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Methylglyoxal Induces Apoptosis in Bovine Retinal Pericytes

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Purpose : Loss of retinal pericytes is the initial deficit in the early stage of diabetic retinopathy. Methylglyoxal is a spontaneous product of glucose metabolism which is known to have cytotoxic actions and to be present in raised concentrations in hyperglycemia. Whether methylglyoxal contributes to the loss of pericytes has not been clear. Thus we have investigated the cytotoxic effects of methylglyoxal on bovine retinal pericytes and whether the antioxidant, N-acetyl cysteine (NAC) provides any protection.

Methods : Primary cultures of retinal capillary pericytes were prepared from isolated bovine retinal microvessels. The cells were incubated under normoglycemic conditions after treatment with 200 - 800 μM methylglyoxal for 6 hours in the presence or absence of 2 mM NAC. Cell viability was assessed by MTT assay. Apoptosis was measured using the Annexin V assay (flow cytometry) and Cell Death Detection ELISA and confirmed by transmission electron microscopy. Caspase 3 protease activity was determined from hydrolysis of DEVD-p-nitroanilide.

Results : Methylglyoxal induced pericyte death in a concentration-dependent manner. ELISA and flow cytometry results showed a 3-fold increase in the level of apoptosis after incubation with methylglyoxal compared with control ($P<0.05$) and NAC protected against pericyte apoptosis. Electron microscopy showed nuclei with apoptotic ultrastructures. Caspase-3 activity significantly increased in cells exposed to 800 μM methylglyoxal ($P<0.05$).

Conclusions : Our findings suggest that elevated methylglyoxal could induce apoptotic cell death and associated oxidative stress possibly contribute to the death of retinal pericytes.

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Mechanism of Glucose-Induced Migration in Vascular Smooth Muscle Cells

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Oxidative stress contributes to vascular diseases in diabetes by promoting vascular smooth muscle cell (VSMC) proliferation and migration, monocyte/macrophage infiltration, endothelial damage and vascular tone alteration. As the mechanism of the development and progression of diabetic vascular complication is poorly understood, this study was aimed to assess the potential mechanism of glucose-induced oxidative stress. Furthermore, the effect of gene transfer of human Cu/ZnSOD against glucose-induced migration of VSMC was also evaluated. Cultured rat aortic VSMCs were incubated for 48 hours in either 5 mmol/L normal glucose(NG) or 30 mmol/L high glucose(HG) conditions. Superoxide production and migration of smooth muscle cells incubated under HG condition were markedly increased compared to normoglycemic condition. Treatment of diphenyleneiodonium(NAD(P)H oxidase inhibitor, 40μM) and SOD(superoxide dismutase, 500 units/ml) significantly suppressed HG-induced VSMC migration and superoxide production, suggesting the role of superoxide derived from NAD(P)H oxidase in HG-induced VSMC migration. After infection of recombinant adenovirus encoding β-galactosidase(LacZ) as a control, expression of LacZ was demonstrated 1 day later and maximized at 7 days in VSMCs. Application of recombinant adenovirus(200 μL of 1×10^{10} pfu/ml) encoding human Cu/ZnSOD to VSMCs significantly inhibited the HG-induced migration. These data suggest that production of NAD(P)H-derived superoxide contributes to HG-induced VSMC migration. Thus adenovirus-mediated transfer of cDNA for human Cu/ZnSOD to VSMCs effectively reduced HG-induced superoxide production and smooth muscle cell migration.