9 SUMMARY

Skeletal muscles are known to serve as the main source of amino acids in various pathological conditions. Severe illnesses as sepsis, burn injury or cancer lead to the inhibition of muscle protein synthesis and particularly to the activation of proteolysis. These mechanisms provide to the organism amino acids that are indispensible for recovery. On the other hand if they persist or occur in exceed, they result in progressive lost of muscle mass, cachexia development and in final to moderate convalescence and increase in mortality.

Ubiquitin-proteasome system (UPS) represents the main intracellular mechanism for protein degradation and thus is essential for many cellular processes including increased degradation of muscle proteins in various pathological conditions. In 2004, the discovery of UPS was awarded with Nobel Price.

Importance of UPS started intensive studies of large spectrum of proteasome inhibitors (IP). To date, the first and only IP in clinical practise is bortezomid, which is used as chemotherapy agent in the treatment of multiple myeloma. This fact concentrates the majority of studies in the field of oncology and less attention is payed to other possibilities of IP exploitation, among them to moderate muscle wasting as a result of severe, long-term ilness.

The aim of my doctoral thesis was to compare synthesis and degradation of proteins in different types of skeletal muscles in two distinct catabolic states and consider the influence of IP belactosin A, C and bortezomib on mentionned processes.

We used isolated muscles of laboratory rat to mesure the direct influence of compounds tested on skeletal muscle. Regarding different structural and biochemical properties of muscles, we used m. soleus (SOL) predominantly composed of oxidative fibres and m. extensor digitorum longus (EDL) composed of glycolytic fibres. We studied the activity of UPS and cathepsins, total and myofibrillar proteolysis, protein synthesis and leucine oxidation. The influence of belactosins A and C was studied only under *in vitro* conditions, whereas the influence of bortezomib also *in vivo*. To evaluate metabolic differences between SOL and EDL we induced the inflammation either by subcutaneous turpentine administration or by endotoxin aplication. To evaluate effect of bortezomib we employed just the more complex endotoxin-based model of sepsis.

Regarding protein metabolism, SOL is considered to be more active than EDL. Turpentine induced inflammation was accompanied by protein synthesis inhibition and proteolysis activation in observed skeletal muscles. CTLA of proteasome and protein synthesis were less affected in SOL compared to EDL. Changes following endotoxin administration were similar but more pronounced. The only exception was CTLA of proteasome which was more activated in SOL compared to EDL. This can be due to differences in both structural and biochemical properties of EDL and SOL.

In agreement with the concept of the substantial role of UPS in skeletal muscles degradation, total proteolysis was decreased, as was CTLA of proteasome after the incubation with both belactosin A and C. We found a decrease in myofibrillar proteolysis in both SOL and EDL incubation with belactosin A, while exposure to belactosin C failed to affect this parametr. Therefore rate of myofibrillar proteolysis is not only a function of UPS. Belactosin A and C affected basic parameters of protein metabolism and the response is both belactosin- and muscle-type-dependent as increase in leucin oxidation occured only in SOL, whereas protein synthesis inhibition in EDL.

Bortezomib had no significant effect in healthy rats. On the other hand, it increases CTLA in muscles of septic rats after *in vivo* as well as *in vitro* administration, thus bortezomib affects proteolysis and protein synthesis mainly via direct interaction with UPS. However total and myofibrillar proteolysis in muscles of septic rats increased, which shows its stimulating effect on other proteolytical mechanisms. Moreover, bortezomib exhibited ambivalent effect on protein synthesis in skeletal muscle of septic rats, thus its protective effect on muscle mass during catabolic conditions could not be confirmed.