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Development of novel hydrogen sulfide and nitric oxide donor compounds to protect against methylglyoxal-mediated endothelial cell dysfunction.

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Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound has been implicated as a mediator of diabetic cardiovascular complications via increased cellular oxidative stress (Brouwers *et al.*, 2010).. The gaseotransmitters, nitric oxide (NO) and hydrogen sulfide (H_2S) are increasingly acknowledged for their cardioprotective properties in the body. Additionally diabetics have been shown to have an altered homeostasis of NO and H_2S production that may be implicated in the increased vulnerability of diabetic patients to vascular complications (Szabo, 2012). The aim of this study is to determine the protective potential of H_2S alone using L-cysteine (endogenous enzyme substrate) and cysteamine (donor compound) against MGO-mediated endothelial dysfunction. Additionally, synthesize novel H_2S/NO generating compounds and explore the use of these compounds for the production of drug-eluting stents.

S-Nitrosothiol compounds, S-Nitroso-L-Cysteine and S-Nitrosocysteamine produced via a chemical reaction between cysteine/cysteamine and sodium nitrite were applied directly (10^{-9} to 10^{-4}M) or eluted from coated (0.2 mg/disc) metal discs (analogous to stents) to e*x vivo* endothelium-denuded aortic rings from male Sprague-Dawley rats to measure NO-dependent relaxation. *In vitro* experiments using a human endothelial cell line, EA.hy926, examined the effects of MGO±L-cysteine/cysteamine on cell viability (MTT assay) and apoptosis/necrosis levels (Hoechst/Propidium iodide staining). L-cysteine and cysteamine coated metal discs were placed in media prior to it being supplemented with MGO and applied to EA.hy926 cells for 24h before measuring cell viability. Data is expressed as mean±SEM and analysed using two-way ANOVA with Bonferroni's correction.

MGO (0.3 mM) exposure increased the IC_{50} for acetylcholine-mediated relaxation from 0.08±0.02 µM to 0.42±0.1 µM ($p<0.05$; n=6), with protection being observed with L-cysteine (0.13±0.02 µM and 0.09±0.03 µM) and cysteamine (0.2±0.04 µM and 0.12±0.02 µM) at 0.3 and 0.5 mM respectively ($p<0.05$ *vs.* MGO alone; n=6). S-Nitroso-L-Cysteine and S-Nitrosocysteamine when applied directly and eluted from discs induced NO-dependent relaxation of aortic rings. To elucidate mechanisms underlying relaxation, a soluble guanylate cyclase inhibitor, 1H-[1,2,4] oxadiazolo [4,3,-a] quinoxalin-1-one (ODQ, 10 µM) was applied, this inhibited relaxation, increasing the EC_{50} from 0.15±0.03 µM to greater than 10.9 µM for S-Nitroso-L-Cysteine ($p<0.05$ *vs.* 0µM OQD; n=4) and from 0.58±0.14 µM to 4.3± 1.3 µM for S-Nitrosocysteamine ($p<0.01$ *vs.* 0µM OQD; n=4). L-cysteine and cysteamine provided dose-dependent protection from MGO-mediated loss of cell viability, returning cell viability from 42.9±3.3% with 0.8 mM MGO alone to 91.5±3.5% and 67.6±4.0% with addition of 500 µM L-cysteine and cysteamine respectively ($p<0.05$ *vs.* MGO alone; n=3 with 6 replicates per experiment). L-cysteine (500 µM) also protected against

MGO (0.6 mM)-mediated increase in apoptosis (reducing it from 7±2% to 1.7±0.3%) and necrosis (reducing it from 20±5% to 6.9±1%) ($p<0.05$ *vs*. MGO alone, n=4 with minimum of 300 nuclei being counted per treatment per experiment). Protection from L-cysteine when coated on discs was maintained, returning cell viability of 0.8mM MGO treated cells from 40.0±1.7% to 65.3±4% with use of 0.2 mg L-cysteine coated disc ($p<0.05$ *vs.* MGO alone; n=3 with 6 replicates per experiment).

In conclusion, H_2S generating compounds either applied directly or eluted from discs made from a stent-like material can effectively protect against MGO-mediated endothelial dysfunction. Our novel H_2S/NO generating compounds caused vasorelaxation when applied directly or eluted from discs made from a stent-like material. NO/H_2S generating drug eluting stents may prove to be effective in treating cardiovascular complications in diabetic patients.

(1) Brouwers O, Niessen PM, Haenen G, Miyata T, Brownlee M, Stehouwer CD, *et al.* (2010). Hyperglycaemia-induced impairment of endothelium-dependent vasorelaxation in rat mesenteric arteries is mediated by intracellular methylglyoxal levels in a pathway dependent on oxidative stress. *Diabetologia* **53**(5)**:** 989-1000.

(2) Szabo C (2012). Roles of Hydrogen Sulfide in the Pathogenesis of Diabetes Mellitus and Its Complications. *Antioxid Redox Signal* **17**(1)**:** 68-80.