Acute hyperglycemia abolishes cardioprotection by remote ischemic perconditioning

Purpose:

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 0.75% (w/v) in saline to maintain a similar osmolality to AHG. Results: Infarct size was significantly reduced by RIPerC in NG, but not in the AHG group (NG 46.27 ± 5.31% vs. NG-RIPerC 24.65 ± 7.45%, p < 0.05; AHG 54.19 ± 4.07% vs. 52.76 ± 3.80%). Acute hyperglycemia per se did not influence infarct size, but significantly increased the incidence and duration of arrhythmias. Acute hyperglycemia inhibited 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 GSH/mg 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.

Deregulation of thio-redoxin system contributes to monocyte dysfunction in diabetes mellitus

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 VEGF1 growth factors. Molecular mechanisms leading to this VEGF-specific signal transduction defect in monocytes is incompletely understood.

Methods: Monocytes from diabetic patients or non-diabetic patients 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 detected 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 Boyden chamber assay (chemotaxtractant: PGF-1). Hindlung 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 was measured in VEGF1 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 hindlung re-perfusion compared to db/db mice receiving placebo.

Conclusions: Deregulated Trx system contributes to higher oxidative stress-related deregulation of PTP activity in hyperglycemic monocytes. Improving Trx function by supplementing Trx mimetic reversed monocyte dysfunction in diabetes. Most importantly, hindlung reperfusion improved 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.

High glucose increases gamma-glutamyltransferase-induced tissue factor expression in human peripheral blood mononuclear cells

Purpose: To assess the effect of an enzymatically inactive human recombinant (hr) GTT on TF antigen (Ag), TF mRNA and TF pro-coagulant activity (PCA) in human peripheral blood mononuclear cells (PBMCs). Experiments were conducted in both normal (NG, 10 mM) and high (HG, 50mM) glucose concentrations, the diabetic hallmark.

Methods: PBMCs obtained from healthy donors (discontinuous Ficoll/Hystopaque density gradient) were incubated with hrGTT (0.5ng/ml) either alone or with anti-hrGTT, a specific polyclonal antibody (2.5ug/ml). Because of the pivotal role played by NF-kB, a redox-sensitive transcription factor encoding TF, we also evaluated the effect Nf-kB inhibition by 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 genes) were the evaluation variables.

Results: hrGTT increased TF PCA (from 0.008 ± 0.007 to 0.37 ± 0.3, ng=14, p<0.001), ag (from 85.5 ± 53.56 to 33 ± 13, p<0.001) and mRNA (from 0.006 ± 0.002 to 0.004 ± 0.04, n=9, p<0.001) an effect inhibited by anti-hrGTT antibody (PCA: from 0.7 ± 0.6 to 0.3 ± 0.3, n=8, p<0.01; Tfg: from 469 ± 394 to 193 ± 65, n=6, p<0.001), HG amplified GTT-induced TF stimulation (PCA: from 0.4 ± 0.3 ± 0.3 ± 0.3, n=27, p<0.001; mRNA: from 0.05 ± 0.04 to 0.05 ± 0.35, n=8, p<0.001). BAY-11-7082 (NG: 0.01 to 0.1 ± 0.08 ± 0.1, n=7; HG: from 4.3 ± 3 ± 0.2 ± 0.1, n=4, p<0.001) and NAC (NG: from 0.3 ± 0.1 ± 0.08 ± 0.1 ± 0.7, n=6, p<0.001; HG: from 3.8 ± 2.9 ± 0.2±0.6, n=4, p<0.001) abolished GTT-induced PCA both NG and HG conditions.

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