THE ROLE OF MYOGLOBIN PROTEOLISYS IN AKI UNDER RHABDOMYOLISYS

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OBJECTIVES

Myoglobine (Mb) is believed to be the major nephrotoxic effector under rhabdomyolysis. Recent data pointed to oxidative stress as a principal mechanism of acute kidney injury (AKI) after myoglobinuria, and the excessive reactive oxygen species (ROS) generation is a result of redox transformations of heme in Mb. ROS-mediated lipid peroxidation leads to nephron damage and renal failure. At the same time the increase of proteases activity in tubular cells after Mb injection was shown. Thus proteolysis of Mb could play an essential role in its toxic effects. The aim of this work was the investigation of the role of Mb degradation in AKI development.

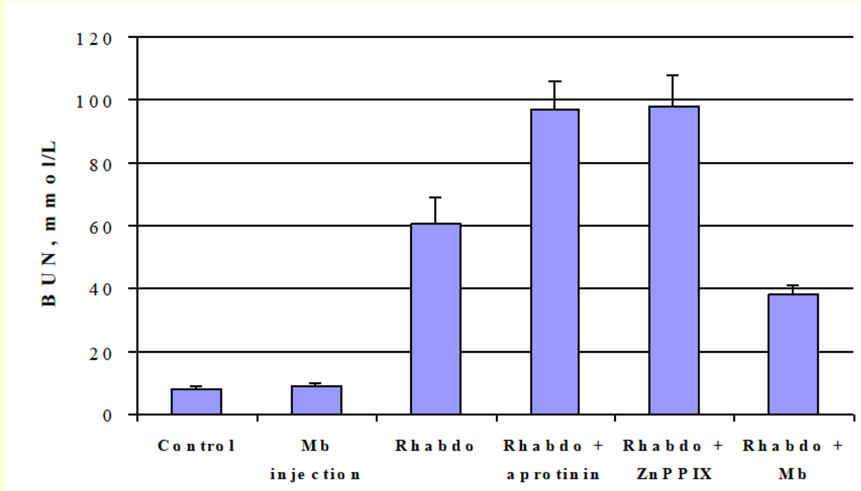

Figure 1. Development of AKI. Elevation of BUN after rhabdomyolisys and effect of MB-degradation inhibitors

METHODS

The glycerol-induced model of rhabdomyolisis in rats was used for in vivo experiments. Cultured tubular cells incubated with myoglobin were used for in vitro experiments. The development of oxidative stress was investigated by colorimetric detection of lipid peroxides products – malonic dialdehyde (MDA). The content of hemeoxygenase-1, myoglobin and carbonylated proteins in kidney was determined by Western blotting with specific antibodies. Activity of proteases in kidney tissue was estimated by zymografy

RESULTS

The development of renal failure was estimated by serum creatinine and BUN elevation (Fig.1). AKI was accompained with by oxidative stress as measured by the increased levels of malonic dialdehyde (MDA, Fig.2) and carbonylated proteins (Fig.3) in kidney tissue under rhabdomyolysis. Furthermore, we observed increased activity of enzymes, involved in Mb degradation in renal cells. Particularly, the rise of hemoxygenase-1 was revealed in kidney tissues 24 h after rhabdomyolysis induction (Fig.4). The same hemoxygenase-1 accumulation occurred in rat kidney after i/v injection of Mb. Interestingly, such Mb injection didn't caused AKI development, as creatinine and BUN concentration were 60 μM and 9 mM, respectively. However, preliminary Mb injection before rhabdomyolysis reduced the severity of AKI, lowering serum creatinine and BUN levels (Fig.3) and similarly prevented oxidized proteins accumulation (Fig.2).

The treatment of animals with experimental rhabdomyolysis with proteases inhibitor aprotinin led to higher MDA level in kidney (Fig.1) and deterioration of kidney function (increased creatinine and BUN (Fig.3)). Inhibition of hemoxygenase-1 by injection of Zn-protoporphyrin IX also caused more severe AKI in rats after rhabdomiolysis (Fig.3).

The same effects were observed in experiments with Mb cytotoxicity on renal tubular cells culture, as incubation with proteases inhibitors induced aggravation of cells death after Mb treatment (Fig.5).

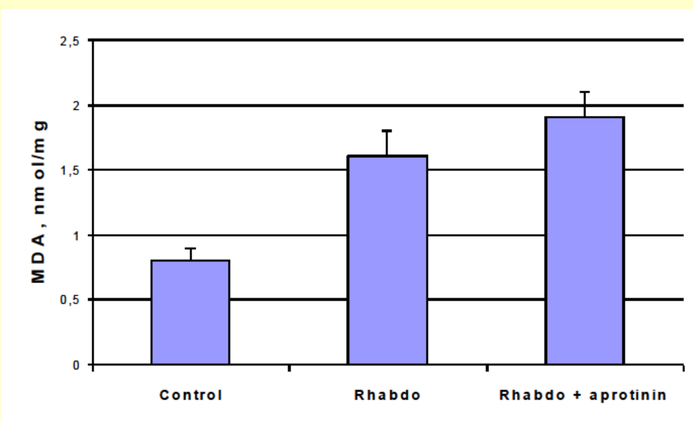

Figure 2. Accumulation of MDA in kidney tissue after rhabdomyolisys, measured by increase in thiobarbituric acid reactive substances

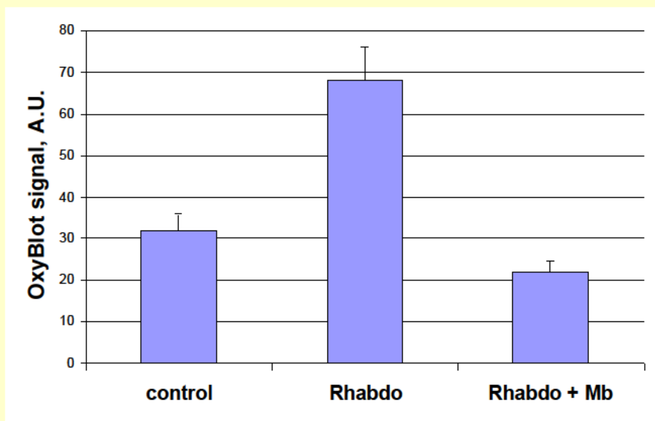

Figure 3. Accumulation of carbonylated proteins in kidney tissue after rhabdomyolisys, measured by OxyBlot kit.

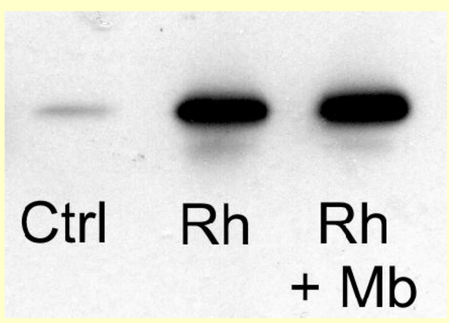

Figure 3. Hemoxygenase-1 levels in kidney tissue after rhabdomyolisys and MB treatment.

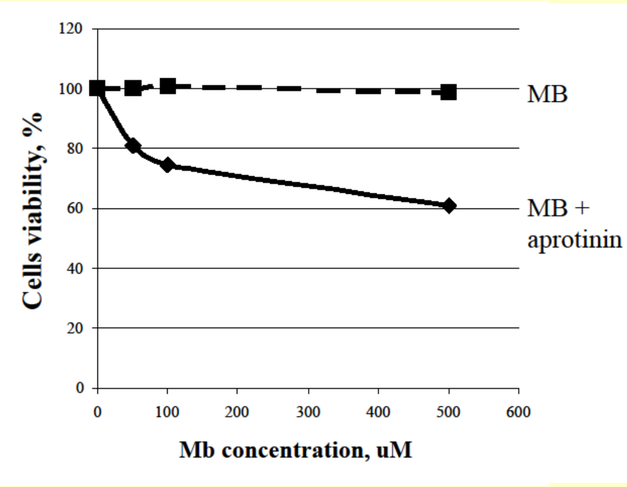

Figure 5. Mb cytotoxicity on renal tubular cells culture, estimated by MTT-test.

CONCLUSIONS

Obviously, Mb proteolysis and following heme degradation are essential steps for Mb elimination from tubules and lowering of its nephrotoxic effects. Activation of enzymes, involved in Mb degradation, could alleviate renal damage.

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