Ras activation modulates methylglyoxal-induced mesangial cell apoptosis through superoxide production.

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Abstract

AIMS: While previous studies have demonstrated that diabetic nephropathy is attributable to glucose-derived dicarbonyl compounds, methylglyoxal (MGO)-inducing apoptosis in renal mesangial cells, the molecular mechanism of upper stream redox signaling modulation, has not been fully elucidated. METHODS: Rat mesangial cells pretreated with or without superoxide dismutase, diphenyloniodium, SB203580, and manumycin A were cultured in methylglyoxal stress-induced apoptosis. Signaling protein expression, flow cytometry, and morphological features of apoptotic cell death were assessed. RESULTS: Methylglyoxal decreased cell viability in mesangial cells. Superoxide mediated methylglyoxal-induced caspase 3 cleavage. Pretreatment with diphenyloniodium, SB203580, and manumycin A reduced methylglyoxal augmentation of superoxide synthesis and caspase-3 activation. Methylglyoxal rapidly enhanced Ras activation and progressively increased cytosolic P38 and nuclear c-Jun activation. Scavenging of superoxide by superoxide dismutase or diphenyloniodium, inhibiting P38 by SB203580, and inhibiting Ras with manumycin A successfully reduced the promoting effect of methylglyoxal on P38 and c-Jun phosphorylation (activation). Furthermore, pretreatment with superoxide dismutase, diphenyloniodium, SB203580, and manumycin A significantly attenuated methylglyoxal induction of apoptosis on the basis of Annexin-V assay and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labelling (TUNEL) staining. CONCLUSIONS: This study has shown that methylglyoxal increased Ras modulation of superoxide-mediated P38 activation and c-Jun activation, which resulted in increased apoptosis.