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

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Abstract:
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 insofar.

Aim: To assess the effect of an enzymatically inactive human recombinant (hr) GGT 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, 50 mM) glucose concentrations, the diabetic hallmark.

Methods: PBMCs obtained from healthy donors (discontinuous Ficoll/Hystopaque density gradient) were incubated with hrGGT (0.5ng/µl) either alone or with anti-hrGGT, a specific polyclonal antibodies (2.5µg/ml). Because of the pivotal role played by NFkB, a redox-sensitive transcription factor encoding TF, we also evaluated the effect NF-κB 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: hrGGT increased TF PCA (from 0.008±0.007 to 0.37±0.3, n=14, p<0.001), ag (from 85±59 to 536±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±0.6 to 0.3±0.3, n=8, p<0.01; TFag: from 489±394 to 193±65, n=6, p<0.01). HG amplified GGT-induced TF stimulation (PCA: from 0.4±0.3 to 4.3±3, n=27, p<0.001; mRNA: from 0.05±0.04 to 0.5±0.35, 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 0.6±0.2, n=6, p<0.001) abolished GGT-induced PCA both NG and HG conditions.

Conclusions: GGT stimulates directly TF expression in PBMNCs 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.