

UBIQUITIN-PROTEASOME SYSTEM CONTRIBUTES TO THE INFLAMMATORY INJURY OF EXPERIMENTAL ACUTE DIABETIC MYOCARDIAL INFARCT

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In the present study we have evaluated whether the ubiquitin-proteasome system, the major pathway for nonlysosomal intracellular protein degradation in eucaryotic cells (1), contributes to the inflammatory injury of experimental acute myocardial infarct induced in streptozotocin-treated rats. These were allocated to one of the four following groups: 1) normoglycemic rats (six sham-operated and six infarcted animals); 2) STZ-hyperglycemic rats (six sham-operated and six infarcted animals); and 3) STZ-hyperglycemic rats pre-treated with bortezomib: six rats were injected through a forepaw vein with 0.05 mg/kg bortezomib; 4) normoglycemic rats pre-treated with bortezomib (six sham-operated and six infarcted animals). Myocardial infarct was induced as previously described (2). Measurement of area at risk (AR) and infarct size (IS) were assessed by staining with Evans blue dye and by computerized planimetry using an image analysis software program. Specimens of the peri-infarct tissue were cut perpendicular to the long axis into 2 halves. The first half was frozen in liquid nitrogen for the following ELISA analyses. A portion of the other half specimen was immediately immersion-fixed in 10% buffered formalin for later immunohistochemistry. For this serial sections were incubated with specific antibodies anti-ubiquitin, anti-proteasome 20S, anti CD68 and anti-CD3; anti-I κ B- β , anti-iNOS; anti-tumor necrosis factor- α (TNF- α). Specific antibodies that selectively recognize the activated form of nuclear factor- κ B (p65 and p50 subunits) were also used. Nitrotyrosine was determined by anti-nitrotyrosine rabbit polyclonal antibody. iNOS quantitation was scored for intensity of immunostaining (0=absent, 1=faint, 2=moderate, 3=intense).

The results showed that ischemic rat lesions during hyperglycemia consistently had higher myocardial damage, ubiquitin-proteasome activity and inflammation. In contrast, the lesions from normoglycemic animals as well as from hyperglycemic rats treated with bortezomib, an inhibitor of ubiquitin-proteasome pathway, had a marked reduction in ubiquitin-proteasome activity, inflammation and myocardial damage. By contributing to the increased inflammation the ubiquitin-proteasome over-activity may enhance the risk of complication during myocardial ischemia in diabetic patients.

1) Hershko A, Ciechanover A, Varshavsky A: Nat Med 6:1073-1081, 2000

2) D'Amico M, Di Filippo C, et al., FASEB J, 14:1867-69, 2000