EFFECT OF A CHRONIC TREATMENT WITH GENTAMICIN ON IN VIVO AND EX VIVO MARKERS OF DYSTROPHIC PROGRESSION IN MDX MOUSE

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Duchenne muscular dystrophy (DMD) is caused by mutations in dystrophin gene, leading to the absence of dystrophin protein in skeletal muscle. Mdx mouse, an animal model for DMD, has a premature stop codons mutation, as occurring in 15% of Duchenne patients. The aminoglycoside gentamicin, reading through premature stop-codon mutations, has potential therapeutic application in DMD. In fact previous studies showed that 14 days of gentamicin treatment in mdx mice, partially restored muscle dystrophin level (1). The aim of this research was to evaluate the potential effectiveness of gentamicin upon a longer period of treatment (32mg/kg/day i.p. for 10-12 weeks) in exercised mdx mice, a more severe model of the pathology. A multidisciplinary approach, involving *in-vivo* evaluation of mouse strength, and *ex-vivo* electrophysiological, biochemical and histological analyses, was used**.** After 8 weeks, the gentamicin-treated mice showed a significantly higher value of normalized strength respect to untreated counterparts (6.48 ± 0.28, n=16 vs. 5.15 ± 0.19, n=6; $P<0.005$). Two microelectrode recordings were used to measure *ex vivo* macroscopic chloride conductance, gCl, and mechanical threshold (MT), a calcium-sensitive parameter. We found that gentamicin counteracted the 30% decrease of gCl, a functional index of degeneration, both in extensor digitorum longus (EDL) and diaphragm muscle fibers. However, MT in EDL muscle was not ameliorated, suggesting no effects on calcium homeostasis. A significant amelioration was observed in histological profile of gentamicin-treated gastrocnemius muscle with a 37% reduction of centronucleated fibers and a 68% decrease in degenerating area compared to untreated exercised mdx mouse muscle. Consequently, the percentage of normal fibers increased by 182% in gentamicin-treated muscle. In parallel, the treatment with gentamicin produced a significant reduction of plasma creatine phosphokinase level (9023 ± 1748 U/liter n=10 vs. 3890 ± 823 U/liter n=14). Immunofluorescence study showed an increase of dystrophin and aquaporin-4 in 20% of fibers in tibialis anterior muscle, suggesting a remodeling of the dystrophin-glycoprotein complex (DGC) by gentamicin. Thus, the long-term treatment with gentamicin maintained the efficiency to increase dystrophin expression. This latter, although partial, can significantly contrast some of the pathological events of dystrophic progression (Telethon GGP05130).

(1) Barton-Davis, Cordier L., Shoturma D., Stuart E. and Sweeney H.(1999). J. Clin. Invest. 104: 375–381.