Iodide reduces cachexia in a BALB/c CT26 mouse tumor model

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December 7th, 2019

12th International Conference on Cachexia, Sarcopenia & Muscle Wasting
Introduction to ERA’s and the MOA of Iodide

• Elemental Reducing Agents (ERA’s)
• Mechanism of Action (MOA) of iodide
Elemental Reducing Agents

Sulfide
\[ \text{Na}_2\text{S} \rightarrow \text{Na}^+ + \text{S}^- \]

Selenide
\[ \text{Na}_2\text{Se} \rightarrow \text{Na}^+ + \text{Se}^- \]

Iodide
\[ \text{NaI} \rightarrow \text{Na}^+ + \text{I}^- \]
Elemental Reducing Agents

- Sulfide (S⁻) has been shown to prevent death in models of lethal hypoxia (1), increase survival during hemorrhagic shock (2) and also prevent cardiac and skeletal muscle damage in models of ischemia/reperfusion (3, 4).

- Iodide (I⁻) can reduce infarct size in mouse (8), rat & pig models (9) of I/R.

- In results recently presented at the 2019 American Heart Association meeting, we showed that administration of FDY-5301 (sodium iodide) decreased median infarct size from 14.9% (placebo) to 8.5% (2 mg/kg FDY-5301) (not statistically significant).
Iodide can inhibit neutrophil chemotaxis (10) and is an effective therapeutic for the treatment of: erythema nodosum & nodular vasculitis (11), as well as acute febrile neutrophilic dermatosis (Sweet's Syndrome) (12).


• Iodide markedly reduces gene expression levels of inflammatory cytokines in a mouse model of SDS induced inflammation (13)


• Iodide reduces inflammation in rat model of carrageenan induced inflammation (14)


• Iodide has recently been shown to reduce markers of intramuscular inflammation (mouse I/R) and prevent muscle cachexia (15)

15. Insko, M.A., Iodide reduces intramuscular inflammation following hind limb ischemia in mice, in 12th international SCWD conference on cachexia, sarcopenia and muscle wasting. 2019: Berlin, Germany.
The catalytic disproportionation of \( H_2O_2 \) by iodide was formally characterized and empirically validated by Abel (16) in 1928 and subsequently verified by Liebhafsky in 1932 (17).


\[
\text{I}^- + \text{H}_2\text{O}_2 \rightarrow \text{IO}^- + \text{H}_2\text{O} \\
\text{IO}^- + \text{H}_2\text{O}_2 \rightarrow \text{I}^- + \text{H}_2\text{O} + \text{O}_2
\]

Net reaction:
\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

In a pig model of cardiac I/R we demonstrated that a 1 mg/kg i.v. bolus of sodium iodide (given 5 min prior to reperfusion) significantly increases peroxidase activity (9)

We have evidence of similar peroxidase activity in human plasma spiked with sodium iodide to mimic the \( C_{\text{max}} \) following a 0.5, 1, or 2 mg/kg i.v. bolus (unpublished)
## Iodide vs ‘sacrificial’ antioxidants

<table>
<thead>
<tr>
<th>Iodide</th>
<th>Vitamin C</th>
<th>Tocopherols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalytically disproportionates H$_2$O$_2$, therefore reducing subsequent emergence of other ROS such as hydroxyl (•OH) and superoxide (O$_2^-$) free radicals (16, 17)</td>
<td>Vitamin C and E (and analogues) are converted from anti-oxidant to pro-oxidant over time (19, 20)</td>
<td></td>
</tr>
<tr>
<td>Endogenous thyroid hormones are de-iodinated in response to injury releasing free iodide therefore increasing availability (18)</td>
<td>endogenous levels are depleted early during critical illness (21)</td>
<td>Vitamin E concentrations in critically ill patients are significantly reduced (24)</td>
</tr>
<tr>
<td>Half life following i.v. administration is approximately 8 hours (healthy volunteers) to ~20 hours (AMI patients) (unpublished)</td>
<td>Half life following i.v. administration ~ 30 minutes (22) to 1.87 hr (23)</td>
<td>Vitamin E delta-tocotrienol (VEDT) Half life of 1.7 – 5.9 hr following p.o. administration (25)</td>
</tr>
</tbody>
</table>

BALB/c CT26 mouse tumor model

- Implications of reactive oxygen species (ROS) in cachexia
- BALB/c tumor model design and results
Role of oxidative stress in cachexia

![Diagram showing the role of oxidative stress in cachexia](image)

Figure 2: Molecular mechanisms involved in cachexia are modulated by oxidative stress. Atrophic factors can generate oxidative stress in skeletal muscle by the activation of different sources of reactive oxygen species, such as the mitochondria, xanthine oxidase (XO), and NADPH oxidase complex with Nox subunit, in addition to the decrease in antioxidant species. Oxidative stress is able to produce mitochondrial dysfunction, increase ubiquitin proteasome system activity, increase myonuclear apoptosis, decrease the protein synthesis pathway, and deregulate autophagy, all of which are involved in cachexia-skeletal muscle atrophy.

Model Design

In-life phase

Day (-15)
Animal Acclimatization

Day (-8)
Cell injection 2 x 10^6 cells/animal

Day 0
Randomization TV 125-150mm^3 Study initiation

Day 21

Tumor volume (mm^3): 0, 3, 6, 9, 12, 15, 18 & 21.

End Points:
Body weight (% body weight loss),
Daily food intake
Clinical observations
Blood sampling – Biochemical & cytokine analysis
Organ & Muscle weight
Histology of muscle
## Treatment Groups

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Cell line</th>
<th>Tumor volume (Study initiation)</th>
<th>No. of Animals</th>
<th>Treatment</th>
<th>Dose</th>
<th>Route</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine colorectal carcinoma</td>
<td>T26</td>
<td>When TV reaches 100mm³</td>
<td>10</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10+3</td>
<td>Vehicle Control 0.5% CMC</td>
<td>-</td>
<td>p.o</td>
<td>QD x 3 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10+3</td>
<td>FDY-5301 2mg/kg i.v</td>
<td>2mg/kg</td>
<td>i.v</td>
<td>QD x 3 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>Bucindolol 2mg/kg p.o</td>
<td>2mg/kg</td>
<td>p.o</td>
<td>QD x 3 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10+3</td>
<td>FDY-5301 40µg/day slow release via Alzet osmotic pump (flow rate-0.11µL/h)</td>
<td>40µg/day s.c</td>
<td>s.c</td>
<td>20 days</td>
</tr>
</tbody>
</table>

- We chose a beta blocker as a positive control as they have shown promise in preventing cachexia related to cancer (27) and heart failure (28).


**Note:** *On day 14, 1 h post dosing, blood sampling will be carried out - 3 animals from group 2, 3 & 5.*
Mean Tumor Volume ($\text{mm}^3$) & Tumor Growth Kinetics

- FDY-5301 & Bucindolol inhibit tumor growth by 23 - 31%

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Tumor volume ($\text{mm}^3$) day 20 Mean ± SEM</th>
<th>% Tumor growth inhibition on day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group -1</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>*Group -2</td>
<td>Vehicle control 0.5 % CMC (10mL/kg, p.o; QD x 3 weeks)</td>
<td>2361 ± 64</td>
<td>-</td>
</tr>
<tr>
<td>*Group -3</td>
<td>FDY-5301 (2mg/kg, i.v; QD x 3 weeks)</td>
<td>1623 ± 83</td>
<td>31***</td>
</tr>
<tr>
<td>Group -4</td>
<td>Bucindolol (2mg/kg, p.o; QD x 3 weeks)</td>
<td>1791 ± 100</td>
<td>24***</td>
</tr>
<tr>
<td>*Group -5</td>
<td>FDY-5301 (Slow release via Alzet osmotic pump flow rate-0.11µL/h)</td>
<td>1825 ± 189</td>
<td>23***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM of 10-13 animals in each group
Statistical analysis carried out by Two-way ANOVA followed by Bonferroni post tests using Graph Pad Prism (Version.5)
*** p<0.001 when respective test groups (FDY-5301 & Bucindolol) were compared with vehicle control group.
% Mean Body Weight Change

- FDY-5301 & Bucindolol help maintain body weight

<table>
<thead>
<tr>
<th>Group</th>
<th>% Mean body weight change (Day20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group -1 – Normal</td>
<td>8% gain</td>
</tr>
<tr>
<td>Group -2 – Vehicle control- 0.5 % CMC (10mL/kg, p.o; QD x 3 weeks)</td>
<td>-8% loss</td>
</tr>
<tr>
<td>Group -3 - FDY-5301 (2mg/kg, i.v; QD x 3 weeks)</td>
<td>19% gain</td>
</tr>
<tr>
<td>Group -4 - Bucindolol (2mg/kg, p.o; QD x 3 weeks)</td>
<td>19% gain</td>
</tr>
<tr>
<td>Group -5 - FDY-5301 (Slow release via Alzet osmotic pump flow rate-0.11µL/h)</td>
<td>27% gain</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM of 10-13 animals in each group.
Based on cage side observations, there were no visible signs of abnormal behavior or clinical symptoms in any of the treated groups.
Percentage change in tumor free body weight of Balb/c mice treated with FDY-5301 & Bucindolol in animals bearing subcutaneous CT26 syngeneic tumor

- FDY-5301 and Bucindolol significantly improve body weight over the course of the study.
- Based on ethical reasons and tumor end points, all animals in all experimental groups were humanely euthanized on day 20.

Values are expressed as Mean ± SEM of 10-13 animals in each group. Based on cage side observations, there were no visible signs of abnormal behavior or clinical symptoms in any of the treated groups.
Mean feed weight of animals during the study

- The feed consumption pattern was similar in all the groups during the experimental period except vehicle control group, where marginal decrease in feed consumption was observed during the last days of experiment.
Mean Tumor weight

- FDY-5301 (i.v.) significantly decreases tumor weight, p<0.001
- FDY-5301 (osmotic pump) & Bucindolol significantly decrease tumor weight, p<0.01

Values are expressed as Mean ± SEM of 10 animals in each group.

Statistical analysis was carried out by one way ANOVA using Graph Pad Prism (Version.5).

*** p<0.001 & ** p<0.01 when treatment groups were compared with Vehicle control (Cachexia) group.
Average Organ Weight

- FDY-5301 (i.v. & osmotic pump) & Bucindolol preserve body weight, liver and heart weight.
- FDY-5301 (i.v.) & Bucindolol prevent lipolysis (preservation of epididymal fat).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight on day 20 (g)</th>
<th>Liver (mg)</th>
<th>Heart (mg)</th>
<th>Lung (mg)</th>
<th>Spleen (mg)</th>
<th>Kidney (mg)</th>
<th>Epididymal Fat (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24 ± 0.5</td>
<td>1282 ± 28</td>
<td>145 ± 5</td>
<td>167 ± 5</td>
<td>111 ± 4</td>
<td>208 ± 10</td>
<td>324 ± 15</td>
</tr>
<tr>
<td>Vehicle</td>
<td>19 ± 0.2</td>
<td>1142 ± 31</td>
<td>113 ± 2</td>
<td>152 ± 2</td>
<td>335 ± 24</td>
<td>214 ± 5</td>
<td>119 ± 4</td>
</tr>
<tr>
<td>FDY-5301 (i.v.)</td>
<td>25 ± 0.5***</td>
<td>1406 ± 47***</td>
<td>129 ± 5*</td>
<td>153 ± 4</td>
<td>274 ± 20</td>
<td>196 ± 7</td>
<td>178 ± 15*</td>
</tr>
<tr>
<td>Bucindolol</td>
<td>25 ± 0.6***</td>
<td>1368 ± 56***</td>
<td>135 ± 3**</td>
<td>165 ± 4</td>
<td>273 ± 24</td>
<td>201 ± 7</td>
<td>186 ± 22**</td>
</tr>
<tr>
<td>FDY-5301 (pump)</td>
<td>26 ± 0.6***</td>
<td>1454 ± 57***</td>
<td>131 ± 5*</td>
<td>170 ± 8</td>
<td>304 ± 13</td>
<td>206 ± 6</td>
<td>167 ± 9</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM of 10 animals in each group.
Statistical analysis was carried out by one way ANOVA using Graph Pad Prism (Version.5).
# (** p<0.01) when normal (Non-tumor bearing healthy control) was compared with vehicle control group.
*** p<0.001 when treatment groups (FDY-5301 (2mg/kg, i.v), Bucindolol (2mg/kg, p.o), and FDY-5301 (40µg/day; Slow release via Alzet osmotic pump flow rate-0.11µL/h)) were compared with Vehicle control (Cachexia) group.

Mean Epididymal fat weight (mg)

- FDY-5301 (i.v. & osmotic pump) & Bucindolol preserve body weight, liver and heart weight.
- FDY-5301 (i.v.) & Bucindolol prevent lipolysis (preservation of epididymal fat).
### Average Muscle Weight

- **FDY-5301 (i.v.) & Bucindolol significantly prevent muscle cachexia, significant preservation of tibialis anterior muscle.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Gastrocnemius (mg)</th>
<th>Tibialis anterior (mg)</th>
<th>Soleus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>148 ± 11</td>
<td>60 ± 2</td>
<td>7 ± 0.5</td>
</tr>
<tr>
<td>Vehicle</td>
<td>107 ± 2</td>
<td>46 ± 2</td>
<td>6 ± 0.3</td>
</tr>
<tr>
<td>FDY-5301 (i.v.)</td>
<td>124 ± 4</td>
<td>61 ± 3**</td>
<td>6 ± 0.3</td>
</tr>
<tr>
<td>Bucindolol</td>
<td>116 ± 5</td>
<td>62 ± 3***</td>
<td>7 ± 0.3</td>
</tr>
<tr>
<td>FDY-5301 (pump)</td>
<td>110 ± 2</td>
<td>57 ± 3*</td>
<td>6 ± 0.2</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM of 10 animals in each group. Statistical analysis was carried out by one way ANOVA using Graph Pad Prism (Version 5).

- # (** p<0.01) when normal (Non-tumor bearing healthy control) was compared with vehicle control group.
- *** p<0.001 when treatment groups (FDY-5301 (2mg/kg, i.v), Bucindolol (2mg/kg, p.o), and Fdy-5301 (40µg/day; Slow release via Alzet osmotic pump flow rate-0.11µL/h)) were compared with Vehicle control (Cachexia) group.
Iodide increases muscle cross sectional area

- Muscle cross sectional area (CSA) is significantly reduced in tumor bearing animals (26.6% reduction).

- Mice treated with 40 μg/day FDY-5301 (delivered by osmotic pump) (A) had a significant increase in tibialis anterior muscle CSA compared to vehicle treatment (B).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1303.2 ± 51.4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>956.9 ± 40.5</td>
</tr>
<tr>
<td>FDY-5301 (i.v.)</td>
<td>1033.5 ± 42.7</td>
</tr>
<tr>
<td>Bucindolol</td>
<td>1073.8 ± 51.3</td>
</tr>
<tr>
<td>FDY-5301 (pump)</td>
<td>1118.4 ± 36.7</td>
</tr>
</tbody>
</table>
Biochemical Analysis of Serum

- FDY-5301 (osmotic pump) & Bucindolol significantly decrease triglycerides and VLDL, while FDY-5301 (osmotic pump) increased LDL.
- No significant change observed in: cholesterol, HDL, glucose, or total protein.
Plasma [iodide]

- Plasma iodide concentration is ~5x to ~30x higher than endogenous iodide levels following osmotic pump or i.v. bolus administration, respectively.
- Iodide was assessed using Ion Chromatography with amperometric detection.

- Plasma [iodide] time curve following i.v. or p.o. bolus (naïve mice, no tumor)
Acknowledgements

This study was run at Syngene International Ltd, (Bangalore, India) and I would like to thank:

• Balaji Ramachandran
• Aravindakshan Jayaprakash
• Rajendiran Satheesh
• Venkidusamy Rajendran

for their contributions on this project.
Conclusions

FDY-5301 (iodide)
- reduces inflammation
- reduce oxidative stress
- catalytically disproportionate hydrogen peroxide

In this model we demonstrate that iodide:
- slows tumor growth progression
- preserves body weight & organ weight
- prevents muscle cachexia
- Increases muscle CSA