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Acute hyperglycemia abolishes cardioprotection by remote ischemic perconditioning T. Baranyai; CT. Nagy; G. Koncos; Z. Ondor; M. Karolyi-Szabo; A. Maklos; ZV. Varga; P. Ferdinandy; Z. Gricz Semmelweis University, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary

Background: Remote ischemic perconditioning (RIPerC) has a promising therapeutic value to improve prognosis of acute myocardial infarction. Chronic comorbidities such as diabetes are known to interfere with conditioning interventions by modulating cardioprotective signaling pathways, such as e.g. autophagy. However, the effect of acute hyperglycemia on RIPerC has not been studied so far. Therefore, here we investigated the effect of acute hyperglycemia on the cardioprotective effect of RIPerC.

Methods: Wistar rats were divided into normoglycemic (NG) and acute hyperglycemic (AHG) groups. Acute hyperglycemia was induced by glucose infusion to maintain a serum glucose concentration of 15 to 20 mM throughout the experimental protocol. NG rats received mannitol infusion of an equal osmolarity. Both groups were subdivided into an ischemic (Isch) and a RIPerC group. Each group underwent reversible occlusion of the left anterior descending coronary artery (LAD) for 40 min in the presence or absence of acute hyperglycemia. After 10 min LAD occlusion, RIPerC was induced by 3 cycles of 5 min unilateral femoral artery and vein occlusion and 5 min reperfusion. After 120 min reperfusion, infarct size was measured by triphenyltetrazolium chloride staining. To study underlying signaling mechanisms, hearts were harvested for immunoblotting after 35 min in both the NG and AHG groups.

Results: Infarct size was significantly reduced by RIPerC in NG, but not in the AHG group (NG: Isch = 46.27 ± 5.31% vs. NG + RIPerC: 24.65 ± 7.45%, p < 0.05; AHG + Isch = 54.19 ± 4.07% vs. AHG + RIPerC = 52.76 ± 3.80%). Acute hyperglycemia per se did not influence infarct size, but significantly increased the incidence and duration of arrhythmias. Acute hyperglycemia induced autophagy, as LC3II/LC3I ratio decreased and phosphorylated S6 increased possibly via the phosphorylation of Akt. Furthermore, acute hyperglycemia significantly elevated the nitrative stress in the heart (0.87 ± 0.01 vs. 0.50 ± 0.04 μmol μg−1 protein, p < 0.05).

Conclusions: This is the first demonstration that acute hyperglycemia deteriorates cardioprotection by RIPerC. The mechanism of this phenomenon may involve an increased nitrative stress and decreased myocardial autophagy induced by acute hyperglycemia.

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Deregulation of thioredoxin system contributes to monocyte dysfunction in diabetes mellitus: Implications for impaired arteriogenesis in type 2 diabetic patients R. Godfrey1; H.M. Schulter1; SK. Shanmugaran1; I. Loeffler2; N. Mueller2; G. Wolf2; UA. Mueller2; G. Koncsos; Z. Onodi; M. Karolyi-Szabo; A. Makkos; ZV. Varga; P. Ferdinandy; Z. Gricz

Semmelweis University, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary

Purpose: Arteriogenesis is a process encompassing the growth of pre-existing collateral blood vessels to form functional arteries. Monocytes play a positive role in this process. Diabetes mellitus (DM) causes monocyte dysfunction. The impaired arteriogenesis seen in DM patients is linked to the reduced ability of monocytes to respond to VEGFR1 agonists. Molecular mechanisms leading to this VEGF-specific signal transduction defect in monocytes is incompletely understood.

Methods: Monocytes from diabetic patients or non-diabetics and monocytes from db/db mice or Wt littermates were analysed. The expression of thioredoxin-1 and -2 (Trx1/2) and Trx-interacting protein (Txnip) were determined by using qPCR and Western Blot. Enzymatic activities of protein tyrosine phosphatase (PTP) and Trx were measured in the monocyte cell lysates. Pharmacological inhibitors were used to inhibit Trx and a Trx Mimetic Peptide (TMP) was used to study the effects of Trx. Ex vivo analysis of monocyte function from db/db and Wt mice was assessed by the modified Boydén chamber assay (chemotaxtant; PGF2α). Hindlimb perfusion in db/db and Wt mice with unilateral hindlimb ischemia (HLI) receiving either TMP or placebo was determined.

Results: DM led to significant downregulation of Trx1/2 and an upregulation of Txnip expression in monocytes. Likewise, Trx activity in diabetic monocytes was impaired resulting in enhanced oxidative stress. As a consequence, the total PTP activity was downregulated in hyperglycemia in a Trx-dependent fashion which resulted in VEGFR1 signal transduction defect. Blockade of Trx activity by pharmacological inhibitors in normoglycemia (non-diabetic patients and Wt mice) evoked VEGF resistance in monocytes ex vivo. On the other hand, improving Trx activity in hyperglycemia (diabetic patients and db/db mice) with the use of TMP significantly reversed monocyte dysfunction in an ex vivo assay. Daily administration of TMP to db/db mice resulted in significantly improved hindlimb reperfusion compared to db/db mice receiving placebo.

Conclusions: Deregulated Trx system contributes to higher oxidative stress-related deregulation of PT activity in hyperglycemic monocytes. Improving Trx function by supplementing Trx mimetic reperfusion in db/db mice with HLI following treatment with Trx mimetic indicating that reconstitution of monocyte function might be an important component in this recovery process. We propose functional complementation of Trx as a novel therapeutic strategy for restoring a proper arteriogenic response in the diabetic environment.

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High glucose increases gamma-glutamyltransferase-induced tissue factor expression in human peripheral blood mononuclear cells V. Scala1; C. Balia1; S. Cianchetti1; V. Carnicelli1; F. Faita1; T. Neri1; R. Zucchi1; A. Corti1; A. Celi1; R. Pedrini1

1University of Pisa, Patologia Chirurgica, Medica, Molecular e dell’Area Critica, Pisa, Italy; 2University of Pisa, Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pisa, Italy

Background: A close association connects Gamma-GlutamylTransferase (GGT) activity to acute thrombotic events that coexisting diabetes increases exponentially. Tissue Factor (TF), the leading factor of acute thrombotic complications, is also coexpressed with GGT in atherosclerotic plaques raising the issue of a direct contribution of GGT to TF activation, a possibility never explored in vivo.

Aim: To assess the effect of an enzymatically inactive human recombinant (hr) GGT on TF antigen expression and PCA, a thrombin-like clotting assay. We also evaluated TF expression in hyperglycemic (HG) conditions, in order to identify a possible coagulation defect raised by hyperglycemia.

Methods: Total mononuclear cells (PBMCs) were isolated from both normal (NG, 10 mM) and high (HG, 50 mM) glucose concentrations, the diabetic hallmark. Enzymatically inactive hrGGT or NG serum was incubated with whole PBMCs for 24 h either alone or with rat anti-human TF antibodies (2.5μg/ml). PBMCs were then washed with medium containing 10% FCS and the cell monolayer was incubated with 10% serum-free medium for 24 h. Cell cultured medium was collected and assessed for cell viability by exclusion of trypan blue (trypan blue exclusion counts). Expression of TF was measured by ELISA (pg/ml) and mRNA (real-time PCR, normalized-fold expression compared to housekeeping gene) were the evaluation variables.

Results: hrGGT increased TF PCA (from 0.008 ± 0.007 to 0.37 ± 0.3, n=14, p<0.001), ag (from 85 ± 59 to 36 ± 32, n=13, p<0.001) and mRNA (from 0.006 ± 0.002 to 0.048 ± 0.04, n=9, p<0.001) an effect inhibited by anti-hrGGT antibody (PCA: from 0.7 ± 0.3 to 0.3 ± 0.3, n=12, p<0.01; mRNA: from 0.05 ± 0.04 to 0.2 ± 0.3, n=8, p<0.001). BAY-11-7082 (10-5M) and N-acetylcysteine (NAC) (10-3M), an antioxidant. TF PCA (1-stage clotting assay, arbitrary units), ag (ELISA, pg/ml) and mRNA (real-time PCR, normalized-fold expression compared to housekeeping gene) were the evaluation variables.

Results: hrGGT increased TF PCA (from 0.008 ± 0.007 to 0.37 ± 0.3, n=14, p<0.001), ag (from 85 ± 59 to 36 ± 32, n=13, p<0.001) and mRNA (from 0.006 ± 0.002 to 0.048 ± 0.04, n=9, p<0.001) an effect inhibited by anti-hrGGT antibody (PCA: from 0.7 ± 0.3 to 0.3 ± 0.3, n=12, p<0.01; mRNA: from 0.05 ± 0.04 to 0.2 ± 0.3, n=8, p<0.001). BAY-11-7082 (NG: from 0.2 ± 0.1 to 0.08 ± 0.1, n=7; HG: from 4.3 ± 3 to 0.2 ± 0.1, n=4, p<0.001) and NAC (NG: from 0.3 ± 0.1 to 0.08 ± 0.1, n=7, p<0.001; HG: from 3.9 ± 2.8 to 0.2±0.2, n=6, p<0.001) abolished GGT-induced PCA both NG and HG conditions.

Conclusions: GGT stimulates directly TF expression in PBMCs through a NF-κB-mediated mechanism and HG amplifies that effect, potentially contributing to the atherothrombotic risk conferred by higher GGT levels, more markedly so in diabetic patients.