The Antiapoptotic Effects of Hominis Placenta Extract

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ABSTRACT

Purpose.
Free radicals are implicated in the pathophysiology of aging, ischemic injury and neurodegenerative disorders. To determine whether Hominis Placenta extract prevents H₂O₂-induced apoptosis, we have performed morphological and biochemical analyses for the detection of apoptotic phenomena in the pineal tumor cell line PGT-β. We have also performed cytochemical and immunocytochemical analyses for the detection of changes in nitric oxide synthase (NOS) activity and estimated the expression of apoptotic genes using reverse transcription–polymerase chain reaction (RT–PCR).

Methods.
PGT-β cells were pretreated with Hominis Placenta extracts (0, 10⁻² μg/ml) for 2 hours and then exposed to H₂O₂ (0, 50 μM) for 3 hours. Appearance of apoptotic characteristics were monitored using 4, 6-diamidino-2-phenylindole dihydrochloride (DAPI) staining assay, terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end

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labeling (TUNEL) assay and flow cytometric analysis. NOS activity was measured by NADPH-diaphorase cytochemistry. Expression of inducible NOS (iNOS) and nuclear factor kappa B (NFκB) was assessed via immunocytochemistry. The expression of apoptotic genes was examined by RT-PCR.

Results.
After 3 hours of exposure to H₂O₂, it was shown that PGT-β cells treated with H₂O₂ (50 μM) exhibit classical apoptotic features and increases in NOS activity and caspase-3 expression. Treatment with Hominis Placenta extract resulted in a reduced occurrence of apoptotic features. DAPI staining, TUNEL and flow cytometric assays revealed decreases in the occurrence of nuclear fragmentation and in the sub-G1 fraction in the PGT-β cells treated with Hominis Placenta extract. Cells treated with Hominis Placenta extract also showed lower activity of NADPH-diaphorase and immunoreactivities of both iNOS and NFκB than those of H₂O₂-treated cells which were not treated with Hominis Placenta extract. By RT-PCR, it was shown that the level of caspase-3 mRNA was decreased in the cells treated with Hominis Placenta extract.

Conclusions.
This study shows that Hominis Placenta extract prevents H₂O₂-induced apoptosis in PGT-β cells; inhibitions of iNOS and caspase-3 are possible mechanisms of the protection against apoptosis.

Keywords: Hominis Placenta extract, Free radicals, H₂O₂, Apoptosis, pineal gland cell line, NADPH-diaphorase, NFκB, iNOS