Giorn. It. Ost. Gin. - Vol. XXXV - n. 1
Gennaio-Febbraio 2013

Expression of TLR1-10 and caspase-3 alfa at women with early miscarriages

LEBEDEVA O., PAKHOMOV S., IVASHOVA O., STARCEVA N., CHURNOSOV M., KUZNETSOVA Y., KUZNICHENKO E.

Belgorod State National Research University, Obstetrics and Gynecology Department, Belgorod, Russia

Introduction

Toll-like receptors (TLR) are the first signal receptors of innate immunity, recognizing bacteria and viruses and promoting development of inflammation through the activation of proinflammatory cytokines secretion (1). In animal models it was shown, that TLRs play role in pathogenesis of miscarriages (2, 3) and can influence to trophoblast apoptosis (4).
The aim of research was to investigate study specialty of mRNA expression of TLR1-10 and caspase-3α in endometrium at patients with early miscarriages.

Materials and methods

mRNA expression of TLR 1-10 and caspase-3 alfa in epithelial cells of endometrium was detected using qPCR according to MIQE guideline (5). Samples were taken from 57 women with early miscarriages (6-10 weeks of gestation) and 57 women with medical abortion (6-10 weeks) as a control group. mRNA was extracted using Trizol ("Invitrogen", USA). First-strand cDNA synthesis was performed using oligodT and Mint reverse transcriptase ("Eurogen", Russia). Quantitative real-time PCR was performed using qPCRmix-HS SYBR kit ("Eurogen", Russia). Results were analyzed using CFX96 ("Bio-rad laboratories", USA). Human beta-actin and peptidylprolyl isomerase A (PPIA) were used as housekeeping genes (Table 1). Amplification was performed using the following cycling conditions: 5 minutes at 95°C, and 45 three-step cycles of 15 seconds at 95°C, 30 seconds of appropriate gene annealing according to the table 1 and 30 seconds at 68°C. Results were calculated as delta-delta cq and estimated by Mann-Whitney criteria.

Results

It was shown that in endometrium at patients with miscarriages significantly higher expression of TLR 3, which ligand is double-stranded viral RNA, was detected (Table 2). On the contrary, expression of TLR4 (ligand - lipopolysaccharides of Gram-negative bacteria), TLR6 (ligand - lipopeptides) and TLR8 (ligand – single-stranded RNA) were significantly lower, than in control group. It was studied mRNA expression of caspase-3 alfa, which can influence on trophoblast apoptosis. Expression

TABLE 1 - PRIMERS FOR QUANTATIVE PCR.

Gene	Forward primer	Reverse primer	Annealing temperature, °C
TLR1	CAGGCACCAGGGCGTGATGG	GATGGAGGGGCCGGACTCGT	57
TLR2	ATCCTGCTCACGGGGGTCCTG	TGCTGGGAGCTTTCCTGGGC	57
TLR3	ACTGATGCTCCGAAGGGTGGC	TGCGTGTTTCCAGAGCCGTGC	56
TLR4	GGAGCCCTGCGTGGAGGTGGTT	GTTGAGAAGGGGAGGTTGTCGGGGA	57
TLR5	GGGTCAGTTCTGGACTTCAGAG	GGCTTCAAGGCACCAGCCATCTC	58
TLR6	ACCCTTTAGGATAGCCACTGC	GACCTGAAGCTCAGCGATGT	59
TLR7	GTGGGGCCAGGAGCACACAAG	ACAGACGTTGGTGGCTCCCCT	57
TLR8	AGGCTACGGCAGCGGATCTGT	GCAGGCCATCCCAGGACAGCA	65
TLR9	AGACCTGAGGGTGGAAGTGT	TCCCCTCTCAGACAGCCTAC	61
TLR10	AGTGCAAGCCGTGGGGGTTT	GTGGCTGGGGTCAAGTCTGCG	60
CASP-3α	GTGCTATTGTGAGGCGGTTG	CACGGATACACAGCCACAGG	55
Beta-actin	CAGGCACCAGGGCGTGATGG	GATGGAGGGGCCGGACTCGT	64
PPIA	CCGCCGAGGAAAACCGTGTACT	TGGACAAGATGCCAGGACCCGT	64

TABLE 2 - EXPRESSION OF TOLL-LIKE RECEPTORS 1-10 AND CASPASE-3 ALFA IN HUMAN ENDOMETRIUM AT PATIENTS WITH MISCARRIAGES AND IN THE CONTROL GROUP.

N°	Gene	Women with early miscarriages (n=57)	Control group (n=57)
1.	TLR1E	7,16 (1,57; 27,66)	4,41 (1,97; 2,48)
2.	TLR2E	0,72 (0,35; 1,46)	0,54 (0,32; 1,15)
3.	TLR3E	**105,42 (55,52; 297,14)**	**63,78 (32,22; 144,01)**
4.	TLR4E	**0,17 (0,06; 0,36)**	**0,26 (0,12; 0,51)**
5.	TLR5E	0,0002 (0,00008; 0,0004)	0,0002 (0,0001; 0,0007)
6.	TLR6E	**0,044 (0,006; 0,129)**	**5,063 (0,105; 9,747)**
7.	TLR7E	131,14 (66,72; 224,41)	153,27(106,52;261,37)
8.	TLR8E	**0,2862 (0,1451; 0,7579)**	0,5230 (0,2793; 1,0353)
9.	TLR9E	0,0434 (0,0073; 0,3322)	0,0209 (0,0009; 0,1713)
10.	TLR10E	1,3803 (0,6417; 3,1059)	1,2527 (0,5141; 2,4708)
11.	CASP-3 alfa	**0,0026 (0,00017; 0,0150)**	0,0004 (0,0001; 0,0039)

of caspase-3α in endometrium was significantly higher versus control group and had moderate negative correlation with expression of TLR6 in endometrium (R=-0,52; p=0,000057).

Discussion

At present it is no doubt, that viral infection plays an important role in the pathogenesis of spontaneous abortions (6). The literature describes the results of a series of experiments on mice, concerning the TLR, activated by viral ligands, in the development of spontaneous abortion. Poly I:C acid, which is ligand of TLR3, in mice induces resorption both syngeneic and allogeneic embryos (7). Blocking of TLR3 by specific antibodies cancel influence of poly I:C on embryo (8). Moreover, viral infection can impact on bacterial infection outcome. It was shown, that in herpes simplex infected mice insertion of lipopolysaccharide led to miscarriages. In control group with absence of viral infection progression of pregnancy was observed (9). It could be suggested, that stimulation of TLR3 by viral ligands can lead to decrease of bacterial TLR expression.

Futhermore, in vitro studies suggest that the pro-apoptotic effect observed following PDG treatment is mediated by TLR1 and TLR2 heterodimers, which then activate caspase-8, caspase-9, and caspase-3 through MyD88/FADD pathway, whereas the presence of TLR-6 may shift the type of response, cell death is prevented and a cytokine response ensues through NFκB activation (10).

Conclusion

According to our data, at patients with early stages miscarriages decrease of TLR6 in endometrium is observed. Therefore, an increase of caspase-3 alfa level probably appears because of absence of its protective effect. Therefore, TLR3 activation is important for early miscarriages development. It could be suggested, that sufficient expression of TLR6 can play protective role in endometrium, preventing miscarriages by avoidance of trophoblast apoptosis.

Acknowledgements

Supported by grant of President of Russian Federation N° MD-936.2012.7 and State Assignment N° 4.3493. 2011.

References

1. Horne A., Stock S., King A. Innate immunity and disorders of female reproductive tract. Reproduction 2008;135:p. 739-749.
2. Wang H., Hirsch E. Bacterially-induced preterm labor and regulation of prostaglandin-metabolizing enzyme expression in mice: the role of toll-like receptor 4. Biology of Reproduction 2003;Vol. 69:p. 1957-1963.
3. Mulla M.J., Brosens J.J., Chamley L.W. Antiphospholipid antibodies induce a pro-inflammatory response in first trimester trophoblast via the TLR4/MyD88 pathway. Am J Reprod Immunol Aug 2009;Vol. 62(2): P. 96-111.
4. Rose J. A., Rabenhold M.M., Parast D.S., Milstone D.S. et al. Peptidoglycan induces necrosis and regulates cytokine production in murine trophoblast stem cells. Am J Reprod Immunol Sep 2011;Vol. 66 (3):P. 209-222.
5. Bustin S. A. et al. The MIQE Guidelines: Minimum information for publication of quantitive Real-Time PCR experiments. Clinical Chemistry 2009; N° 55, Vol. 4:p. 611-622.
6. Anzivino E., Fioriti D., Mischitelli M., Bellizzi A., et al. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention// Virology journal 2009; N° 6:P. 40.
7. Lin Y., Zeng Y., Di J., Zeng S. Murine CD200+ CK7+ trophoblasts in a poly (I:C)-induced embryo resorption model. Reproduction 2005;Vol. 130 :P. 529-537.
8. Lin Y., Liang Z., Chen Y., Zeng Y. TLR3-involved modulation of pregnancy tolerance in double-stranded RNA-stimulated NOD/SCID mice. J Immunol 2006;Vol. 176:P. 4147-4154.
9. Nansen A., Thompsen A.R. Viral infection causes rapid sensitization to lipopolysaccharide: central role of IFN-alpha beta. J. Immunol 2002;Vol. 166:P. 982-988.
10. Abrahams V.M., Aldo P.B., Murphy S.P., Visintin I. et al. TLR6 modulates first trimester trophoblast responses to peptidoglycan. J. Immunol 2008;Vol 180:P. 6035-6043.