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Muscle Fiber Type Switch as a Potential Therapeutic Target for ALS.

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Study Description

In ALS, the motoneurons that control muscle movement become dysfunctional and die. Muscle denervation is the cause of muscle weakness, one of the first diagnostic signs of ALS. From physiological studies, large motor neurons that innervate fast-type muscle fibers are the most vulnerable in ALS. Endurance athlete's muscles are characterized by a higher percentage of slow-type fibers innervated by motoneurons thought to be resistant in ALS. Some of the physiological adaptations to endurance exercise are: increased mitochondria size and number, increased vasculature, and higher proportion of slow type fibers. From what we understand about ALS, these adaptations should be beneficial to promoting motoneuron health and function. One would then expect that endurance athletes are less susceptible to ALS; however, this is not the case. One possible explanation for this dichotomy could be that exercise induced-injury could be an explanation for the lack of exercise benefit, where the effects of this injury negate muscle adaptations that promote motoneuron health. *A possible therapeutic approach for ALS could be to induce the positive effects of exercise, (increase mitochondria number and size, switch fiber type towards a more resistant phenotype, prevent atrophy, and increase vasculature) but without any of the possible negative effects (exercise induced-injury).*

AICAR is a chemical that was given to mice that did no exercise. These mice could then run 44% farther on a treadmill than those that did not receive the drug (1). The same study showed that AICAR treated mice had more muscles with characteristics slow-type fibers than the untreated mice. While there is much controversy regarding the use of AICAR to substitute for exercise and its use in athletes, this chemical could provide the endurance exercise benefits to ALS patients while allowing them to avoid the exercise induced-injury. Furthermore, AICAR has been tested in humans for a variety of conditions.

AICAR activates AMPK. AMPK is a fuel-sensing serine/threonine kinase in cells that is activated under conditions of energetic demands, such as exercise, to restore energy balance (2). Chronic administration of AICAR activates AMPK increasing the expression of genes implicated in oxidative metabolism, mitochondrial biogenesis and in muscles a switch to slow-type fibers.

We hypothesize that activation of AMPK through AICAR administration to the $SOD1^{G93A}$ mouse model of ALS will:

a) switch muscle fiber phenotype from vulnerable (fast-type fiber) to a more resistant phenotype (slow-type fiber), and
b) increase the mitochondria content in muscle fiber and motor neurons.

As a result we expect to see an increase in muscle performance and delay in the onset and/or progression of the disease based on:

Delayed denervation and/or increased re-innervation by healthy motor units in the fibers innervated by large motor neurons (FF motor units) in the ALS mice.

Problem

This initial study was designed to determine an effective dose of AICAR. There are several reports in the literature indicating different effective doses of AICAR [1, 3, 4]. Additionally, personal communication with other investigators also indicates that the source of AICAR is critical, as one company's purity is less than another.

We had preformed initial experiments prior to this proposal using AICAR from Toronto Research Chemical (TRC). Toronto Research Chemical (TRC) sells AICAR at a more affordable price ($120.00/1g) compared to Sigma ($3140.00/1g). However, using the 250mg/kg/day dose, we failed to detect increases in AMPK activation, suggesting that the agent does not work as expected. Although in our pilot study we only used a single-dose (250mg/kg/day), data obtained from Dr. Goldberg's lab using higher doses of AICAR (500mg/kg/day) from TRC, also failed [3]. These findings raised our concern about the purity of AICAR from TRC.

Proposal and Initial Results

Determine if quality and purified AICAR induces AMPK activation in muscle and the effective dose to achieve this.

We proposed to use AICAR from Sigma instead of Toronto Research Chemical (TRC). We negotiated a generous 50% reduction in price from Sigma. The Blazeman Foundation sent funds in October 2011 to WFUSM to allow us to begin this project. Due to an unexpected delay in processing the funds, the Milligan lab did not gain access to necessary funds until mid-December 2010. We then ordered the AICAR, and learned that Sigma would have to synthesize it for us. The synthesis would require 2-4 months. We received AICAR and began experiments in March 2011. We had to breed animals to generate those that would be treated. All animals were treated and tissue collected by June 2011.

Mice were treated with different doses of AICAR (Sigma; Cat#9978; 100, 250, 500 and 1000 mg AICAR) at 30 days postnatal (P30) for 4 weeks. AICAR was freshly prepared everyday before injection, and remained on ice prior to the injection as suggested by Dr. Vihang A. Narkar. We should note that treatment with the 1 g dose was lethal in one animal. Another animal was then treated and added to that group. Following treatment, animals were killed and the tibialis anterior (TA) muscle was isolated. We analyzed the TA muscle because in mice this is composed primarily of type 2b muscle fibers. Furthermore, this muscle undergoes significant denervation by the first postnatal month in the $SOD1^{G93A}$ mouse model of ALS. Activation and increased expression of AMPK in this muscle would provide evidence that an effective dose of AICAR was achieved.

As described in the initial proposal, AICAR administration is reported to induce an increase in PGC1–α levels 24 hours after administration in muscle, presumably in response to AMPK activation. We isolated protein from the TA muscle and performed Western Blot analysis. We initially examined if there was an increase in PCG1-α; however, the antibody to this protein did not recognize a specific protein in Western Blots and as such we could not interpret results. While we could not confirm an increase in PCG1- α protein, we did however, detect an increase in RNA levels of PCG1-α in muscle of AICAR-treated mice with maximum levels detected in the 250-500 mg doses. These results suggest that treatment with AICAR results in the expected increases of the biochemical and metabolic components in muscle that has been previously reported.

To confirm that AICAR treatment was effective, we then performed Western Blot analysis to determine if treatment with AICAR resulted in activation of AMPK. As shown in Figure 1, treatment with AICAR resulted in an apparent dose-response increase in AMPK. These results, together with the lethality observed with the 1 g dose suggest that the effective dose is 500 mg.

Mitochondrial dysfunction and damage is a hallmark of ALS both in patients and animal models. AICAR administration is reported to also result in increased mitochondria biogenesis, and this would be expected to improve mitochondrial function in ALS. We are currently investigating if there is increased expression of cytochrome C, a mitochondrial protein in muscle from AICAR-treated mice. Increases in mitochondrial proteins would be further indication that AICAR administration is having the biological effects expected. We had also proposed to investigate if there were alterations in mitochondrial activity following treatment with AICAR. We

have more limited material from these initial experiments than we expected, and therefore the extent of the mitochondrial activity assays will have to be reduced.

Nonetheless, these results so far indicate that the 500 mg dosage of AICAR induces the greatest increase of AMPK protein and PGC1-α RNA expression. We expect that higher levels of AMPK and PGC1-α will have a therapeutic effect in the $SOD1^{G93A}$ mouse model of ALS. Thus, determination of this effective dose of AICAR provides "proof of concept."

We believe that these critical preliminary data provide the foundation for future experiments that will determine 1) the effect of AICAR on preventing muscle denervation and 2) if administration of AICAR can delay symptom onset and extend survival in the $SOD1^{G93A}$ mouse model of ALS.

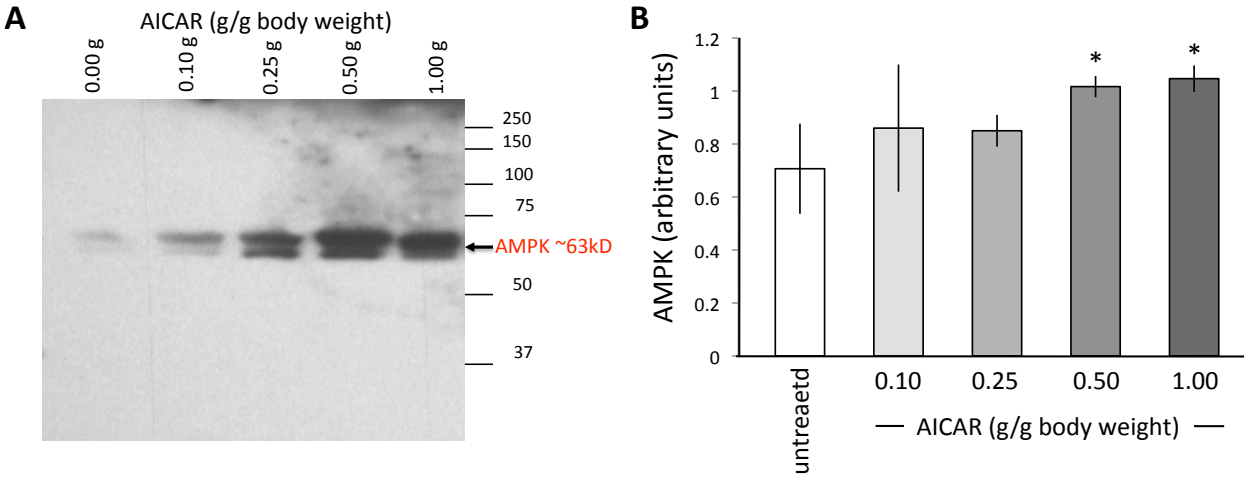

Figure 1. Treatment with AICAR increases AMPK expression in the tibialis anterior (TA) muscle. Protein was isolated from the TA muscles of untreated and AICAR-treated animals and separated on SDS-PAGE. After the protein was transferred to a PVDF membrane (Millipore), the membrane was blocked overnight at 4°C in 5% milk prepared in TBS-T. The following day the membrane was incubated with primary antibody overnight, at 4°C. After washing, the membrane was probed with an HRP-conjugated secondary antibody and the reaction product detected by ECL. Mouse anti-actin (MAB1501, Calbiochem) was used to verify equal loading. A. Shown in a representative Western Blot. B. Quantification of AMPK expression was determined by densitomitry analysis. Levels of AMPK were normalized to the level of actin for each condition. Results are expressed as the mean + SD AMPK/actin. n=3 animals/treatment group. Protein from individual mice was analyzed as an n=1. * $p<0.05$, as compared to untreated; repeated measures ANOVA followed by post-hoc test for linear trend.

References

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