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

Poster 9-16

Michael A. Insko & Stephen A. Hill, Faraday Pharmaceuticals, Seattle, WA (USA)

Introduction: Faraday Pharmaceuticals is focused on the research and development of elemental reducing agents (ERAs) and their potential applications for the treatment of critical care diseases with a focus on cardiac and skeletal muscle disease. In this study we investigated the utility of FDY-5301 (sodium iodide) or Bucindolol (as a positive control) in reducing cachexia in a CT26 mouse tumor model.
Methods: Male Balb/c mice were injected subcutaneously with a CT26 tumor cell line. Once the tumor volume reached ~125 mm3 the animals were randomized into four treatment groups: vehicle control, 2 mg/kg/day FDY-5301 i.v., 2 mg/kg/day Bucindolol p.o., and 40 µg/day FDY-5301 delivered continuously by subcutaneous osmotic pump. Clinical signs, body weight and food consumption were monitored daily, and tumor volume was monitored every 3 days. A subset of animals was sacrificed on day 14 for plasma iodide analysis. At the end of the experimental period (day 20) the remaining animals were sacrificed, and blood was collected for biochemical analysis of glucose, total protein, a lipid panel, and cytokine analysis. Various tissues and organs were weighed including: tumor, liver, heart, lung, spleen, kidney, and epididymal fat. The muscles: gastrocnemius, tibialis anterior, and soleus, were weighed and then fixed, H&E stained and analyzed morphometrically.
Results: Administration of FDY-5301 and Bucindolol significantly increased overall body weight, liver, heart, and tibialis anterior muscle weight compared to vehicle treatment, and significantly inhibited tumor growth. The group receiving continuous delivery of FDY-5301 showed a significant increase in tibialis anterior muscle fiber area compared to vehicle control. FDY-5301 (i.v.) and Bucindolol helped preserve epididymal fat weight, while FDY-5301 (continuous delivery) and Bucindolol stemmed the rise in triglycerides and VLDL.
Conclusions: Administration of FDY-5301 and Bucindolol helped retain the body weight of animals, prevented organ and skeletal muscle cachexia, and reduced tumor growth.

Background

Cachexia resulting from cancer is a complex disease with evidence pointing towards unbalanced reactive oxygen species (ROS) [1-3], and inflammation [4, 5] ultimately resulting in a loss of skeletal muscle [6] and adipose tissue [7]. Faraday has shown that FDY-5301 (sodium iodide) reduces cardiac muscle damage in animal models of acute myocardial infarction [8, 9], and reduces intramuscular inflammation [10]. The mechanism is multifactorial but includes the ability of FDY-5301 to catalytically destroy peroxide [9] and reduce inflammation [8-11]. In this study we investigated the utility of FDY-5301 to reduce cachexia in a murine cancer model.

Materials & Methods

- Male Balb/c mice (~7-8 weeks old) from Envigo, were group housed in individually ventilated cages (IVC) with 15-20 fresh air changes per hour and maintained in a controlled environment with a temperature of 22 ± 3 °C, humidity of 50 ± 20%, and a 12 hour light/dark cycle.
- Autoclaved corncob (Sparconn Life Sciences, Bangalore, India) was used as a bedding material. The animals were fed, ad libitum, with certified irradiated laboratory rodent diet (Nutrilab brand, Tetragon Chemie Pvt. Ltd., Bangalore) and had access to fresh, RO filtered water, during the study period.
- CT26 (murine colon carcinoma) cells with a viability of >90% were injected subcutaneously into the right flank of male Balb/c mice (~ 2x106 cells/ animal), and monitored for tumor growth. Once the tumor volume reached ~125 mm^3 the animals were randomized into groups.

- Group I - non-tumor bearing healthy control
- Group II – vehicle control, p.o. administration of 0.5% carboxy methylcellulose (CMC)
- Group III – FDY-5301 i.v., 2 mg/kg/day (saline as the vehicle)
- Group IV – Bucindolol p.o., 2 mg/kg/day (0.5% CMC as the vehicle)
- Group V – FDY-5301, 40 ug/day delivered via Alzet slow release osmotic pump.

- The alzet® pump, model 1004, from DURECT (Cupertino, CA) was filled with FDY-5301 and soaked overnight in saline to prime prior to sterile implantation into the left flank on the day of randomization
- Clinical signs, body weight and food consumption were monitored daily. Tumor volume was monitored every 3 days using a Vernier caliper to determine length (L) and width (W). TV (mm^3) = (LxW2)/2)
- A subset of animals were sacrificed on day 14 for plasma iodide analysis. Iodide concentration was determined using ion chromatography with amperometric detection.
- At the end of the experimental period (day 20) the remaining animals were sacrificed, and blood was collected for biochemical analysis of glucose, total protein, a lipid panel, and cytokine analysis. Various tissues and organs were weighed including: tumor, liver, heart, lung, spleen, kidney, and epididymal fat.
- The muscles: gastrocnemius, tibialis anterior, and soleus, were weighed and then fixed, H&E stained and analyzed morphometrically.

- For the evaluation of the statistical significance of organ and feed weight one-way ANOVA and for tumor growth inhibition two-way ANOVA followed by Bonferroni post hoc test was performed using Graph Pad Prism v5.0. p values <0.05 indicate statistically significant differences between groups.

- Animals were taken care as per the regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines.

Results

- Administration of FDY-5301 and Bucindolol significantly increased tumor free body weight and inhibited tumor growth (Figure 1)
- FDY-5301 and Bucindolol improved: tumor free body weight, liver, heart, and tibialis anterior muscle weight compared to vehicle treatment (Table 1) and significantly reduced tumor weight (Figure 1).
- The group receiving continuous delivery of FDY-5301 showed a significant increase in tibialis anterior muscle fiber area compared to vehicle control. (Figure 2)
- FDY-5301 (i.v.) and Bucindolol helped preserve epididymal fat weight, while FDY-5301 (continuous delivery) and Bucindolol stemmed the rise in triglycerides and VLDL. (Figure 3)

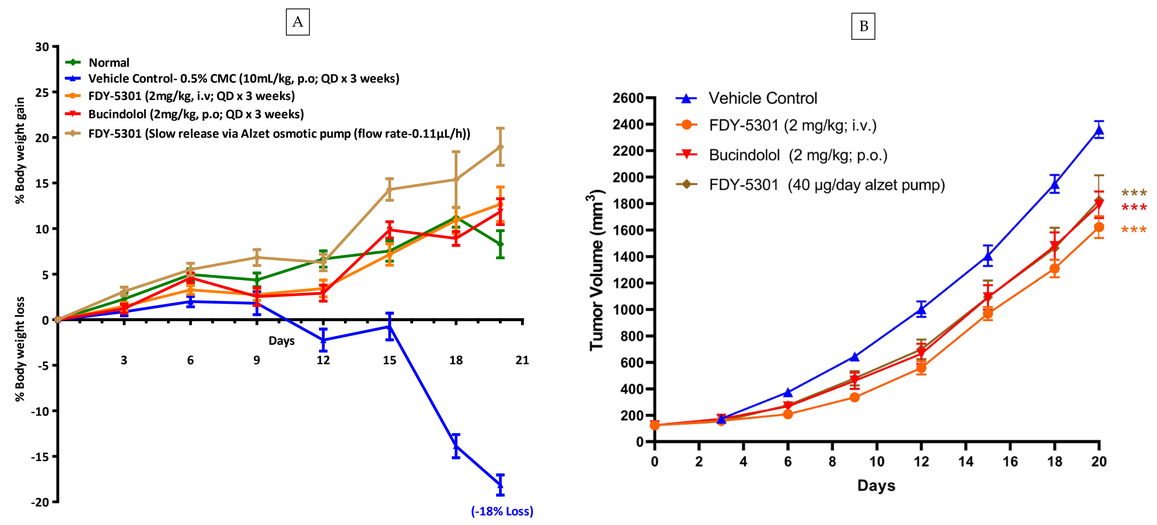

Figure 1: Iodide increases tumor free body weight and inhibits tumor growth. Mice treated with FDY-5301 or Bucindolol had significant increases in tumor free body weight (A), and a decrease in tumor growth kinetics (B) when compared to vehicle treated animals. Values are mean ± SEM (n=10-13/group). *** p<0.001, two-way ANOVA followed by Bonferroni post hoc test. FDY-5301 and Bucindolol also significantly reduced tumor weight (day 20) (C) ** p<0.01, ***p<0.001 one way ANOVA compared to vehicle.

Group	Body Weight on day 20 (g)	Liver (mg)	Heart (mg)	Lung (mg)	Spleen (mg)	Kidney (mg)	Epididymal Fat (mg)	Gastrocnemius (mg)	Tibialis anterior (mg)	Soleus (mg)
Normal	24 ± 0.5	1282 ± 28	145 ± 5	167 ± 5	111 ± 4	208 ± 10	324 ± 15	148 ± 11	60 ± 2	7 ± 0.5
Vehicle	19 ± 0.2	1142 ± 31	113 ± 2	152 ± 2	335 ± 24	214 ± 5	119 ± 4	107 ± 2	46 ± 2	6 ± 0.3
FDY-5301 (i.v.)	25 ± 0.5***	1406 ± 47***	129 ± 5*	153 ± 4	274 ± 20	196 ± 7	178 ± 15*	124 ± 4	61 ± 3**	6 ± 0.3
Bucindolol	25 ± 0.6***	1368 ± 56***	135 ± 3**	165 ± 4	273 ± 24	201 ± 7	186 ± 22**	116 ± 5	62 ± 3***	7 ± 0.3
FDY-5301 (pump)	26 ± 0.6***	1454 ± 57***	131 ± 5*	170 ± 8	304 ± 13	206 ± 6	167 ± 9	110 ± 2	57 ± 3*	6 ± 0.2

Table 1: Iodide preserves whole body, organ and muscle weight. Mice treated with FDY-5301 or Bucindolol had significant increases in: body weight, liver, heart, and epididymal fat mass, as well as an increase in tibialis anterior weight. Values are mean ± SEM (n=10/group). * p<0.05, **p<0.01, ***p<0.001 one way ANOVA compared to vehicle.

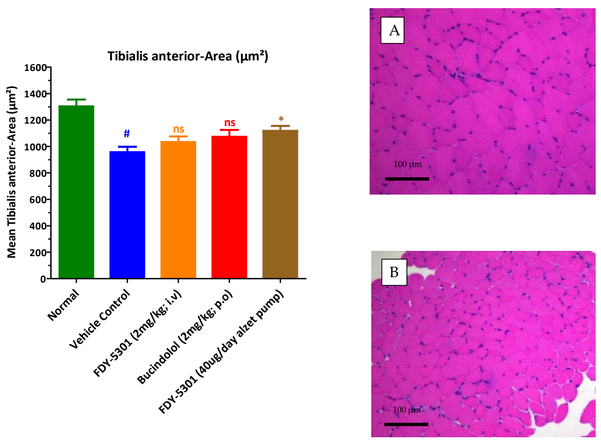

Figure 2: Iodide increases muscle cross sectional area. Muscle cross sectional area (CSA) is significantly reduced in tumor bearing animals (26.6% reduction). Mice treated with 40 µg/day FDY-5301 (delivered by osmotic pump) (A) had a significant increase(s) in tibialis anterior muscle CSA compared to vehicle treatment (B). Values are mean ± SEM (n=10/group). * p<0.05, one way ANOVA compared to vehicle.

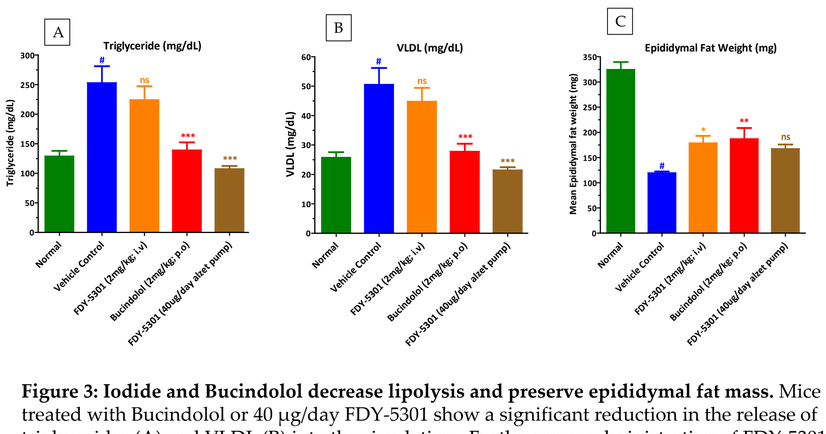

Figure 3: Iodide and Bucindolol decrease lipolysis and preserve epididymal fat mass. Mice treated with Bucindolol or 40 µg/day FDY-5301 show a significant reduction in the release of triglycerides (A) and VLDL (B) into the circulation. Furthermore, administration of FDY-5301 (i.v.) or Bucindolol help to prevent the loss of epididymal fat. Values are mean ± SEM (n=10/group). * p<0.05, ** p<0.01, *** p<0.001, one way ANOVA compared to vehicle.

Conclusions

- Administration of FDY-5301 and Bucindolol helped preserve the body weight of animals, prevented organ and skeletal muscle cachexia, and reduced tumor growth.

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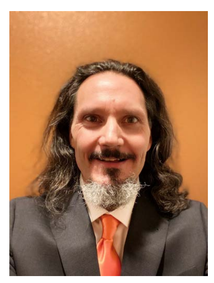
Acknowledgements

This study was run at Syngene International Ltd, (Bangalore, India) and I would like to thank: Balaji Ramachandran, Aravindakshan Jayaprakash, Rajendiran Satheesh, and Venkidusamy Rajendran for their contributions on this project.



Michael A. Insko