European Review for Medical and Pharmacological Sciences 2018; 22: 4762-4767

IFN-γ induces upregulation of TNF-α, downregulation of MMP-2 and MMP-9 expressions in abortion rat

Y. HE, Q. SUN

Department of Obstetrics, Jinan Maternity and Child Care Hospital, Jinan, Shandong, China

Abstract. – OBJECTIVE: Tumor necrosis factor-α (TNF-α) participates in the regulation of the whole process of pregnancy. Matrix metalloproteinases-2 and -9 (MMP-2 and MMP-9) play important roles in the process of trophoblast invading decidua. This research aims to determine the role of IFN-γ in TNF-α, MMP-2, and MMP-9 of abortion rats.

MATERIALS AND METHODS: The rats were divided into control, abortion model, gestation, and IFN-γ group. Abortion rats model in IFN-γ group were treated by IFN-γ at low and high doses upon abortion model. Serum and tissue TNF-α, MMP-2, and MMP-9 expressions were detected by ELISA and immunohistochemistry.

RESULTS: The level of TNF-α was significantly elevated, while MMP-2 and MMP-9 were statistically declined in the serum and decidual tissue of rat from model group ($p < 0.05$). Of note, the expression of TNF-α was further increased, whereas MMP-2 and MMP-9 reduced in IFN-γ high dose group ($p < 0.05$).

CONCLUSIONS: We demonstrated that in abortion rats, TNF-α was overexpressed, while MMP-2 and MMP-9 were reduced declined in the serum and decidual tissue. The treatment with a high dose of IFN-γ further upregulated TNF-α expression and decreased MMP-2 and MMP-9 levels, aggravating the severity of rat abortion.

Key Words:
IFN-γ, Abortion, TNF-α, MMP-2, MMP-9.

Introduction

Abortion is a type of common complication of pregnancy affected by a variety of factors, such as chromosomal abnormalities and immune response. A large number of studies showed that cytokines were involved in the pathogenesis of early abortion[1,2]. IFN-γ is characterized as a sort of Th1 cytokine. A small dose of IFN-γ represents as one of the indispensable cytokines in the process of normal pregnancy. Injection with a low dose of IFN-γ significantly enhanced the immunocompetence of pregnant rat on parasitic disease, whereas excessive IFN-γ may adversely affect the normal pregnancy[3,4]. TNF-α is involved in the whole process of pregnancy by regulating embryo implantation and affecting trophoblast cells proliferation, differentiation, and apoptosis[5,6]. MMPs regulate the process of trophocytes invading endometrium. MMP-2 and MMP-9 degrade type IV and V collagen, which contributes to an important part of trophocytes invasion[7,8]. This study investigated the impact of IFN-γ abortion rat and determined the related mechanism.

Materials and Methods

Experimental Animal

A total of 50 female Wistar rats at 8-week old with the weight of 180 ± 20 g were provided by Shandong University (Jinan, Shandong, China). Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Jinan Maternity and Child Care Hospital.

Reagents and Instruments

TNF-α, MMP-2, and MMP-9 ELISA kits were bought from TaKaRa (Otsu, Shiga, Japan). TNF-α, MMP-2, and MMP-9 primary antibodies, and rabbit anti-mouse secondary were purchased from Abcam (Cambridge, MA, USA). Paraffins and hematoxylin were got from Changzhou Shanfeng Chemical Co., Ltd., (Changzhou, Jiangsu, China).

Rat Abortion Model Establishment

Male and female rats were mated at 1:1. Vaginal suppository or sperm was found at the vaginal orifice of pregnant rats. The rats were randomly divided into six groups. Experimental group: Mifepristone solution (Xianju Pharmaceutics,

Corresponding Author: Qian Sun, MD; e-mail: lzweivxsxzj@sina.com

Hangzhou, Zhejiang, China) was intragastrically admitted at 4 mg/(kg·d) on the 7th or 8th day of pregnancy to establish rat abortion model. Low-dose IFN-γ group: a total of 10 rats were used to establish abortion model. Afterwards, IFN-γ was intramuscular in into the vaginal orifice at 1 IU/kg on the second day of pregnancy. High-dose IFN-γ group: similar to Low-dose IFN-γ group, but IFN-γ was intramuscular injected into the vaginal orifice at 10 IU/kg on the second day of pregnancy. Model group: a total of 10 rats were used to establish abortion model. An equal amount of normal saline was intramuscular injected into the vaginal orifice on the second day of pregnancy. Pregnancy group: a total of 10 normal pregnant rats were enrolled. An equal amount of normal saline was intramuscular injected into the vaginal orifice on the second day of pregnancy. Control: a total of 10 healthy non-pregnant rats were enrolled. An equal amount of normal saline was intramuscular injected into the vaginal orifice on the second day of pregnancy.

Sample Collection

The capillary tube was inserted into the rat retinal vein plexus to collect the blood. The placenta tissue was cut into 0.5 cm×0.5 cm and preserved at -80°C.

ELISA

The venous blood was centrifuged at 1500 g and the supernatant was collected. Then, the sample was added to the plate and treated by a color-developing agent. At last, the plate was read at 450 nm.

Immunohistochemistry

The tissue was fixed, embedded, and sectioned. Next, the slice was dewaxed and thermal remediated. After being blocked, the slice was incubated in primary antibody (1:200) for 1 h and secondary antibody (1:500) for 10 min. At last, the slice was treated by development, dehydration, sealing, and observed under the microscope. The positive staining of TNF-α, MMP-2, and MMP-9 were defined as brown particles appeared in the membrane or cytoplasm but not the nucleus. Positive cell number was defined as negative (-) ≤ 10%, weak positive (+) 11-25%, positive (++) 26-50%, and strong positive (+++) > 50%.

Statistical Analysis

SPSS 17.0 software (SPSS Inc. Chicago, IL, USA) was adopted for data analysis. The enumeration data were analyzed by chi-square test. The measurement data were depicted as mean ± standard deviation and compared by ANOVA with Tukey's post-hoc test. $p < 0.05$ was considered as statistical significant.

Results

ELISA Detection of Serum TNF-α, MMP-2, and MMP-9 Levels in the Rat

Our result showed that the level of TNF-α was significantly elevated, while the expressions of MMP-2 and MMP-9 were statistically declined in the rat serum from model group, compared to normal control ($p < 0.05$), suggesting they might be involved in the pathogenesis of abortion. Consistently, the expression of TNF-α was even statistically increased, whereas the levels of MMP-2 and MMP-9 were significantly reduced in IFN-γ high dose group, compared to model group ($p < 0.05$) (Table I), indicating IFN-γ treatment can increase TNF-α expression but decrease MMP-2 and MMP-9 expressions.

Immunohistochemistry Detection in Decidual Tissue

Immunohistochemistry detection indicated that TNF-α positive rate was significantly increased in model group, compared to that in normal control ($p < 0.05$). Furthermore, TNF-α expression in high-dose IFN-γ group was even obviously higher than that in low-dose group and model group ($p < 0.05$) (Table II, Figure 1). MMP-2 and MMP-9 positive rates were markedly declined in model group in comparison to those in normal control ($p < 0.05$). MMP-2 and MMP-9 expressions in high-dose IFN-γ group were apparently lower than that in low-dose group or model group ($p < 0.05$) (Table III, Figures 2-3), indicating IFN-γ can induce the decrease of MMP-2 and MMP-9 expressions in abortion rat.

Discussion

Inappropriate stimulation may cause repeat abortion, fetal growth restriction, and preeclampsia[9,10]. It was reported that trophoblast cells exhibited similar biological characteristics with some malignant cells. However, the process of trophoblast cell invading to the endometrium presents as the abstinent invasion. A variety of cytokines and multiple signaling pathways are involved in

Table I. Serum TNF-α, MMP-2, and MMP-9 levels in the rat.

Group	Cases	TNF-α (ng/ml)	MMP-2 (ng/ml)	MMP-9 (ng/ml)
Experimental group				
Low-dose IFN-γ group	10	4.16±0.03$^{*\#\&}$	6.47±0.02$^{*\#\&}$	6.58±0.01$^{*\#\&}$
High-dose IFN-γ group	10	9.03±0.02$^{*\#\&@}$	3.02±0.05$^{*\#\&@}$	2.23±0.03$^{*\#\&@}$
Model group	10	10.26±0.04$^{\#\&}$	4.47±0.07$^{\#\&}$	4.58±0.04$^{\#\&}$
Pregnant group	10	3.05±0.03$^{\&}$	10.04±0.02$^{\&}$	10.01±0.01$^{\&}$
Control	10	1.03±0.01	0.02±0.01	0.01±0.02

$^{*}p < 0.05$, compared with model group. $^{\#}p < 0.05$, compared with pregnant group. $^{\&}p < 0.05$, compared with control. $^{@}p < 0.05$, compared with low-dose IFN-γ group.

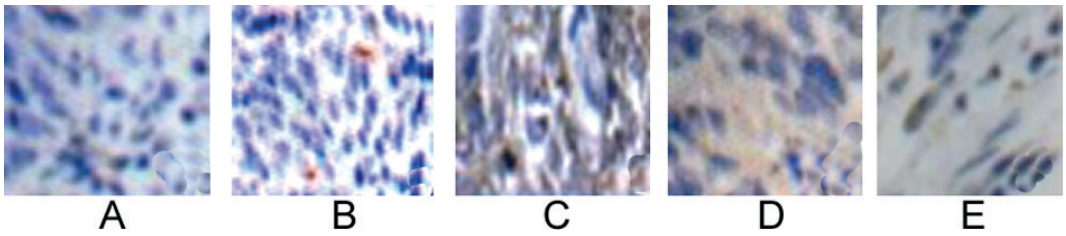

Figure 1. TNF-α expression in decidual tissue (×400). ***A,*** low-dose IFN-γ group; ***B,*** high-dose IFN-γ group; ***C,*** model group; ***D,*** pregnant group; ***E,*** control.

the regulation of this process[11]. For instance, IFN-γ participates in anti-inflammation, antivirus, and immune response. Decidua and placental syncytiotrophoblasts were able to synthetize moderate IFN-γ, which is conducive to the normal pregnancy[12]. Based on the previous finding, this study evaluated the impact of IFN-γ abortion with rat model.

The pregnancy embryo is an allogeneic transplant. The embryo avoids the maternal immunological rejection because of the immune tolerance produced by the matrix through unique immunological recognition. Th1/Th2 type cytokines play a critical role in the immune response during this process. Th2 type cytokines promote pregnancy, while on the contrary, Th1 cytokines, including INF-γ and TNF-α, lead to adverse effects on the gestation process and embryonic development. INF-γ has a high biological activity and a wide range of functions. Moreover, it can induce or inhibit other cytokines expression. Baren et al[13] indicated that IFN-γ exerted an inhibitory effect on P secretion, induced placental cells apoptosis, and increased MHC II expression. Low level of IFN-γ may cause negative effect by activating decidual macrophages and secreting a serious cytokines that promote abortion[14]. A normal pregnant rat can secrete a small amount of TNF-α to degrade extracellular matrix. Then, the trophocytes participate in the maintenance of normal pregnancy[15]. The trophocytes autocrine secrete MMPs to

Table II. TNF-α expression in decidual tissue.

Group	Cases	Expression intensity				Positive rate (%)
		-	+	++	+++	
Experimental group						
Low-dose IFN-γ group	10	7	2	1	0	30$^{*\#\&}$
High-dose IFN-γ group	10	4	2	2	3	60$^{*\#\&@}$
Model group	10	2	3	2	3	80$^{\#\&}$
Pregnant group	10	8	1	1	0	20$^{\&}$
Control	10	9	1	0	0	10

$^{*}p < 0.05$, compared with model group. $^{\#}p < 0.05$, compared with pregnant group. $^{\&}p < 0.05$, compared with control. $^{@}p < 0.05$, compared with low-dose IFN-γ group.

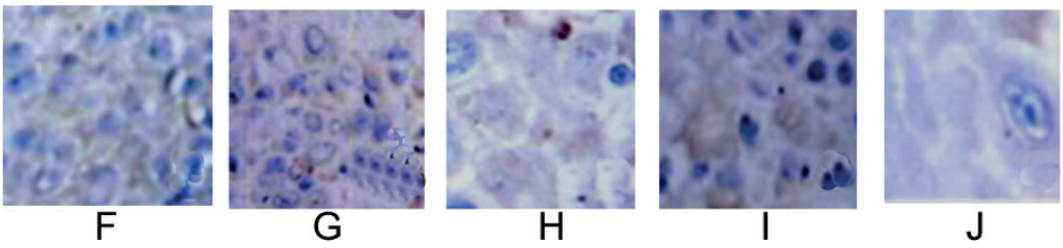

Figure 2. MMP-2 expression in decidual tissue (×400). ***F,*** low-dose IFN-γ group; ***G,*** high-dose IFN-γ group; ***H,*** model group; ***I,*** pregnant group; ***J,*** control.

Table III. MMP-2 and MMP-9 expressions in decidual tissue.

		MMP-2 expression intensity				MMP-9 expression intensity			
Group	Cases	-	+-++	+++	Positive rate (%)	-	+-++	+++	Positive rate (%)
Experimental group									
Low-dose IFN-γ group	10	4	6	0	$60^{*\#\&}$	5	5	0	$50^{*\#\&}$
High-dose IFN-γ group	10	8	2	0	$20^{*\#\&@}$	8	2	0	$20^{*\#\&@}$
Model group	10	7	3	0	$30^{\#\&}$	7	3	0	$30^{\#\&}$
Pregnant group	10	2	5	3	$80^{\&}$	3	5	2	$70^{\&}$
Control	10	9	1	0	10	9	1	0	10

$^{*}p < 0.05$, compared with model group. $^{\#}p < 0.05$, compared with pregnant group. $^{\&}p < 0.05$, compared with control. $^{@}p < 0.05$, compared with low-dose IFN-γ group.

induce themselves to invade the decidua. Autocrine and paracrine simultaneously regulate this process. MMP-9 is the "speed limit" enzyme of the invasion of trophocytes to decidua, which can degrade the basement membrane and extracellular matrix[16,17]. Consistently, in this work, we found that TNF-α was increased, whereas MMP-2 and MMP-9 were reduced in the rats abortion model.

In addition, TNF-α was kept in high level, whereas MMP-2 and MMP-9 were declined after IFN-γ intervention. High dose of IFN-γ exhibited the stronger effect. TNF-α was increased under infection or hypoxia stimuli, leading to corpus luteum function suppression and progestational hormone synthesis reduction. Besides, a high concentration of TNF-α induces the release of inflammatory cells and collagen enzyme, damages the endothelial cells in decidua and umbilical vein, thus leading to villi dysplasia, trophocytes apoptosis, and high rate abortion[18]. In our research, with the treatment of high dose of IFN-γ, we propose that IFN-γ and TNF-α synergistically inhibit the normal embryo and fetus growth and development. IFN-γ strengthens the role of TNF-α to further induce trophoblast cells apoptosis. Also, IFN-γ and TNF-α synergistically promote adhesion molecules expression on the surface of trophocytes, endothelial cells, and epithelial cells.

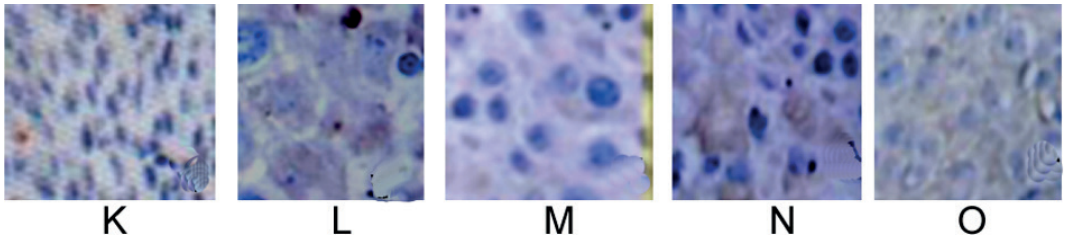

Figure 3. MMP-9 expression in decidual tissue (×400). K, low-dose IFN-γ group; L, high-dose IFN-γ group; M, model group; N, pregnant group; O, control.

Leukocyte adhesion is further activated to damage the trophoblastic cells. A previous report indicated that MMP-2 and MMP-9 were detected in trophocytes in the first 12 weeks. The level of MMP-9 reached the peak in the first three months of pregnancy[19]. Downregulation of MMP-2 and MMP-9 may affect the decidualization of the endometrium and the angiogenesis of endometrium and placenta, leading to the blockage between matrix and fetus[20]. Clinical research pointed out that MMP-9 was significantly enhanced in villi and decidua of the aborted female. It has been found that, in cultured trophocytes from pregnant mice, cells can secrete MMP-9 especially in the window phase of embryo implantation. The application of MMPs inhibitor to pregnant mice led to endometrial decidual dysplasia, embryo implantation difficulty, and embryo death rate was increased. It was speculated that MMPs expression deficiency may cause placenta dysplasia and stillbirth. Our results indicate that high dose of IFN-γ may aggravate the occurrence of abortion. By contrast, low dose of IFN-γ intervention may have a nutritious recovery effect on abortion rat, and in-depth investigation ought to be performed with a large amount of patients in clinic, which is associated with possible signaling pathways[21].

Conclusions

Our data on rat model demonstrate that abortion causes overexpression of TNF-α, reduction of MMP-2 and MMP-9, while high dose of IFN-γ intervention even promoted the change of TNF-α, MMP-2, and MMP-9 in abortion rat, resulting in the aggravation of rat abortion. This provides new insights for the strategy of gestation protection.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol 2008; 29: 357-365.
2) Kwak-Kim J, Gilman-Sachs A. Clinical implication of natural killer cells and reproduction. Am J Reprod Immunol 2008; 59: 388-400.
3) Bose P, Kadyrov M, Goldin R, Hahn S, Backos M, Regan L, Huppertz B. Aberrations of early trophoblast differentiation predispose to pregnancy failure: lessons from the anti-phospholipid syndrome. Placenta 2006; 27: 869-875.
4) Soares H, Waechter H, Glaichenhaus N, Mougneau E, Yagita H, Mizenina O, Dudziak D, Nussenzweig MC, Steinman RM. A subset of dendritic cells induces CD4+ T cells to produce IFN-gamma by an IL-12-independent but CD70-dependent mechanism in vivo. J Exp Med 2007; 204: 1095-1106.
5) Kent LN, Konno T, Soares MJ. Phosphatidylinositol 3 kinase modulation of trophoblast cell differentiation. BMC Dev Biol 2010; 10: 97.
6) Jiang BH, Liu LZ. PI3K/PTEN signaling in angiogenesis and tumorigenesis. Adv Cancer Res 2009; 102: 19-65.
7) Qiu Q, Yang M, Tsang BK, Gruslin A. EGF-induced trophoblast secretion of MMP-9 and TIMP-1 involves activation of both PI3K and MAPK signalling pathways. Reproduction 2004; 128: 355-363.
8) Zhu R, Huang YH, Tao Y, Wang SC, Sun C, Piao HL, Wang XQ, Du MR, Li DJ. Hyaluronan up-regulates growth and invasion of trophoblasts in an autocrine manner via PI3K/AKT and MAPK/ERK1/2 pathways in early human pregnancy. Placenta 2013; 34: 784-791.
9) Liu Z, Chen Y, Yang Y, Peng JP. The effect on MHC class II expression and apoptosis in placenta by IFNgamma administration. Contraception 2002; 65: 177-184.
10) Sugama S, Fujita M, Hashimoto M, Conti B. Stress induced morphological microglial activation in the rodent brain: involvement of interleukin-18. Neuroscience 2007; 146: 1388-1399.
11) Raghupathy R, Kalinka J. Cytokine imbalance in pregnancy complications and its modulation. Front Biosci 2008; 13: 985-994.
12) Si LF, Zhang SY, Gao CS, Chen SL, Zhao J, Cheng XC. Effects of IFN-gamma on IL-18 expression in pregnant rats and pregnancy outcomes. Asian-Australas J Anim Sci 2013; 26: 1399-1405.
13) Baren JP, Stewart GD, Stokes A, Gray K, Pennington CJ, O'Neill R, Deans DA, Paterson-Brown S, Riddick AC, Edwards DR, Fearon KC, Ross JA, Skipworth RJ. mRNA profiling of the cancer degradome in oesophago-gastric adenocarcinoma. Br J Cancer 2012; 107: 143-149.
14) Hagemann C, Anacker J, Ernestus RI, Vince GH. A complete compilation of matrix metalloproteinase expression in human malignant gliomas. World J Clin Oncol 2012; 3: 67-79.
15) Jiao YB, Rui YC, Li TJ, Yang PY, Qiu Y. Expression of pro-inflammatory and anti-inflammatory cytokines in brain of atherosclerotic rats and effects of Ginkgo biloba extract. Acta Pharmacol Sin 2005; 26: 835-839.
16) Sonderegger S, Haslinger P, Sabri A, Leisser C, Otten JV, Fiala C, Knofler M. Wingless (Wnt)-3A induces trophoblast migration and matrix metalloprotein-

ase-2 secretion through canonical Wnt signaling and protein kinase B/AKT activation. Endocrinology 2010; 151: 211-220.

17) Li X, Yang Z, Song W, Zhou L, Li Q, Tao K, Zhou J, Wang X, Zheng Z, You N, Dou K, Li H. Overexpression of Bmi-1 contributes to the invasion and metastasis of hepatocellular carcinoma by increasing the expression of matrix metalloproteinase (MMP)2, MMP-9 and vascular endothelial growth factor via the PTEN/PI3K/Akt pathway. Int J Oncol 2013; 43: 793-802.

18) Nissi R, Talvensaari-Mattila A, Kotila V, Niinimaki M, Jarvela I, Turpeenniemi-Hujanen T. Circulating matrix metalloproteinase MMP-9 and MMP-2/TIMP-2 complex are associated with spontaneous early pregnancy failure. Reprod Biol Endocrinol 2013; 11: 2.

19) Moore CS, Crocker SJ. An alternate perspective on the roles of TIMPs and MMPs in pathology. Am J Pathol 2012; 180: 12-16.

20) Haslinger P, Haider S, Sonderegger S, Otten JV, Pollheimer J, Whitley G, Knofler M. AKT isoforms 1 and 3 regulate basal and epidermal growth factor-stimulated SGHPL-5 trophoblast cell migration in humans. Biol Reprod 2013; 88: 54.

21) Sun Q, Zhang XL. Research on apoptotic signaling pathways of recurrent spontaneous abortion caused by dysfunction of trophoblast infiltration. Eur Rev Med Pharmacol Sci 2017; 21: 12-19.