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Commentary

A Potential New Therapeutic Approach to DMD: PKC Theta Inhibition

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Duchenne muscular dystrophy (DMD) is an X-linked muscle disease affecting approximately 1 in 3600–6000 live male births (Mendell et al., 2012) characterized by the lack of dystrophin, progressive skeletal muscle degeneration and cardiorespiratory failure. Although DMD is one of the best studied muscle disorders, no cure is still available. Gene replacement therapy constitutes the best hope for treating DMD. However, gene therapies are still under development (Muntoni et al., 2016) and the only medication so far able to retard the progression of DMD are corticoids. (Matthews et al., 2016)

Lack of dystrophin is the primary defect in DMD. In the absence of dystrophin, there is sarcolemma instability, increased calcium influx and myonecrosis (Deconinck and Dan, 2007). Cycles of muscle degeneration-regeneration promotes a chronic inflammation, which is a secondary event that further contributes to the pathogenesis of dystrophy (Villalta et al., 2009). The beneficial effects of corticoids are to reduce inflammation. However, the secondary adverse effects of corticoids stimulate the search for new drugs. In this scenario, the importance of the report made by Marrocco and colleagues (Marrocco et al., 2017) is that they offer evidence for a new potential therapy to DMD, by testing a specific inhibitor of protein kinase C theta, named compound 20 (C20), in the *mdx* mice model of DMD.

Previously, it had been shown that ablation of protein kinase C (PKC) theta ameliorated *mdx* dystrophy (Madaro et al., 2012). In the present study, Marrocco and colleagues (Marrocco et al., 2017) showed that C20 prevented T cell activation by concanavalin A, in peripheral T cells in wild-type mice, thus demonstrating the inhibitory activity of C20 on PKC theta, which is an essential factor to promote T cell activation. In the dystrophic gastrocnemius, C20 reduced myofiber degeneration (IgG-positive fibers were decreased), inflammation (decrease the levels of NF-kB and of CD45-positive cells) and improved regeneration. These results suggest that C20 was able to ameliorate a limb dystrophic muscle, at an early stage of dystrophy. Interesting, PKC theta activity seems to be required for normal muscle development, but only during earlier ages than those studied here (Madaro et al., 2012). Diaphragm and cardiac muscles are important muscles to be studied under a clinical point of view, with the diaphragm being the most affected muscle in *mdx*. Considering the cardiac muscle, C20 did not induce fibrosis in the normal heart and did not change the expression of TGFβ, a pro-fibrotic factor, in the 4 week-old wildtype mice (Marrocco et al., 2017). In the *mdx* mouse, cardiac changes, such as fibrosis and ECG abnormalities, are first seen in aged *mdx* mice (Bostick et al., 2008). Therefore, it would be interesting to investigate the effects of C20 at later stages of the disease, to verify whether C20 therapy could also counteract fibrosis formation in diaphragm and heart, since fibrosis is responsible for the functional loss of skeletal and cardiac muscles, in DMD.

An intriguing finding of the present study was the observation that C20 leads to an increase in tibialis anterior muscle mass that seemed to result from an increase in the number of myofibers, in the wildtype mice. This is a very interesting finding, deserving further studies about the mechanisms involved. Increasing the number of muscle fibers points out for the potential effects of C20 on satellite cells, considered the stem cells of skeletal muscles. Due to the cycles of degeneration and regeneration, there is exhaustion of the satellite cell pool and defects in muscular regeneration. Notch and Wnt pathways, which are involved in satellite cell renewal and myogenesis, seem to be impaired in dystrophy (Jiang et al., 2014) and whether PKC theta signaling is involved in these pathways remains to be elucidated.

The functional evaluations performed in this study need to be highlighted. Treadmill protocols are useful to exacerbate muscle damage in the dystrophic muscle, due to contraction-induced injury related to running, and are widely used in proof-of-concept studies to verify the benefits of pre-clinical drugs in vivo (Radley-Crabb et al., 2012). They performed ex vivo analysis of limb muscle contraction force (C20 prevented loss of EDL muscle force) and in the treadmill exercise, they verified that C20-treated *mdx* performed better than the untreated *mdx*, running longer distances and taking more time to reach exhaustion. The better functional performance was accompanied by a morphological improvement in both gastrocnemius and, very important, in the diaphragm muscle due to C20 therapy, as demonstrated by a significant decreased in IgG-positive fibers, an indicative of myonecrosis.

While PKC theta inhibition and C20 emerge as a potential new therapeutic approach to be tested in future clinical trials in DMD patients, we must be careful when extrapolating data from mice to humans. Several aspects still to be investigated include dosage, toxicity, long-term

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therapy, and the effects of the combination corticoid and PKC theta inhibition to the patients that are already under corticoid therapy. Another important issue to highlight for future work would be the characterization of the PKC theta -dependent immune response, since Marrocco and colleagues (Marrocco et al., 2017) only showed reduction of CD45-positive cells, but the major players in these events are still elusive, and how PKC theta inhibition may modulate immune response remains unknown. Overall, Marrocco and colleagues (Marrocco et al., 2017) work opens new perspectives in the management of muscular diseases and in the molecular pathways involved in muscle regeneration and inflammation.

Disclosure

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