Adiponectin pathway in a murine model of disuse muscle atrophy: an in vivo study

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Introduction

Skeletal muscle deconditioning is an important issue for patients managed in Intensive-Care Unit (ICU) where prolonged bedrest results in the development of a Disuse-mediated Muscle Atrophy (DMA) associated to impaired regeneration potential compromising muscle recovery (Muthuvel et al., Acta Physiologica, 2009). Despite exercise training (ET) is best only effective treatment against DMA, ET tolerance limits rehabilitation. As well, mechanisms implicated in its beneficial effects must be clarified.

Skeletal muscle is an endocrine organ that secretes myokines and weakens those that results from ET. Among those myokines, adiponectin (Ad) is an adipocyte/myokine with anti-inflammatory, antioxidant, and pro-myogenic properties. While increasing evidence highlights its positive role in skeletal muscle, Ad pathway was found altered in a DMA murine model (Goto et al., PLoS ONE, 2013).

We hypothesize that muscle deconditioning is associated to Ad pathway alterations which could reinforce (i) the loss of muscle mass and (ii) the impairment of regeneration potential in a vicious circle. A murine model of Hindlimb Unloading and immobilisation (HULU) was therefore developed in our laboratory to investigate Ad pathway in a context of DMA.

Aims

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Methods

Ad protective role against DMA ?

Ad in vivo DMA murine model

Figure 2. Effect of 14 days Hindlimb Unloading coupled with immobilisation (HULU) on Adiponectin (Ad) pathway in mice. (A) Plasma Ad was measured in plasma by using the QuantiKaivit ELISA kit - Human Adiponectin/Adipose Tissue/Plasma Immunoassay. One way ANOVA, NS. (B-F) Ad-mers distribution determined by Western blot analyses. (B) S Index corresponds to HMW on total Ad-mers (Ad circulating forms) ratio (HMW/(LMW+MMW+HMW)). One Way ANOVA: NS. (C) Representative blot. (D) Western blot densitometric analysis were performed with the Image J software and each circulating form signal was normalized to total Ad-mers signal. *: p<0.05; t-test. Data represented as histogram.

Figure 3. Effect of 14 days Hindlimb Unloading coupled with immobilisation (HULU) on Adiponectin (Ad) pathway in mice. (A) Plasma Ad was measured in plasma by using the QuantiKaivit ELISA kit - Human Adiponectin/Adipose Tissue/Plasma Immunoassay. One way ANOVA, NS. (B-D) Ad-mers distribution determined by Western blot analyses. (B-D) Representative blot. (D) Western blot densitometric analysis were performed with the Image J software and each circulating form signal was normalized to total Ad-mers signal. *: p<0.05; t-test. Data represented as histogram.

AdipoR1 / Red Ponceau

AdipoR2 / Red Ponceau

T-Cadherin / Red Ponceau

Laminin

Gastronemius

Soleus

Muscle components of Ad pathway in the DMA model

Conclusions

In conclusion, we optimized a model allowing to mimetic DMA in mouse hindlimbs in the Gastrocnemius. Indeed, the decrease in muscle CSA and modifications in fibre size distribution indicate an atrophic phase. The slow-twitch fibres (Type I) were the most affected in the DMA model, which is consistent with a reduction in the muscle number. Type I myofiber size distribution was also modified in favour of fibres with medium area. This latter aspect was captured by Cross-sectional area (µm²) measured in type I fibres of soleus.

Regulation of the plasma soluble components of Ad pathway in our murine model of DMA, proportion of Low Molecular Weight (LMW) circulating forms is decreased in favor of Medium MW molecules although total plasma levels is unchanged.

Ad pathway was also found altered in disused muscles. Ad receptors and T-Cadherin co-receptor were unchanged at the mRNA and protein level in the Gastrocnemius muscle, as well as AMPK activity. However, the Soleus muscle showed a reduction in AdipoR1 protein level suggesting that disuse-deconditioned Ad pathway might not be a significant factor in reducing muscle mass in this muscular model.

Ongoing studies aim to further determine whether Ad may constitute a good therapeutic target to improve muscle mass and regeneration potential in DMA through gain and loss of function experiments.

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Figure 1. Effect of 14 days of Hindlimb Unloading coupled with immobilisation (HULU) on muscle. Soleus muscle. Cross-sectional Area (CSA) and type I and IIa fibre size distribution. Each myofiber CSA was measured on Soleus muscle cryosections following type I Ila and Iib immunofluorescence detection and morphometrical analyzes with the ImageJ software. (A) Representative field. (B) CSA was determined in all fibres (B), in type I (E) and in type Ila fibres (D). All fibres were classified in clusters according to their area (µm²) (% type I fibres (F) and % type Ila fibres (G). Chi-square: p<0.05; C, F and G. (H) Cumulative type I myofibre % and cumulative type IIa myofibre % of type I myofibres

Figure 3. Effect of 14 days Hindlimb Unloading coupled with immobilisation (HULU) on Adiponectin (Ad) pathway in mice. (A) Plasma Ad was measured in plasma by using the QuantiKaivit ELISA kit - Human Adiponectin/Adipose Tissue/Plasma Immunoassay. One way ANOVA, NS. (B) Ad-mers distribution determined by Western blot analyses. (B) S Index corresponds to HMW on total Ad-mers (Ad circulating forms) ratio (HMW/(LMW+MMW+HMW)). One Way ANOVA: NS. (C) Representative blot. (D) Western blot densitometric analysis were performed with the Image J software and each circulating form signal was normalized to total Ad-mers signal. *: p<0.05; t-test. Data represented as histogram.