

The Antiapoptic Effects of Hominis Placenta Extract

Jung-Chul Seo¹ · Joo-Ho Chung² · Byoung-Choul Ahn³

¹ Department of Acupuncture & Moxibution, College of Oriental Medicine, Kyunghee University

² Department of Pharmacology, College of Medicine, Kyunghee University

³ Ahn Byoung-Choul Oriental Clinic

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 μ g/ml) for 2 hours and then exposed to H₂O₂ (0, 50 μ M) 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

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 (NF κ B) 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 μ M) 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 NF κ B 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, NF κ B, iNOS