Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING I

Loss of FGF21 Aggravates Muscle Weakness in a Mouse Model of Critical Illness

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Introduction Critically ill patients frequently develop muscle weakness during their stay in the intensive care unit (ICU), which contributes to adverse outcome. Muscle atrophy and insufficient autophagy activation, which hampers removal of cellular damage, have been implicated. Fibroblast growth factor 21 (FGF21) mediates fasting-induced loss of muscle mass and force, in part by increasing (mitochondrial) autophagy (1). Interestingly, critically ill patients show increased serum FGF21 levels throughout ICU stay (2). We investigated whether preventing the critical illness-induced rise in FGF21 attenuates or aggravates ICU-acquired weakness and underlying pathways. **Methods** We used a clinically relevant, catheterized mouse model of critical illness evoked by sepsis (cecal ligation and puncture). Mice were fluid resuscitated for 24 hours, followed by parenteral nutrition to 40% of healthy caloric target, and received analgetics and antibiotics. Ad random, $fgf21^{+/+}$ and $fgf21^{-/-}$ mice (3) were allocated to a sepsis or a healthy (pair fed) group. After 5 days, the force of the extensor digitorum longus was measured *ex vivo*. Tibialis and gastrocnemius muscles were taken for quantification of mitochondrial enzyme activities and of expression of markers of autophagy, endoplasmic reticulum stress and atrophy. The study was continued until 15 animals per group with successful muscle force measurement had been included. **Results** Both $fgf21^{+/+}$ and $fgf21^{-/-}$ mice with sepsis had a lower muscle force and mass than the respective healthy (pair fed) mice after 5 days of caloric restriction ($p \leq 0.0001$). However, an interaction was observed ($p=0.03$) between the effects of sepsis and loss of FGF21 on muscle force. Whereas muscle force was higher in healthy (pair fed) $fgf21^{-/-}$ than in $fgf21^{+/+}$ mice ($p=0.0004$), muscle force was comparable for both groups in mice with sepsis, indicating a more pronounced effect of sepsis in $fgf21^{-/-}$ than in $fgf21^{+/+}$ mice. No such interaction was observed for muscle mass as loss of FGF21 resulted in a higher muscle mass in both healthy and septic mice. Mitochondrial enzyme activities were reduced by sepsis whereas LC3-II and the autophagy substrate SQSTM1 increased with sepsis ($p \leq 0.006$), to a similar extent in both genotypes. Sepsis increased markers of the ATF4 stress pathway and of atrophy ($p \leq 0.05$), to a higher extent in $fgf21^{-/-}$ than in $fgf21^{+/+}$ mice ($p \leq 0.05$). **Conclusion** Genetic inactivation of FGF21 aggravated sepsis-induced loss of muscle force. Increased activation of the ATF4 pathway and atrophy, rather than modulation of autophagy or mitochondrial function, may play a role. These findings contrast with the protection against muscle weakness when muscle FGF21 is absent under healthy fasting. Whether supplementation with FGF21 can improve muscle function during sepsis remains to be investigated.

1 Oost et al JCSM 2019; 2 Thiessen et al JCEM 2015; 3 Potthoff et al PNAS 2009

Neuroendocrinology and Pituitary

PITUITARY TUMORS I

Effect of Silibinin on ACTH Synthesis and Secretion in Human Adenomatous Corticotropes in Vitro

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Silibinin, a milk thistle extract with known hepatoprotective effects, has recently been shown to act upon tumoral corticotropes and revert the Cushingoid phenotype in an allograft mouse model (Riebold et al 2015). Silbinin is known to inhibit HSP90 -a chaperone to the glucocorticoid receptor- thereby restoring sensitivity to glucocorticoid negative feedback in tumoral corticotropes. **Aim of the present study** was to assess the effect of silibinin on ACTH synthesis and secretion by human corticotrope adenomas *in vitro*. **Methods**: Eight human ACTH-secreting pituitary adenomas were collected during surgery and established in culture as per our protocol (Pecori Giraldi et al 2011). Specimens were treated with 10 - 50 μM silibinin for up to 72 hours. ACTH medium levels were measured by Elisa; *POMC* expression was assessed by RT-PCR (Cassarino et al 2017). **Results**. Silibinin reduced spontaneous ACTH secretion to a variable extent in individual adenomas: from 32 to 79% of baseline at 4h, and 54 - 85 % of baseline at 48 and 72h. Silibinin was also effective in reinstating or enhancing sensitivity to steroid negative feedback: ACTH decreases during 10–50 μM silibinin incubation ranged from 10 to 63% of dexamethasone-treated wells at 4 hours, 70 -80% at 48 hours and 36 to 80% at 72 hours, indicating long-lasting effect on glucocorticoid sensitivity. Silibinin induced a variable decrease in *POMC* expression, both as regards expression in control and dexamethasone-treated wells; some specimens exhibited a marked sensitivity to the inhibitory effect, with *POMC* expression decreasing to less than 50% of control. **Conclusions**:, this data suggests that silibinin can inhibit ACTH secretion and *POMC* synthesis and restore sensitivity to negative glucocorticoid feedback. **References**: Cassarino et al (2017) *Endocrine* **55**: 853–860. Pecori Giraldi et al (2011) *Journal of Neuroendocrinology* **23**:1214–21. Riebold et al (2015) *Nature Medicine* **21**:276–280.