Statin Adverse Effects in Skeletal Muscle are Exacerbated by Aging Process: a Biophysical and Gene and Protein Expression Study

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Skeletal muscle is a target of statin side effects, indeed patients in therapy complain muscle disorders ranging from myalgia to severe myopathy. Advanced age patients, suffering from senile muscle atrophy and loss of performance, experience an increased occurrence of statin-induced muscle disorders. Statin administration to adult rats reduces resting chloride conductance (gCl), sustained by the muscular ClC-1 chloride channel and regulated by Protein Kinase C (PKC) (Pierno et al., 2009). Resting gCl stabilize resting membrane potential and sustain muscle function. Moreover, statin treatment modifies the proteomic profile in adult rats (Camerino et al., 2011). Resting gCl is also reduced in skeletal muscle of aged rats (Pierno et al., 1999). Here we investigated if statin therapy has higher influence on skeletal muscle performance of aged subjects. Thus, we evaluated the resting gCl, by two-intracellular microelectrode technique and the resting intracellular calcium (restCa) by FURA-2 in the extensor digitorum longus (EDL) muscle of 28-months-old aged rats treated with 10 mg/kg/day atorvastatin for 1-month in comparison to the adults. Gene and protein expression were also analyzed by qPCR and Western Blot (WB). Resting gCl was reduced in the aged treated rats with respect to the adult treated ones. Indeed, it was 1319±70 $\mu S/cm^2$ (n=42) and 1789±77 $\mu S/cm^2$ (n=57) in treated and untreated aged rats and was 1884±60 $\mu S/cm^2$ (n=45) and 2534±59 $\mu S/cm^2$ (n=26) in treated and untreated adult rats, respectively. Muscle excitability was accordingly modified demonstrating a functional worsening due to statin treatment during aging. The *in vitro* application of chelerythrine, a PKC inhibitor, prevents the reduction of gCl caused by atorvastatin, demonstrating that this protein has a crucial role either in the adult or in the aged muscles.

The electrophysiological data were supported by the qPCR and WB analysis. Indeed, a more potent reduction of ClC-1 mRNA expression was observed in aged rats treated with atorvastatin with respect to the adults and for the first time we found a reduction of ClC-1 protein expression in both adult and aged treated rats. Interesting results demonstrated that the PKCα mRNA was increased in EDL muscle of aged animals but not in adult, suggesting its role in the modification of gCl due to aging process. In contrast PKCθ was found to be increased by statin treatment, in adult and aged EDL muscles. Preliminary results showed that the PKCθ protein level was accordingly modified, while the PKCα protein was not increased in aged animals. As expected from the typical sarcopenia occurring in aged animals we found an increase of atrogin-1 and MURF-1, but atrogin increased also in treated adult animals. In contrast, autophagic genes were not involved. Transcription factors related to ClC-1 channel activity (Camerino et al., 2014) were also evaluated. Indeed, an increase of Myocyte enhancer factor (MEF-2) in accord with calcineurin (CN) has been found during statin treatment in adult and aged muscles. However, Histone deacetylase (HDAC) was not modified. Since the restCa was increased by statin treatment in adult rats (Liantonio et al., 2007) we evaluated this parameter in EDL muscle of atorvastatin-treated aged rats. However, restCa was not further modified in treated aged rats as well as the RyR-1 mRNA and protein expression. Interestingly, parvalbumin mRNA slightly increased likely to buffer the higher calcium release due to statin treatment and to aging process. In this study we identified new biomarker useful to better understand the causes of increased muscle damage in elderly patients under statin therapy.

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