Procaine local effects on skeletal muscles in dysferlin-deficient Bla/J mice.

O.N.Chernova¹, A.A. Titova¹, M.O. Mavlikeev¹, A.K. Shafigullina¹, A.K. Zeynalova¹, F.A. Faizrahmanova¹, A.P.Kiyasov¹, R.V.Deev²,³

1 Kazan (Volga region) Federal University. Kazan, 420008, Russia
2 Human Stem Cell Institute, Moscow, 129110, Russia
3 Ryazan State Medical University named after academician I.P.Pavlov, Ryazan, 390026, Russia

Dysferlin is 230kDa transmembrane protein involved in repair of sarcolemma. Mutations in DYSF gene lead to dysferlinopathies. Dysferlinopathies are often studied on transgenic mice B6.A-Dysf<sup>prmd</sup>/GeneJ (Bla/J), that we used to demonstrate regenerative potential of dysferlin after chemical injury by procaine intramuscular injection.

Gastrocnemius muscle of 5 months old Bla/J and C57Bl/6 (control) mice was injected with 100µl of 0,1% procaine (myotoxic agent). Calf muscles were obtained at 2,4,10,14 days after injection and paraffin sections were stained with H&E, immunohistochemically with antibodies against α-SMA (capillary density), myogenin (terminal myogenic differentiation), Ki-67 (proliferation marker), MHC fast/slow (muscular functional activity).

Necrotic muscle fibers (MF) with leukocytes infiltration were found at all time points after injection with gradual reduction (35,1±9,7% vs 8,7±5,4%, respectively, p<0,001), in C57Bl/6 this parameter was significantly lower. Percentage of centrinucleated MF in Bla/J was significantly lower at 4 day (11,6±1,18% vs 22,5±4,19% in control, p=0,03), remained till 10 days. In Bla/J mice myogenin+ MF maximum was on 4<sup>th</sup> day after injection (4,4±3,9% vs 9,5±10,01% in C57Bl/6 mice, respectively, p=0,046) but significantly lower at all time points comparing with control, which is an indication of activated but incomplete terminal myogenic differentiation. Capillary density was significantly lower in Bla/J mice only on 4<sup>th</sup> day (0,15±0,04 vs 0,18±0,07 in control, p=0,03). Proliferative activity was maximal on 2<sup>nd</sup> day in both groups (13,77±11,08% in Bla/J vs 19,06±19,7% in C57Bl/6, p=0,97) and then decreased till 14<sup>th</sup> day (0,7±1,09% vs 0,8±1,10%, p=0,74). MHC slow/fast staining demonstrated higher ratio of slow MF in Bla/J in compare with control group at all data point with maximum on 10<sup>th</sup> day (19,6±22,2% vs 0,07±0,4% in control, p<0,001).

Conclusion. Procaine injection leads to severe myotoxic lesions of Bla/J mice skeletal muscles and regeneration is slower than in control C57Bl/6 mice. Work supported by Program of Competitive Growth of KFU.