CONFOCAL MICROSCOPIC ANALYSIS AS A RAPID EFFECTIVE TOOL FOR STUDIES OF EXPERIMENTAL MUSCULAR DYSTROPHY

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The organogenesis and differentiation of striated tail musculature in *Xenopus laevis* (Amphibia, Anura) are an effective experimental model for studies on muscular dystrophy pathogenesis induced by oxidative stress [1]. Stage 37/38 embryo histopathological diagnosis reveals myoblasts with defects in contractile apparatus mainly localized at cell extremities and, in stage 47 larvae, myocyte myofibrils are separated from sarcolemma and showily shrinking. Stage 37/38 embryo electron microscopic analysis shows great myofibril disorder at cell extremities and a delay in intersomitic junction membrane differentiation. These alterations are most evident in stage 47 larva myocytes [2-4]. In many muscular dystrophies, the principal defect is the loss of a mechanical link between the contractile apparatus and the extracellular matrix which somehow leads to muscle fragility, contraction-induced damage and cell necrosis [5]. Confocal microscopy allows to analyze the link among myofibril actin, the sub-sarcolemmal protein dystrophin, the intermediate filament desmin and the sarcolemma dystrophin glycoprotein complex. Actin stained with phalloidin-TRITC labelled confirms the shrinking of myofibril extremities. Desmin immunofluorescence staining (anti-desmin monoclonal antibody) shows the loss of its normal structural organization especially at the Z-disc level. Dystrophin staining (anti-dystrophin monoclonal antibody) is less intense or absent at the altered intersomitic junction membrane level. Double immunofluorescence staining with anti α, δ-sarcoglycan-TRITC labelled and with anti dystrophin-FITC labelled shows the loss of a dystrophin-actin link at intersomitic junction membrane levels responsible for the detachment and subsequent shrinking of contractile apparatus due to oxidative stress. The results confirm that confocal microscopic analysis is a rapid effective tool for studies on experimental muscular dystrophy.

References

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