DEVELOPMENT OF A MECHANISTIC MODEL FOR NRF2 AND OXIDATIVE STRESS IN THE CONTEXT OF THE AOP FRAMEWORK

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OUTLINE OF TALK

• Background of project and objective for the development of the oxidative stress AOP
• Scope of the systems model
• Approaches for translation of model for decision making
MAIN AIMS OF USING THE OXIDATIVE STRESS SYSTEM AS A CASE STUDY

• Build capability in understanding how to apply this approach to a general stress mechanism

• Understand the behaviour of an integrated system centred on homeostasis control to enable chemical risk assessment

• Define molecular events that lead to adverse effects and select appropriate biomarkers and pathways based on relevant biology

» so that adverse effects measured in *in vitro* systems could be correctly interpreted in the context of risk for human health
OXIDATIVE STRESS - OVERVIEW

Balance between ROS production and ROS removal by scavenging mechanisms

Cellular / Environmental Sources, Cytosolic / Mitochondria

Catalase, Thierodoxin, GPx
Non-enzymatic eg. Glutathione, Ascorbate

Transcriptional Adaption eg. Nrf2

Downstream consequences
Altered cell signalling, Protein oxidation, Lipid Peroxidation, DNA damage, Mitochondria damage

Removal

Altered Cellular processes
Apoptosis, Necrosis, Proliferation, Differentiation, Inflammation,
An existing biochemical circuit in the cell that, when sufficiently perturbed, is expected to result in an adverse health effect.
OXIDATIVE STRESS AOP UNDERSTANDING
COMPLEX INTERACTIONS

Modified network of oxidative stress as depicted by sbv improver.
https://sbvimprover.com/
SCOPE OF LIVER OXIDATIVE STRESS SYSTEMS MODEL
SCOPE OF LIVER OXIDATIVE STRESS SYSTEMS MODEL – NRF2 SUBMODULE
IN VITRO MEASUREMENTS - REDOX IMPACT

Key Parameters – Inputs; Modulators
Outputs; Signal transducer

- Measurement of intracellular Reactive Oxygen Species in HaCaT cells treated with hydrogen peroxide for 2 hours
- Measurement of intracellular Reactive Oxygen Species in HaCaT cells treated with quercetin for 3 hours
- Measurement of intracellular Reactive Oxygen Species in HaCaT cells treated with 100uM and 200uM hydrogen peroxide over time
- Measurement of intracellular Reactive Oxygen Species in HaCaT cells treated with 100uM, 500uM and 1000uM quercetin over time

Graphs showing the ratio of GSH/GSSG over time for different treatments:
- Curcumin (10 μM)
- tBHQ (50 μM)
- H₂O₂ (0.5 mM)
- Quercetin (10 μM)
Current data does not show a robust protein carbonylation response in HaCat cells at exposure concentrations that have been shown to induce other markers of oxidative stress.
IMPACT OF NRF2 ON KEY PARAMETERS

20 µM Cur

50 µM tBHQ

10 µM Que

500 µM H2O2

NQO1

HO1

TNFα

GCLC
IN SILICO SIMULATION NRF2 AND KEAP1 KNOCKOUT STUDIES COMPARISON

Results from literature [Wu]

Simulation results

Kai Connie Wu et al, Beneficial Role of Nrf2 in Regulating NADPH Generation and Consumption, TOXICOLOGICAL SCIENCES 123(2), 590–600 (2011)
## IN SILICO SIMULATION PROTEIN THIOL BUFFERING

<table>
<thead>
<tr>
<th>Type of thiol</th>
<th>description</th>
<th>Experiment results</th>
<th>Simulation Results</th>
</tr>
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<tbody>
<tr>
<td>PSH</td>
<td>% of total Protein thiols</td>
<td>56 +/-10</td>
<td>55%</td>
</tr>
<tr>
<td>PSSG</td>
<td>% of total Protein thiols</td>
<td>19 +/-4</td>
<td>20%</td>
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<tr>
<td>PSSP</td>
<td>% of total Protein thiols</td>
<td>25 +/-11</td>
<td>25%</td>
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<tr>
<td>GSH</td>
<td>% of total GSH equivalents</td>
<td>37 +/-14</td>
<td>31%</td>
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</table>

## STRESS CONDITIONING SIMULATION – REPEAT DOSE

<table>
<thead>
<tr>
<th>Stage of expt</th>
<th>Parameter</th>
<th>Fold change in reference [JZC]</th>
<th>Fold change in our simulations</th>
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<tbody>
<tr>
<td>After first pulse</td>
<td>MDA</td>
<td>Approximately 2</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>4-HNE</td>
<td>Approximately 2</td>
<td>1.945</td>
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<tr>
<td></td>
<td>GST protein at 2 hrs</td>
<td>2.7</td>
<td>2.82</td>
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<tr>
<td></td>
<td>SH-HNE elimination rate at 2 hrs</td>
<td>2.9</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>GSH at 2 hrs</td>
<td>Not shown</td>
<td>0.85</td>
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<tr>
<td>After challenge dose</td>
<td>4-HNE any point of time</td>
<td>&lt;1.5</td>
<td>1.31</td>
</tr>
</tbody>
</table>

*Ji-Zhong Cheng et al, Accelerated Metabolism and Exclusion of 4-Hydroxynonenal through Induction of RLIP76 and hGST5.8 Is an Early Adaptive Response of Cells to Heat and Oxidative Stress J. Biol. Chem. 2001, 276:41213-41223*
STRESS CONDITIONING SIMULATION – REPEAT DOSE

Cytosolic MDA

Cytosolic 4-HNE

Elimination of 4-HNE

Cytosolic GSH
DETERMINATION OF REDOX SENSITIVE COMPONENTS OF THE MODEL – GLUTATHIONE REDOX POTENTIAL
TRANSLATION – CONTEXT VIA CORRELATE LEVELS OF CELL DAMAGE AND RECOVERING TO PHYSIOLOGICAL PROCESS

From S. Mrakic-Sposta et al. OxiMed2012-973927
TRANSLATION – NEW TOOLS FOR IMPROVED QUANTITATIVE TIME RESOLVED RESOLUTION
SUMMARY/NEXT STEPS

• Have used the AOP approach as a framework to describe the relationship between key events across scales for oxidative stress.

• Developed a model to quantify the relationships between the events and understand the homeostatic control of the system. Reiterative approach to assess and refine model.

• Have tools in place to determine some of the key parameters however there are still further required.

• Looking at how we can use the outputs to understand the adaptive/adverse continuum to enable decision making.

• Further additional work is necessary to finalise the model, eg. biokinetics to aid translation to risk assessment.
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