Possible involvement of nitric oxide in lipopolysaccharide-induced apoptosis in trophoblasts

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Introduction
Chorioamnionitis is a significant cause of preterm premature rupture of membrane, which leads to preterm labor and low birth weight infants, and it is also involved in life-threatening placental abruption. Lipopolysaccharide (LPS) concentration is reported to be high in amniotic fluid of chorioamnionitis [1] and LPS is also known to induce generation of NO and superoxide. These molecules or peroxynitrite, which is formed by a reaction of NO and superoxide and one of potent and relatively long-lived oxidants, can be toxic to placental tissue. We has reported that expression of NO synthase-2 (NOS-2, inducible NOS) and apoptosis were induced in placentae from patients suffering from chorioamnionitis.

In the present study we treated trophoblasts isolated from human placenta with LPS to elucidate the involvement of NO in apoptosis seen in trophoblasts.

Materials and Methods

Culture of Trophoblasts
Placentae were obtained from the patients, who submitted to elective caesarean section at term. Trophoblasts were isolated as described previously [2], and cultured in 6-wells multidishes or chamber slides (Nunc, Inc. Naperville, IL) up to 7 days.

Treatment and Collection of Samples
Various doses of LPS (B E. coli 0111:B4, DIFCO laboratories, Detroit, MI) with or without aminoguanidine (Sigma chemicals, St. Louis, M) were added into culture media on the day of isolation of trophoblasts (Day 0). Medium was collected at Day 1, 3, 5, and 7. Cells were fixed in suspension by 1% glutaraldehyde or fixed on chamberslides by 4% formaldehyde at Day 5.

Immunocytochemistry
Immunocytochemical detection was performed using DAKO LSAB kit and DAB chromogen (DAKO corporation, Carpinteria, CA) with specific polyclonal antibodies following the manufacturer’s instructions. To determine the formation of peroxynitrite, nitrotyrosine was detected by immunocytochemical techniques because peroxynitrite caused nitration of tyrosine residues in protein [3].

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measurement of nitrite/nitrate

Medium was treated with nitrate reductase and the nitrite concentrations were determined by Griess reagents in duplication.

detection of apoptosis

Apoptosis was identified morphologically under fluorescent microscope after staining with Hoechst 33258 (Molecular Probes, Eugene, OR). It was also confirmed by detection of DNA degradation by in situ nick end labeling technique using ApopTag Plus kit (Oncor, Gaithersburg, MD) following the manufacturer's instructions.

results and discussion

Immunocytochemical detection indicated that NOS-2 was induced in trophoblasts by the treatment with LPS. Accumulation of nitrite/nitrate, stable metabolites of nitric oxide, was observed in the medium of untreated trophoblasts, which suggested that NOS was present in trophoblasts constitutively. Nitrite/nitrate accumulation was enhanced by LPS to 5-10 fold of that of control and it was suppressed by aminoguanidine, a relatively selective inhibitor of NOS-2. This indicated that NOS-2 was responsible to nitrite/nitrate elevation by LPS.

Ratio of apoptotic cell in trophoblasts treated by LPS was also 10 times higher than that of control. Moreover, it was suppressed by aminoguanidine. These results suggested that NO generated by NOS-2 was involved in apoptosis induced by LPS.

Nitrotyrosine was detected in trophoblasts treated by LPS, which indicated generation of peroxynitrite. Aminoguanidine also suppressed formation of nitrotyrosine. Peroxynitrite, which is known to cause cell death including apoptosis in various cells, can be another candidate for the apoptosis detected in trophoblasts. Suppression of NOS-2 could protect trophoblasts against LPS-induced apoptotic cell death. These results suggest that NOS-2 may be a novel target of therapy of chorioamnionitis.

references