The inhibitory effects of heparin on proinflammatory cytokines (TNF-alpha/IFN-gamma)-induced cellular reduction and sFlt-1 (soluble fms-like tyrosine kinase-1) release in trophoblast-derived cell line

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Objectives (목적)

To examine regarding the inhibitory effect of heparin on the proinflammatory cytokines-induced reduction of the cellular proliferation and release of sFlt-1 in the trophoblast-derived cell line.

Methods (연구 방법)

1)A trophoblast-derived cell line (ATCC CRL-1584) was treated with TNF-alpha/IFN-gamma. A trophoblast-derived cell line (100,000 cells/mL;100μL/well) was plated onto 96-well plates, and incubated for 24hours (37°C/5%CO_2). Cell line was treated with each TNF-alpha/IFN-gamma (0.1 ng/mL;1ng/mL;10ng/mL;100ng/mL) for 72hours. Cellular proliferation was measured with the CCK-8 (Cell Counting Kit-8), and the cell supernatant was assayed for sFlt1 using ELISA;2)A trophoblast-derived cell line prepared by the same method as experiment-1 was treated with various concentrations of TNF-alpha/IFN-gamma (0.1 ng/mL;1ng/mL; 10ng/mL;100 ng/mL) and heparin (1 ng/mL; 10ng/mL;100ng/mL;1,000ng/mL;10,000ng/mL) for 72hours. Cellular proliferation was measured with the CCK-8;3)After the investigation of the concentration of heparin that can inhibit reduction of the cellular proliferation in a trophoblast-derived cell line by all concentrations (0.1 ng/mL;1ng/mL;10ng/mL;100ng/mL) of TNF-alpha/ IFN-gamma in experiment-2, the cell supernatant was assayed for sFlt1 using ELISA in the concentrations of heparin with the inhibitory effect;4)Experiments were repeated more than three-times, and nonparametric methods were used for statistical analysis.

Results (결과)

1) The cellular proliferation was significantly lower and the supernatant sFlt-1 concentrations were significantly higher in the cases with treatment of TNF-alpha/IFN-gamma than in those without of TNF-alpha/IFN-gamma, and the cellular proliferation was decreased and the supernatant sFlt-1 concentrations were increased, dependently according to the increased concentration of TNF-alpha/IFN-gamma;2) The inhibition of cellular proliferation in a trophoblast-derived cell line by TNF-alpha/IFN-gamma was significantly more attenuated in cases with treatment of heparin 1 ng/mL, 10 ng/mL and 100 ng/mL than in cases without of heparin (for each $p<.05$), but there are no significant differences in cellular proliferation of a trophoblast-derived cell line between the cases with and without treatment of heparin more than 1,000 ng/mL; 3) The release of sFlt-1 among cell line with the stimulation of TNF-alpha/IFN-gamma 10 ng/mL is significantly lower in cases with treatment of heparin 1ng/ml ($p<.05$), not lower in those with treatment of heparin 10 ng/mL, and significantly higher in cases with treatment of heparin 100 ng/mL ($p<.05$) than in those without treatment of heparin.

Conclusions (결론)

The inhibition of cellular proliferation and the release of sFlt-1 in a trophoblast-derived cell line by TNF-alpha/IFN-gamma was more attenuated in cases with treatment of specific concentration of heparin (1 ng/mL) than those without treatment of heparin. However, these effects were disappeared in cases of the treatment of heparin more than certain concentration. These findings suggest that heparin may be considered as agent for treatment and prevention of preeclampsia, and the selection of adequate concentration of heparin in clinical practice may be very important.