ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 8, pp. 19298-19301, August, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

THE EFFECT OF RECOMBINANT VEGF-121 ON DECIDUAL NATURAL KILLER CELLS EXPRESSION IN PREECLAMPSIA MICE MODEL

Sri Sulistyowati*., Alexander Indra Humala and Supriyadi Hari Respati

Department of Obstetrics & Gynecology Faculty of Medicine Sebelas Maret University / Dr. Moewardi General Hospital Surakarta, Indonesia

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0808.0664

ARTICLE INFO

Article History:

Received 15th May, 2017
Received in revised form 25th June, 2017
Accepted 28th July, 2017
Published online 28th August, 2017

Key Words:

Preeclampsia, dNK/ CD56 Cells, Recombinant VEGF-121

ABSTRACT

Background: Decidual Natural Killer (dNK) cells are expressed slightly in the maternal fetal interface and serve the trophoblast to invaded decidual tissue in normal pregnancy. In preeclampsia, Decidual Natural Killer (dNK) expression increases as a result of maternal immune maladaptation, causing the decreased of VEGF expression. Recombinant VEGF-121 therapy is thought to decrease the expression of dNK cells. This study aims to determine the effect of recombinant VEGF-121 on dNK cell expression in preeclampsia mice model.

Methods: Experimental. The sample size was 30 pregnant mice divided into 3 groups: 10 normal pregnant mice (N), 10 preeclampsia pregnant mice models, (PE) and 10 preeclampsia mice model with recombinant VEGF-121 therapy (VEGF). Preeclampsia mice model was made with injection of an intra venous 10 ng anti-Qa-2 which would eliminate the expression of Qa-2 (homologous HLA-G in humans) from day 1 to 4th of pregnancy. Recombinant VEGF-121 therapy is administered on the 12th to 15th day of pregnant mice with an intravenaous injection of 125 mg / kgBW / day. On day 16, all samples were examined of dNK/ CD56 by using Immunohistochemical methods. Data were analyzed by using Kruskal Wallis and Mann Whitney test.

Result: Mean of dNK/ CD56 cell in N group =1.20±0.62; PE group =2.32±1.33 with p value =0.01. Mean of dNK/ CD56 cells in VEGF group =2,25±0,94, with p value =0.02.

Conclusions: Recombinant VEGF-121 therapy had an effect on decreasing dNK/ CD56 cells in preeclampsia mice model.

INTRODUCTION

Preeclampsia is one of the complication of pregnancy, characterized by high blood pressure and proteinuria that appear after 20 weeks of gestation, and it affects 5 to 10% of all pregnancy. According to the World Health Organization (WHO), 20% of 15 million premature births are related to preeclampsia. In developing countries, the incidence of preeclampsia is higher, and the mortality for both the mother and the premature baby are 20 times higher than in developed country.[1]

The etiology of preeclampsia until now has not been known for certain, but many believe their hypotheses about placentation failure during early pregnancy because of the failure of trophoblast invasion and spiral artery remodeling lead into the placenta ischemia then endothelial dysfunction that is the hallmark/ pathognomonic in preeclampsia.[2]

In normal pregnancy, trophoblast invaded well into the decidua and myometrium because of their adaptation to the maternal immune tolerance both locally during early pregnancy and systemic.[3] Maternal immune tolerance occurs because the trophoblasts express the HLA class 1b (HLA-C, E and G) to be recognized and considered as part of "self", not to trigger maternal immune reactions. In preeclampsia, immune maladaptation occurs because of the trophoblast considered as a foreign object/ non self that causes invasion of trophoblast and inadequate spiralis arteria remodeling that will trigger the occurrence of hypoxia and inflammation in the placenta that ends up as preeclampsia.[4,5]

Immune cells in the decidual in early gestation are the decidual Natural Killer cells (dNK cells), macrophages, T cells and dendritic cells. The majority of these immune cells are NK cells accounted for 70% of all leukocytes in the decidua.[3] Decidual Natural Killer cells are a part of immune cells that are Large Granular Lymphocytes (LGL) that play a role in the

Corresponding author:* **Sri Sulistyowati
Department of Obstetrics & Gynecology Faculty of Medicine Sebelas Maret University / Dr. Moewardi General Hospital Surakarta, Indonesia

innate immune system of the human reproductive system by recognizing and destroying foreign cells. Decidual Natural Killer phenotype cells are different from the peripheral NK cells there are $CD56^{bright}$ CD^{-16}. Natural Killer cells also expressing Killer cells Immuno Receptor (KIRS) and produce cytokines that are important and has angiogenic factors such as TNF-α, IFN-ɤ, VEGF, IL-8, chemokines, and also ligand 10 which are essential for trophoblast invasion, angiogenesis, spiralis artery remodeling and successful placentation.[2]

At the time of implantation, dNK cells will react and form a receptor called Killer Immunoglobulin Receptor (KIR) to recognize trophoblast cells. The KIR will then bind to the HLA gene expressed by Extra Villous Trofoblast (EVT) cells so that EVT cells will be recognized and considered part of "self". KIR will then give a signal to dNK cells to inhibit cytolysis function of dNK cells so that the EVT implantation and invasion process can take place. If the process of the recognition is going well, the dNK cells will then generate a proangiogenic factors such as angiopoietin (Ang-2), Vascular Endothelial Growth Factor (VEGF), Placental Growth Factor (PlGF), and TNFα, IL, IFN, TGFβ cytokine to help implantation and placentation process.

From several studies it was found that in preeclampsia, dNK cells produced a lower proangiogenic VEGF than in normal pregnancies.[6,7] This suggests that dNK cells also have an important role in angiogenesis and the occurrence of preeclampsia.

Recombinant VEGF-121 is an exogenous VEGF that can be used in research, the proangiogenic protein that has role to process vasculogenesis and angiogenesis which have receptor in endotel wall such as VEGFR-1. The provision recombinant VEGF-121 as a treatment of preeclampsia in experimental animals has the ability to decrease the sFlt-1 level in blood circulation, clinically lowers blood pressure, histopathologically improves the endothelial function and reduces placental hypoxia.[8,9]

This study used experimental animals, that are Mus Musculus mice due to a short reproductive and developmental period, their ability to adapt well in a laboratory environment and genetically resemble humans.[10]

This study aims to determine the effect of recombinant VEGF-121 on the expression of dNK cells on preeclampsia, and it is expected to be one of the solution to reduce the incidence of preeclampsia.

Subjects and Methods

This research is an experimental analytical research based in In Vitro & Biomedical Laboratory of Veterinary Faculty of Airlangga University Surabaya, Indonesia. The study sample was decidual tissue of 30 female pregnant mice (Mus musculus) divided into 3 groups: 10 normal pregnant of mice group (N), group of preeklampsia pregnant mice model (PE) as many as 10 and group of preeclampsia mice model with Recombinant VEGF-121 therapy (VEGF) as many as 10 mice. Recombinant VEGF-121 therapy was performed by intra venous injection through a mice tail vein of 125 mg / kgBW / day on day 12 to -15 gestational mice followed by termination and decidual tissue sampling on day 16.

Preeclampsia Mice Model

The way to make a pregnant mice is to synchronized lust, 3-month-old adults mice weighing 20-25 grams injected by 5 IU Pregnant More Serum Gonadotropin (PMSG) hormone, 48 hours later injected by using 5 IU Human Chorionic Gonadotropin (hCG). Female mice synchronized lust married with male mice aged 7 months weighing ± 60 grams, 17 hours after mated can be diagnosed pregnant when there is copulatory plug (a plug covering the vaginal mice from the cervix to the vulva).

On the 1st day of pregnancy, the whole sample was divided into three groups, namely: 10 mice in normal group of pregnant mice (N) was kept without intervention, 20 pregnant mice with preeclampsia model (on day 1 to day 4 of pregnancy given anti-Qa-2 as much as 10 ng iv to be a preeclampsia model that will be divided into PE 10 and VEGF 10 mice of groups. On the day of 12-15 in the gestation of mice in the VEGF group was given Recombinant VEGF-121 125 mg/ kgBW. On the 16th day, all the pregnancy was terminated, then the mice then tested by using ketamine and necropsy was followed after opening the abdominal cavity, the uterus is taken and inserted into a pot containing 10% Formalin Neutral Buffer, then the Immunohistochemistry is performed by using reagents/ kits, the anti-Qa2 antibody kit (5K44), Recombinant Kit VEGF-121 (Recombinant mouse) 125 mg/ kgBW Xeno-free™, Pregnant More Gonadotropin Serum (PMSG) PG 600, Human Chorionic Gonadotropin (hCG) Chorulon 1500 iu and Anti CD56 monoclonal antibody.

dNK cell examination was performed by counting the number of dNK surface cell expression, CD56 in samples that had been immunohistochemically stained with anti-CD56 monoclonal antibodies. Determination of the number of immunopositive CD56 expression was calculated by Remmele Method modification.[12] Data were analyzed by using Kruskal Wallis Test followed by Post Hoc Mann Whitney test with significance level of 0.05 (CI-95%).

Ethical Clearence

Ethical eligibility was obtained from the Research Ethics Commission of the Faculty of Veterinary Medicine of Airlangga University. 419-KE, March 18th, 2015

RESULT

Table 1 Dyscription of dNK Cells/ CD56

Group	Total	Mean	Deviation standard	CI-95%	Minimum value	Maximum value
N	10	2,25	0,94546	1,574 - 2,926	0,90	3,60
PE	10	2,32	1,33733	1,363 - 3,277	0,50	5,40
VEGF	10	1,20	0,62716	0,751 - 1,649	0,30	2,30

Table 1 presents dNK cell surface / CD56 cell positive data in each group. Mean of CD56 expression in control group (N) or group of normal pregnant mice is 2,25±0.94. Mean CD56 expression in group (PE) or preeclampsia model is 2,32±1.33. Mean CD56 expression in the group (VEGF) or group of

preeclampsia pregnant mice who received recombinant VEGF-121 injections is 1,20±0.62.

Table 2 Post Hoc Mann-Whitney Test of dNK Cells / Positive CD56 in Normal Pregnant model, Preeclampsia Model, and Preeclampsia Model with Recombinant VEGF-121 Therapy

Pair group	Probability
(N) group – (PE) group	0,01*
(PE) group – (VEGF) group	0,02*
(N) group – (VEGF) group	0,85

*Significant p<0,05

Table 2 indicates that there are 2 pairs of groups with significant difference of CD56 values in group pairs of (N) with (PE) group, p = 0.01 and group pairs of (PE) with (VEGF) group, p = 0.02, so it can be concluded that recombinant VEGF-121 administration had an effect on decreasing expression of dNK cells in pregnant mice of preeclampsia model. Pair group of (N) with (VEGF) group was not significant with p = 0.85 indicating that recombinant VEGF-121 therapy decreased the NK decidual cell just nearly normal. CD56 examination of dNK cells by using Mixon Eclip CY1 light microscope with 400x magnification on 10 randomly selected fields from each sample on the glass object. Immunopositive CD56 expression in dNK cells was demonstrated by chromogenous brown cytoplasmic membrane features.

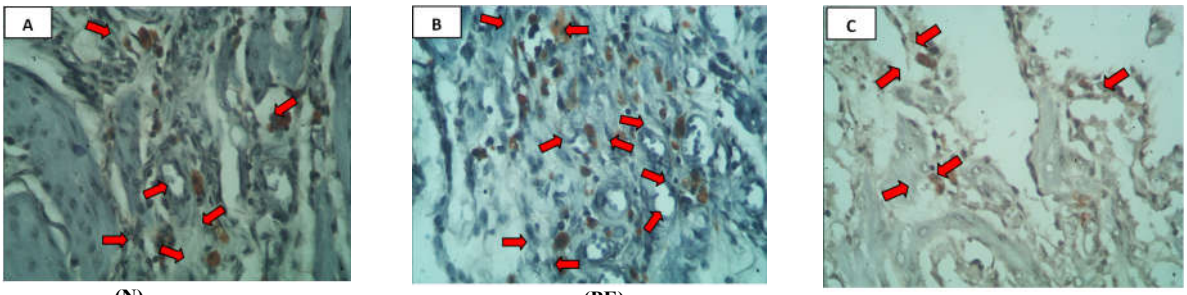

Figure 1. **A**. CD56 microfotographic representation in dNK cells (appointed by arrows) in normal group of pregnant mice (N). Figure **1. B**. Group of preeclampsia model (PE) and figure **1. C**. Group model of preeclampsia with VEGF therapy (VEGF).

DISCUSSION

dNK cells play an important role in the implantation and placentation process. In normal pregnancy, dNK cells in the endometrium at 8-10 weeks gestation produce proangiogenic VEGF (C), PLGF and Ang-2 factors. Furthermore, at 12-14 weeks gestational age, dNK cells will produce cytokines to regulate the ongoing process of trophoblast invasion and angiogenesis.[13,14,15]

dNK cell activation in preeclampsia occurs because of failure of cell tolerance against the products of conception so as to increase the number of DNK cells / CD56 + compared to normal pregnant cause implantation trophoblasts failure that result in decreased of VEGF level .[6,7]

VEGF proangiogenic factor in humans consists of several subtypes such as VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF. Recombinant VEGF121 is an external VEGF included in the VEGF-A subtype. Recombinants VEGF-121 have activity such as VEGF that will bind to the VEGFR-1 receptor in the endothelial wall. Provision of recombinant VEGF-121 can trigger the process of vasculogenesis and angiogenesis at the time of invasion and implantation of trophoblast cells.[16,17] In several studies, we found the provision of recombinant VEGF-121 in pregnant mice with preeclampsia models will provide improvements to the pre-eclampsia syndrome in the form of a decrease in systolic blood pressure, decreased glomerular proteinuria and renal histological improvement without significant side effects in mice.[8,18] This is possible because recombinant VEGF-121 can reduce the activation of dNK cells, as not to trigger the mother's immune response.

In this study, the expression of dNK cell/ CD56 increased in the group of pre-eclampsia compared to normal pregnant mice because their dNK cell activation that will reduce VEGF levels. The results are consistent with the concept that the failure of trophoblast invasion in preeclampsia is caused by a failure of the maternal immune cell tolerance, in this regard, dNK cells against the products of conception. Increased expression of cytotoxic dNK cells destroys the trophoblast. Provision of recombinant VEGF-121 125 mg/ kgBW/ day in the group of pre-eclampsia will cause a significant decrease in the expression of dNK / CD56 in this study, so VEGF-121 therapy will provide improvement in the process of angiogenesis and implantation of the trophoblast.

CONCLUSION

Recombinant VEGF-121 therapy decreases the expression of dNK cell / CD56 in preeclampsia model mice.

Limitations of Research

The dNK cells in humans have $CD56^{bright}$ $CD16^{-}$ surface markers that are less cytotoxic and inhibitory.[19] In mice, the isolated dNK cells are CD3-CD122+. Although the CD56 antibody can be used as a marker for both humans and mice, Hayakawa Y (2006) found that the use of CD27 as a marker to describe mature cell NK in the decidua will give more specific results.[20]

Similarly, VEGF proangiogenic factor in humans consists of several subtypes, namely VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF. VEGF-121 has a protein belongs to the VEGF-A subtype. In this study we have not been able to investigate the VEGF subtypes produced by dNK cells early in the implantation period and the effect of recombinant VEGF-121 on these VEGF levels and the effect of recombinant

VEGF-121 administration in different doses and modes of administration.

Conflict of Interest Statements

The authors state that there is no conflict of interest with respect to the research, authors and / or publications of this article.

Thank-you note

Thank you to the animal creation team of preeclampsia model at the Faculty of Veterinary Medicine of Airlangga University.

References

1. Sánchez-Aranguren LC, Prada CE, Riaño-Medina CE, Lopez M. Endothelial dysfunction and preeclampsia: role of oxidative stress. *Front Physiol.* 2014; 5: 372
2. Dey P. Role of decidual natural killer cells & macrophages in pre-eclampsia. *Indian J Med Re*s. 2016 Dec; 144(6): 793–795
3. Munoz-Suano A1, Hamilton AB, Betz AG. Gimme shelter: the immune system during pregnancy. *Immunol Rev*. 2011 May;241(1):20-38
4. Eastabrook G, Hu Y, Dadelszen P. The Role of Decidual Natural Killer Cells in Normal Placentation and in the Pathogenesis of Preeclampsia. *J Obstet Gynaecol Can*. 2008 Jun;30(6):467-76
5. Redman CWG, Sargent IL. Review Article: Immunology of Pre-Eclampsia. *American Journal of Reproductive Immunolog*i. 2010;63(6):534-543
6. Rieger L, Segerer S, Bernar T, Kapp M, Majic M, Morr AK, *et al*. Specific subsets of immune cells in human decidua differ between normal pregnancy and preeclampsia - a prospective observational study. *Reprod Biol Endocrinol*. 2009;7:132.
7. Akhlaq M, Nagi AH, Yousaf AW. Placental morphology in preeclampsia and eclampsia and the likely role of NK cells. *Indian J Pathol Microbiol*. 2012 Jan-Mar;55(1):17-21
8. Li Z, Zhang Y, Ma JY, Kapoun AM, Shao Q, Kerr I, *et al*. Recombinant Vascular Endothelial Growth Factor 121 Attenuates Hypertension and Improves Kidney Damage in a Rat Model of Preeclampsia. *Hypertension*. 2007 Oct;50(4):686-92
9. Mateus J, Bytautiene E, Lu F, Tamayo EH, Betancourt A, Hankins GD, *et al*. Endothelial growth factor therapy improves preeclampsia-like manifestasions in a murine model induced by over expression of sVEGFR-1. *Am J Physiol Heart Circ Physiol.* 2011 Nov;301(5):H1781-7
10. Carson E, Clifton D and Lee T. Mus Musculus. Dr House Mouse in The House. [internet] 2008
11. Sulistyowati S, Abadi A, Wijiati. Low Class Ib (HLA-G/Qa-2) MHC Protein Expression against Hsp-70 and VCAM-1 Profile on Preeclampsia. An observation on experimental animal Mus Musculus with Endothelial Dysfunction model. *Indones J Obstet Gynecol*. 2010; 34(3):103-107
12. Nowak M, Madej JA, Dziegiel P. Intensity of Cox2 expression in Cell of Soft Tissue Fibrosarcomas in Dog As Related to Grade of Tumor malignation. Bull Vet Inst Pulawy. 2007;51:275-279
13. Tang AW1, Alfirevic Z, Quenby S. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. *Hum Reprod.* 2011 Aug;26(8):1971-80
14. Engert S, Rieger L, Kapp M, Becker JC, Dietl J, Kammerer U. Profiling chemokines, cytokines and growth factors in human early pregnancy decidua by protein array. *Am J Reprod Immunol*. 2007 Aug; 58(2):129-37.
15. Kalkunte SS, Mselle TF, Norris WE, Wira CR, Sentman CL, Sharma S. VEGF C facilitates immune tolerance and endovascular activity of human uterine NK cells at the maternal-fetal interface. *J Immunol.* 2009 Apr 1; 182(7): 4085-4092.
16. Maynard SE, Karumanchi SA. Angiogenic Factors and Preeclampsia. *Semin Nephrol*. 2011 Jan;31(1):33-46.
17. Siddiqui AH, Irani RA, Zhang Y, Dai Y, Blackwell SC, Ramin SM, *et al*. Recombinant Vascular Endothelial Growth Factor 121 Attenuates Autoantibody-Induced Features of Pre-eclampsia in Pregnant Mice. *Am J Hypertens.* 2011 May; 24(5):606-12
18. Gilbert JS, Verzwyvelt J, Colson D, Arany M, Karumanchi SA, Granger JP. Recombinant Vascular Endothelial Growth Factor 121 Infusion Lowers Blood Pressure and Improves Renal Function in Rats With Placental Ischemia-Induced Hypertension. *Hypertension*. 2010;55(2):380-385
19. Fukui A, Yokota M, Funamizu A, *et al*. Changes of NK cells in preeclampsia. *Am J Reprod Immunol*. 2012 Apr;67(4):278-86
20. Hayakawa Y, Huntington ND, Nutt SL, Smyth MJ. Functional subsets of mouse natural killer cells. *Immunol Rev*. 2006 Dec;214:47-55

How to cite this article:

Sri Sulistyowati *et al*.2017, The effect of Recombinant Vegf-121 On Decidual Natural Killer Cells Expression in Preeclampsia Mice Model. *Int J Recent Sci Res.* 8(8), pp. 19298-19301. DOI: http://dx.doi.org/10.24327/ijrsr.2017.0808.0664

****