Sildenafil (Viagra) improves cardiac and diaphragm muscle function in the dystrophic mouse
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Until genetic correction approaches are routinely available for treatment of muscular dystrophies, treatments that extend longevity and improve quality of life will be important alternatives. Based on our studies of one isoform of nitric oxide synthase (nNOSβ) which localizes on the Golgi with guanylyl cyclase and protein kinase G, we proposed that increasing cGMP levels by inhibition of specific phosphodiesterases (PDEs) would improve cardiac and skeletal muscle function in the mdx mouse. We examined the effect of sildenafil (Viagra), a selective inhibitor of PDE5 which is present in cardiac and diaphragm muscle. Our published results (Adamo et al., 2010, Proc. Natl. Acad. Sci. 107:19079) showed that chronic administration of sildenafil via the drinking water reduces functional deficits in the cardiac performance of aged mdx mice. Furthermore, when sildenafil treatment was started after cardiomyopathy had developed, the established symptoms were reversed within a few days. Chronic administration of sildenafil to mdx mice beginning at 3 weeks of age improved diaphragm function. At 5 months of age, diaphragms from mdx mice treated with sildenafil exhibited less Evans Blue dye uptake and markedly reduced levels of fibrosis, compared to untreated mdx diaphragms. Functional measurements showed an increase in specific force. Quantitative PCR analyses of pro-fibrotic and pro-inflammatory gene expression revealed that transcript levels for TNFα and MMP-13 were upregulated in dystrophic diaphragm. Sildenafil treatment reduced the transcripts of TNFα and MMP-13 to near normal levels. Our results suggest that sildenafil treatment of individuals with muscular dystrophies and other muscle fibrotic diseases may preserve muscle function and integrity.

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Combination anti-inflammatory and anti-fibrotic treatment in muscular dystrophy – The more the better?
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Congenital muscular dystrophy is an incurable disorder with no effective treatment. Anti-inflammatory and anti-fibrotic agents have been suggested as potential therapies. As previously demonstrated by our group, Glatiramer acetate (GA), an anti-inflammatory agent and the anti-fibrotic agent Losartan exert their effect through different pathways and have distinct beneficial effects on strength, mobility and fibrosis. Thus, the aim of the present study was to evaluate the effect of combination therapy of GA with Losartan in the dy2J/dy2J mouse model of congenital muscular dystrophy. Fore and hind limb muscle strength, fibrosis and mobility parameters were assessed. dy2J/dy2J mice receiving the combination of GA/Losartan or GA alone showed mild improvement in forelimb muscle strength (11%) in contrast to treatment with Losartan alone (74%). Hind limb muscle strength was unchanged in dy2J/dy2J mice receiving the combination therapy, while the two drugs alone showed significant improvement in strength (GA: 52.7% vs. Losartan: 74%). The combined treatment showed only mild reduction in muscle fibrosis (20%) compared with Losartan alone (42.3%). No significant change for GA; GA/Losartan combination therapy or Losartan alone showed no change in mobility parameters. However, treatment with GA alone resulted in a marked improvement in mobility parameters. In conclusion, the improvements seen with GA/Losartan combination therapy were less noticeable than the improvements of each of the two agents administered as a single medication. We conclude that combination therapy should be administered with caution. Combining two medications with theoretical synergistic effect and previous positive effect may result in a reduction of the therapeutic effect compared to the use of each of these agents separately.

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Induction of SMN protein by combination of STAT5 and p38 kinase activating, clinic ready compounds for the treatment of SMA
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Small molecule compounds correct alternative splicing of the SMN2 gene and restore SMN protein expression and function
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Spinal muscular atrophy (SMA) is caused by the reduced expression of the survival motor neuron (SMN) protein due to the loss of functional SMN1 gene and alternative splicing of exon 7 in the SMN2 gene. We are pursuing innovative drug discovery strategies aimed at restoring the production of the SMN protein by modulating SMN2 alternative splicing. Panels of cell based assays and animal models have been established and optimized to assess the effects of compounds on the splicing of SMN mRNA and the production and function of SMN protein. Using these assays, small molecules have been identified and developed that increase the inclusion of exon 7 into SMN2 mRNA and efficiently correct the splicing defect of SMN2. As a result of the increase of exon 7 inclusion, SMN protein level in SMA patient cell lines and mouse models is elevated by several fold and can even exceed that in healthy SMA carriers. These small molecules extend the lifespan of severely affected delta7 SMA mice tenfold (>150 days) and result in striking gains in motor function relative to untreated mice that live an average of 14 days. Moreover, these molecules demonstrate efficacy when treatment of delta7 SMA mice is initiated after disease onset. Lead compounds from this program are undergoing further characterization and chemical optimization with the ultimate goal of identifying molecules for preclinical and clinical development.

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Spinal muscle atrophy (SMA) is an autosomal recessive neurodegenerative disease which is characterized by the loss of α motor neurons resulting in progressive muscle atrophy. Loss of functional survival motor neuron (SMN) protein due to mutations or deletion in the SMN1 gene is the cause of SMA. A potential treatment strategy for SMA is to upregulate levels SMN protein originating from the SMN2 gene compensating in part for the absence of functional SMN1 gene. Sodium valproate, TSA and aclarubicin, which effectively enhance SMN2 expression, have been shown to activate STAT5 in vitro. Given that Prolactin is also known to activate the STAT5 signalling pathway, we elected to test its impact on SMN levels. In this manner we have demonstrated a significant induction in SMN levels in vitro upon treatment with Prolactin. We have demonstrated that activation of STAT5 pathway by Prolactin is necessary for this transcriptional upregulation of SMN gene. We have found that Prolactin treatment induces SMN expression, improves motor neuron function, ameliorates disease phenotype and increases survival in SMAA7 mice. We have previously documented that activation of p38 pathway stabilizes and increase SMN mRNA levels in vitro. We have identified and demonstrated a significant induction in SMN protein levels upon treatment with various clinic ready compounds which also activates p38 pathway. The impact of these compounds along with Prolactin on SMA mouse model is currently under investigation. This study will help in the identification and characterization of combination of potential therapeutic compounds for the treatment of SMA.

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T.P.9 Sialyllactose reversed myopathic phenotype in symptomatic GNE myopathy model mice
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GNE myopathy (GM) is caused by mutations in GNE gene, encoding an essential enzyme in sialic acid biosynthesis. We previously provided evidence that sialic acid given to clinically asymptomatic GM mice prevented the development of the myopathic phenotype, indicating that hyposialylation is one of key factors in the pathomechanism underlying GM. In this study, we examined the effect of sialic acid administration on muscle atrophy and weakness in symptomatic, older GM mice to mimic the stage of disease in symptomatic GM patients, using free sialic acid (NeuAc) and the less-metabolized sialic acid conjugate, 6′-sialyllactose (6′SL). Fifty-week-old GM mice and littersmates were given either water, NeuAc, or 6′SL for about 30 weeks. Voluntary exercise on running wheel was evaluated longitudinally at 10 week interval, while the size, force generation, and pathology of gastrocnemius muscle (GC) were evaluated at the end of the study. Voluntary exercise in non-treated model mice markedly decreased with aging, but was maintained in 6′SL group, including one mouse that showed marked recovery to non-affected control level. The NeuAc group, on the other hand, exhibited marked decrease in voluntary wheel running similar to non-treated mice. The response in running performance correlated with findings in analysis of GC, i.e., the GC size and force production in 6′SL group was recovered to levels measured in control littersmates, while those in the NeuAc group responded poorly and showed similar findings with non-treated GM mice. We show that 6′SL can ameliorate muscle atrophy and weakness in symptomatic GM mice, providing a proof of principle in the use of this compound in the clinical trial of GM patients with progressive or advanced stage of disease.

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T.P.10 The acute effects of curcumin exposure on skeletal muscle contractile function
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Curcumin is a component in the Asian spice turmeric which has demonstrated anti-inflammatory, anti-oxidant and anti-cancer effects in animal models of human disease. It has also been shown to alleviate the dystrophopathology in mdx mice. However, in vitro studies on isolated sarcoplasmic reticulum (SR) vesicles indicate that curcumin has inhibitory effects on SR function which may adversely affect muscle contraction. The aim of this study was to investigate the specific effects of curcumin on skeletal muscle contractile function. These experiments, conducted on isolated extensor digitorum longus (EDL) muscles from 8 week old ARC mice, were approved by the animal ethics committee of the University of Western Australia. Mice were anaesthetized (pentobarbitone, 40 mg/kg, IP) and the muscles surgically removed and mounted in an in vitro muscle test system containing mammalian Ringer solution bubbled with carbogen. Measures of contractile function were performed before and after 60 min exposure to either 100 μM curcumin (dissolved in DMSO) or control solution containing the equivalent DMSO concentration (0.05%). The effect of curcumin on the susceptibility to and recovery from fatigue was also assessed. Curcumin exposure significantly decreased maximum specific force in EDL muscles by 14% compared to control muscles exposed to DMSO alone (curcumin: 19.7 ± 0.7 N/cm², n = 6, control: 23.0 ± 1.0 N/cm², n = 6, P < 0.05). Curcumin had no significant effect on peak twitch force, the time to peak or the 1/2 relaxation time of twitch contractions. The rate of muscle fatigue was significantly reduced after curcumin exposure compared to controls (P < 0.01, n = 6). This study shows for the first time that (100 μM) curcumin exposure significantly affects skeletal muscle contractile function. As the twitch contraction and relaxation times are sensitive to changes in SR function, these results are not consistent with previous findings that curcumin inhibits SR Ca2+ handling in SR vesicles.

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T.P.11 Effect of formoterol, a selective β2 adrenoceptor agonist, against nerve injury-induced muscle disuse atrophy
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Skeletal muscle atrophy is common, debilitating and without acceptable treatment options. In humans, skeletal muscle atrophy occurs under various physiological and disease conditions, such as injury resulting in immobilization, critical illness, burns, cancer, COPD, liver disease, AIDS, congestive heart failure, diabetes. The reduction in strength and endurance associated with the involuntary loss of muscle mass results in functional limitations, loss of independence, reduced quality of life, increased disability, and increased mortality. Selective β2 adrenoceptor agonists, such as formoterol, elicit skeletal muscle hypertrophy, which is associated with increased force producing capacity in both fast- and slow-twitch muscles.