P0707 UREMIC TOXINS IMPAIR SKELETAL MUSCLE REGENERATION PROCESS INDUCING CELL CYCLE ARREST AND APOPTOSIS IN CULTURED MYOBLASTS

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Background and Aims: The loss of muscle mass and function has been related to chronic kidney disease (CKD). About 37% of dialysis patients show symptoms of sarcopenia and this has been related to an increased risk of mortality. Changes in sarcopenic muscle include the loss of its regenerative capacity due to a reduction in the number and function of satellite cells, the muscle stem cells. The concentration of serum uremic toxins (UT) increases in parallel to a decline in the glomerular filtration rate in patients with CKD and this uremia may be involved in the development of sarcopenia. Previous studies showed as serum concentration of UT found in the early stages of CKD inhibits myogenic differentiation of cultured myoblasts. Nevertheless, the effect of those concentrations found in the advanced stages of CKD has not been described. The study aimed to analyse whether UT affect the muscular regeneration process by modifying the proliferation capacity of myoblasts (activated satellite cells).

Method: Cultured mouse myoblasts C_2C_{12} cells were used for all experiments. Cells were grown with 0% or 10% FBS culture media in the presence or absence of indoxyl sulphate and para-cresol at doses of 100μg/ml each one, which are similar to ones found in the advanced stages of CKD. Proliferation was evaluated by scratch wound healing and cell cycle by flow cytometry with propidium iodide and the fluorescent probe CFSE, an intracellular protein binding dye that is divided equally between daughter cells, allowing the discrimination of successive rounds of cell division. Chromosome condensation was assessed by immunofluorescence staining by confocal microscopy. Apoptosis was analysed by annexin V staining.

Results: C_2C_{12} cells treated with UT shown a significant decrease in the proliferation rate. A significant delay in wound closure was observed in cells treated with UT compared to control cells. Myoblasts treated with UT suffered a significant decrease in the proliferation rate since the probe remained higher than in the vehicle-treated cells. Proliferating cells treated with UT suffered a dramatic cell cycle arrest between the phases S and G2/M. Chromosome condensation was also analysed, finding that in the presence of colcemid, vehicle-treated cells condensed their chromosomes, as expected, whereas UT-treated cells did not, suggesting that UT stop the cell cycle at any point before the entry of cells in the mitosis phase. Besides, there was strong phosphorylation of cdc2 in the presence of UT indicating that cdc2 and the complex cdc2-cyclin B were inactive. This result explains why cells did not enter in the mitosis phase under UT exposition. Finally, UT induced the death of proliferating C_2C_{12} cells by apoptosis.

Conclusion: In the advanced stages of CKD, uremic toxins concentration increases, thereby inducing a dramatic arrest in the cell cycle of myoblasts, inactivating the cdc2-cyclin B complex, interrupting their proliferation and leading them towards cell apoptosis. These results point to a role of uremic toxins impairing the skeletal muscle regeneration process, which could be involved in CKD-related sarcopenia and frailty.