammer et al. (2014) postulate:

“Modulation of ROS generates regulated, intrinsic stress in a population of cells that has sustained some form of external stress.

Ultimate goal of this process is to select cells that are healthy and thus fit to become part of the healing process; weak or genomically altered cells may be removed by apoptosis - a tissue clearance process that reduces the risk for cancer induction.”

Recall Naviaux’s suggestion (2012):

“The machinery of oxidative shielding evolved from pathways of innate immunity designed to protect the cell from attack and limit the spread of infection.”
Adaptive Response, Evidence of Cross-Resistance and Its Potential Clinical Use

*Int. J. Mol. Sci. 2012, 13, 10771-10806*

Irina Milisav, Borut Poljsak and Dušan Šuput

Table 1. Examples of cross-resistance to stressors.

<table>
<thead>
<tr>
<th>Stressor</th>
<th>Cell Type/Organism</th>
<th>Cross-Resistance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O$_2$</td>
<td>CHO cells</td>
<td>Heat shock, $N$-methyl-$N'$-nitro-$N$-nitrosoguanidin (MNNG), $\gamma$-ray irradiation, X-ray irradiation</td>
<td>[143, 144, 144, 145]</td>
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<tr>
<td></td>
<td>Human lymphocytes</td>
<td></td>
<td></td>
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<tr>
<td>Heat</td>
<td>CHO cells</td>
<td>$H_2O_2$</td>
<td>[143]</td>
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<tr>
<td></td>
<td>Human skin fibroblasts</td>
<td>Delayed aging</td>
<td>[146]</td>
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<td></td>
<td>Renal epithelial cells</td>
<td>Cyanide</td>
<td>[147]</td>
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<td></td>
<td><em>C. elegans</em></td>
<td>Increased lifespan</td>
<td>[148]</td>
</tr>
<tr>
<td>Cold</td>
<td><em>Drosophila melanogaster</em></td>
<td>Heat stress, Increased lifespan, delayed aging</td>
<td>[149, 150]</td>
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<tr>
<td>Chemicals</td>
<td>CHO cells</td>
<td>$H_2O_2$ and $\gamma$-irradiation</td>
<td>[144]</td>
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<td></td>
<td>Rat hepatoma cells</td>
<td>$H_2O_2$ and $\gamma$-irradiation</td>
<td>[144]</td>
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<td></td>
<td>Rat brain cells</td>
<td>Ischemia</td>
<td>[151]</td>
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<td></td>
<td>Hepatocytes</td>
<td>Improved survival</td>
<td>[152]</td>
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### Oxidative Stress Adaptation Promotes Organism Survival from Other Forms of Stress

Milisav et al., 2012

<table>
<thead>
<tr>
<th>Stressor</th>
<th>Cell Type/Organism</th>
<th>Cross-Resistance</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Exercise</td>
<td>Rat skeletal muscle</td>
<td>Oxidative stress</td>
<td>[153]</td>
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<tr>
<td></td>
<td>Rat heart</td>
<td>Ischemia</td>
<td>[154]</td>
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<td></td>
<td>Rats</td>
<td>Delayed aging</td>
<td>[155]</td>
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<tr>
<td></td>
<td>Humans</td>
<td>Ischemia</td>
<td>[156,157]</td>
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<td></td>
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<td>Delayed aging</td>
<td>[158]</td>
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<tr>
<td></td>
<td></td>
<td>Ischemia</td>
<td>[159]</td>
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<tr>
<td>Caloric restriction</td>
<td><em>Aeromonas hydrophila</em></td>
<td>Lowered temperature, sodium, and ethanol stresses</td>
<td>[160]</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>Heat stress and H$_2$O$_2$</td>
<td>[161]</td>
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<td></td>
<td><em>Lactococcus lactis</em> subsp.</td>
<td>Heat, ethanol, acid, osmotic, and oxidative stresses</td>
<td>[162]</td>
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<td></td>
<td><em>Lactis</em></td>
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<td></td>
<td>Rhesus monkeys</td>
<td>Delayed aging</td>
<td>[163]</td>
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<tr>
<td></td>
<td>Humans</td>
<td>Delayed aging</td>
<td>[164]</td>
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<tr>
<td>Hypergravity</td>
<td><em>Drosophyla sp.</em></td>
<td>Thermotolerance</td>
<td>[150]</td>
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<td></td>
<td></td>
<td>Longevity, delayed aging</td>
<td>[150,165]</td>
</tr>
<tr>
<td>Hydrostatic pressure</td>
<td>Mouse blastocysts</td>
<td>Improved survival</td>
<td>[166]</td>
</tr>
<tr>
<td></td>
<td>Pig oocytes</td>
<td>Improved survival</td>
<td>[167,168]</td>
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<tr>
<td></td>
<td>Bull, boar spermatozoa</td>
<td>Improved semen quality</td>
<td>[169,170]</td>
</tr>
<tr>
<td>Shear forces</td>
<td>Liver tissue</td>
<td>Improved survival</td>
<td>[171]</td>
</tr>
</tbody>
</table>
How Might Air Plasma Mimic Innate Immunity?

Following damage or infection:

*respiratory burst* (animals)

*oxidative burst* (plants)

Innate Immune System Chemistry

Dedon and Tannenbaum, 2004
Hypothesis: *Plasma medicine mimics the innate immune system and induces cellular RONS/redox as key signaling agents and acts therapeutically by inducing adaptation and apoptosis*

“Immuno-mimetic, oxy-nitroso burst stress model”
What is our *Model* for Plasma Cancer-Therapy?

![Diagram of plasma cancer therapy model]

- **Plasma device**
  - Gas Phase RONS: O, OH, O3, O2-, NO, NO2
  - Liquid Phase RONS (products)

RONS gas species: transfer into liquid

(Liquid: water, salt, proteins, lipids; RONS react to form products)

Surface cells: RONS-macromolecule products induce stress that causes signaling to bulk tissue: *tumor apoptosis*

- Cell-cell communication: 'bystander effect' leading to tumor apoptosis?
- Immune cell involvement?
- Plasma-induced increased blood flow/O2?

Surface Tissue/Tumor
Huge Literature on Pro-Oxidant Anti-Tumor Mechanism.....for example

The emerging role of reactive oxygen species in cancer therapy

Markus F. Renschler *

ROS stress in cancer cells and therapeutic implications

Helene Pelicano a, Dennis Carney a, b, Peng Huang a, *


Oxidative stress and apoptosis: a new treatment paradigm in cancer

Ryan H. Engel, and Andrew M. Evens

[Frontiers in Bioscience 11, 300-312, January 1, 2006]
We propose to target the antioxidant mechanism of tumor adaptation by an anticancer therapy... by treating cancer cells either with ROS-inducing therapies or with antioxidant inhibiting therapies.
“The vast majority of all agents used to directly kill cancer cells (ionizing radiation, most chemotherapeutic agents and some targeted therapies) work either through directly or indirectly generating ROS that block key steps in the cell cycle....”

“A common ROS-mediated way through which almost all anti-cancer agents induce apoptosis explains why cancers that become resistant to chemotherapeutic control become equally resistant to ionizing radiotherapy.”

*Plasma RONS-based therapy must offer advantages over existing therapies!*

Can plasma therapy overcome resistance??
NO\textsubscript{x} Cancer Therapy: A Possible Mechanism for Plasma to Overcome Resistance?

Need Reliable Targeting in Tumors at High Concentration*

*Targeting nitric oxide for cancer therapy
David Hirst and Tracy Robson
JPP 2007, 59: 3–13
Air plasma (making various nitrogen oxides and OH) adjacent to water will form solutions of nitrite (NO$_2^-$), nitrate (NO$_3^-$) and hydrogen peroxide (H$_2$O$_2$), among others.

Unbuffered solutions will become acidic: pH $\sim -\log (C_{NO_3^-})$.

These react to form NO, NO$_2$, HO$_2$ / O$_2^-$, ONOOOH / ONOOO$^-$ and OH in solution.
Air Plasma - Water: NO, NO$_2$, ONOO$^-$

\[
\text{NO}_2^{(aq)} + \text{NO}_2^{(aq)} + \text{H}_2\text{O}_{(l)} \rightarrow \text{NO}_2^- + \text{NO}_3^- + 2\text{H}^+
\]

\[
\text{NO}^{(aq)} + \text{NO}_2^{(aq)} + \text{H}_2\text{O}_{(l)} \rightarrow 2\text{NO}_2^- + 2\text{H}^+.
\]

\[
\text{NO}_2^- + \text{H}^+ \rightarrow \text{HNO}_2
\]

\[
2\text{HNO}_2 \rightarrow 2\text{N}_2\text{O}_3 + \text{H}_2\text{O}
\]

\[
\text{N}_2\text{O}_3 \rightarrow \cdot\text{NO} + \cdot\text{NO}_2
\]

\[
\text{NO}_2^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{O}==\text{NOOH} + \text{H}_2\text{O}
\]

\[
\text{O}==\text{NOOH} \leftrightarrow \text{OH}^- + \text{NO}_2^-.
\]

\[
\text{O}_2^- + \text{NO}^- \rightarrow \text{O}==\text{NOO}^-
\]

\[
\text{OH}^- + \text{NO}_2^- \rightarrow \text{O}==\text{NOO}^- + \text{H}^+.
\]

(Lundberg et al., 2008; Lukes et al., 2014)
NOx leads to radio- & chemo-sensitization or apoptosis: can plasma do the same?
Summary of Ideas

• Atmospheric pressure air plasmas create large quantities of reactive oxygen and nitrogen species, as well as photons, electric fields and charges; RONS effects probably dominate biochemically.

• $\text{H}_2\text{O}_2/\text{OH}$ are key ROS; NO/NO$_2$/NO$_2^-$/ONOO$^-$ are key RNS

• Plasma can deliver locally high concentrations of key RONS to cells at tissue surface; RONS-based cell-cell communication probably transmits effects to adjacent tissue.
Summary of Ideas, continued

• RONS are also *generated within cells* as a generalized stress response: cells subject to RONS undergo either adaptation and survive or apoptosis and die.

• This is thought to make the tissue/organ/organism stronger.

• This concept has been discussed in terms of lifespan effects as well as radiation biology and oncology.
Implications for Plasma Cancer Therapy

- Generally accepted that chemotherapy and radiotherapy for cancer operate via oxidative stress generation; often followed by tumor resistance to treatment (an example of stress adaptation??)

- However, NO and related compounds have been shown to re-sensitize tumors to chemo- and radiation-therapy.

- Suggest that plasma might generate useful fluxes of these RONS to tumors, leading to more effective therapy.
Concluding Remarks

1. Oxidative stress might be better thought of as *oxidative shielding*: evolved from innate immunity and designed to protect the cell from attack and limit the spread of infection (Naviaux, 2012).

2. Endogenous or exogenous ROS ‘generates regulated, intrinsic stress in a population of cells that has sustained some form of external stress.’ (Klammer et al. 2014)

3. Goal is ‘to select cells that are healthy and thus fit to become part of the healing process; weak or genomically altered cells may be removed by apoptosis - a tissue clearance process that reduces the risk for cancer induction.’ (Klammer et al. 2014)
Concluding Remarks

Plasma application to surface cells creates and/or amplifies a natural form of stress - oxynitroso stress - that can be communicated to adjacent (and perhaps distant) cells.

By forcing cells to either adapt and get stronger or die through apoptosis, oxynitroso stress acts therapeutically. It mimics the immune system and the natural protective mechanisms that have evolved over hundreds of millions of years.

“Immuno-mimetic, oxy-nitroso burst stress model”
A well-known quote from a Professor of Medicine to medical students:

“50% of what we teach you is lies....

....we just don’t know which 50%!”