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Downregulates of ICAM1 expression in Myometrium from pregnant *Rattus norvegicus* infected with *Tachyzoite* of *Toxoplasma gondii* with Hyperbaric Oxygen Therapy

ARIF RAHMAN NURDIANTO[1*], ARYATI[2], MOHAMMAD GURITNO SURYOKUSUMO[3], MUFASIRIN[4]

[1]*STIKES Anwar Medika Hospital*
[2]*Faculty of Medicine, Universitas Airlangga, Indonesia*
[3]*Aquatic and Hyperbaric Medicine Academie Nationale de Medicine, Paris, France*
[4]*Faculty of veterinary medicine, Universitas Airlangga, Indonesia*
Corresponding email address: didins99@gmail.com

Abstract

Background: ICAM1 in pregnancy with *Toxoplasma gondii* infection can increase the number of *Tachyzoite* infections into the placenta and fetus. Hyperbaric Oxygen Therapy (HBOT) is often used and is safe in the treatment of anemia in pregnancy, besides HBOT can reduce ICAM1 through the induction of eNOS. It is hoped that HBOT therapy in pregnancy with Toxoplasmosis can prevent *T. gondii* from infecting the fetus.
Material and Methods : This is an experimental study with a post-test only design on 37 pregnant *Rattus norvegicus Sprague Dawley*, then the rats were divided into 4 groups. The group A is pregnant rats infected with tachyzoite received Hyperbaric Oxygen Therapy (HBOT); the group B is Pregnant, not infected with *tachyzoite* and get HBOT; the group C is Pregnant and infected with *tachyzoite T.gondii* but not received HBOT, and group D consisted of normal pregnant rats that were not infected with tachyzoite and were not given HBOT. Rats were killed and the myometrial ICAM1 levels were measured with Immunohistochemistry examination. All data were analyzed with the ANOVA test with SPSS 21 Program.
Results: The results show that HBOT can downregulate ICAM1 in the administered of HBOT 2.4 ATA for 3x30 minutes in 10 sessions over 5 days of therapy.
Conclusion: The study concludes that HBOT can downregulate the expressions of ICAM1 in the myometrium, in the provision of HBO 2.4 ATA for 3x30 minutes in 10 sessions.
Keywords: HBOT, ICAM1, *Tachyzoite Toxoplasma gondii*

Introduction

Tachyzoite T. gondii infection can harm the fetus and often causes abortion and congenital disability (McAuley et al., 2014). T. gondii infection can cause an increase in ICAM1 and 5-HT and the migration of mast cells to the site of infection. This results in decreased submucosal thickness and impaired extra cellular matrix formation (ECM) over a period of 12-24 hours after infection (Pastre et al., 2019).

Tachyzoite T. gondii can easily invade cells that are far from the site of infection by infecting monocytes and multiplying in these cells then through ICAM1, infected monocytic cells will attach to other cells. After that a *Tachyzoite* process will infect cells which are attached by infected monocytes to cause damage when lysis of infected cells occurs. (Ueno et al., 2014)

HBOT can reduce ischemia-reperfusion due to neutrophil-ICAM1 adhesion through CD18 polarization barriers. It is expected that with the inhibition of adhesion, *Tachyzoite T.gondii* potential to select myometrial cells can be reduced. (Uzun Gunalp et al., 2010; Lavrnja et al., 2015). HBOT can also reduce ICAM1 formation (Uzun Gunalp et al., 2010; John et al., 2008).

Therefore, the administration of HBOT is expected to reduce ICAM1 production so that the spread of *Tachyzoite T. gondii* can be prevented or reduced so that there will be no spread in all organs, especially myometrium during pregnancy.

Materials and Methods

This is experimental study with a post test only design on 37 pregnant *Rattus norvegicus Sprague Dawley*, then the rats were divided into 4 groups. The HBOT group A is pregnant rats infected with tachyzoite received therapy 10 sessions of HBOT 2.4 ATA in 3x30 minutes; the group B is Pregnant, not infected with *tachyzoite* and get HBOT; the group C is Pregnant and infected with *tachyzoite T.gondii* but not received HBOT; and group D consisted of normal pregnant rats that were not infected with tachyzoite and were not given HBOT. Each infected pregnant rats were given a 10^3 Tachyzoite of *Toxoplasma gondii* via intraperitoneal. Examinations of ICAM1 expressions were performed on day-5 after HBOT (twice a day). The sacrificed rats will be taken myometrium tissue and immunohistochemistry is examined to see the expression of ICAM1 (ICAM1 SANTA CRUZ Biotechnology Inc, Dallas, Texas, United States Of America). The data obtained were then tested by ANOVA test with SPSS 21. This experiment has an ethical clearance No.777_KE from Animal Care and Use

Committee (ACUC) Faculty of veterinary medicine, Universitas Airlangga.

ICAM1 expression from each sample was assessed semiquantitatively technique according to the modified Remmele method (Novak et al., 2007), where the Remmele scale index (ImmunoReactive Score / IRS) was the result of multiplication of the percentage of positive immunoreactive cells with color intensity scores on immunoreactive cells. Data for each sample is the average IRS value observed in 5 (five) different Fields of View at 400x magnification. The ICAM1 examination use an ordinary light microscope, the Nikon H600L brand, which is equipped with a 300 megapixel Fi2 DS digital camera and Nikkon Image System image processing software.

Results

The mean results of calculating ICAM1 expression in the four groups obtained ICAM1 values in the pregnant *Rattus norvegicus* group infected with *Tachyzoite T. gondii* without obtaining therapy or group C experienced a significant increase of 8,525 while in group A HBOT expression could decrease ICAM1 expression in myometrium. Whereas in group D normal pregnant without infection and group B (normal and given HBOT) a decrease in ICAM1 expression was obtained.

Table 1. ICAM1 Expression in Myometrium

No	Group	Average	Standar Deviation
1.	A	7.67	2.8
2.	B	7.5	2.81
3.	C	8.53	3.11
4.	D	6.82	1.88

The mean ICAM1 expression in group B *Rattus norvegicus* is lower than group A which is from the table above we can see that the expression in group A is lower than the group C, then the lowest is in group D. The lowest ICAM1 expression in group D is in the control group. The results of the ANOVA test using SPSS 21 obtained the results of the significance between groups of p = 0.652. Then the LSD test was obtained by group A relationship with group B group at p = 0.896, group A with group C at p = 0.503, group A with group C at p = 0.465, Group B and C at p = 0.438, Group B and D for p = 0.470 and group C and D for p = 0.161.

Discussion

Increased penetration of *Tachyzoite T. gondii* to the myometrium of *Rattus norvegicus* is influenced by the high IFNγ (Aboubacar et al., 2004a; Pfaff et aI., 2005; Nurdianto et al., 2018; Nurdianto et al., 2019). Increased ICAM1 due to high IFNγ stimulation can facilitate the migration of *Tachyzoite T.gondii*-infected monocytes to myometrial cells (Abbas et al., 2000; Pfaff et al., 2005). Monocytes are cells that are often infected with *Tachyzoite T. gondii* and are used as vehicles to infect myometrial cells (Channon et al., 2000). IFNγ if neutralized can also cause tachyzoite transmission to myometrial cells (Abou-bacar et al., 2004b) because IFNγ is needed in the optimum amount to eliminate *T.gondii tachyzoite* (Nurdianto et al., 2018).

The results of this study indicate that ICAM1 myometrium levels in group A were lower than those infected and did not get HBOT namely group C. This is in accordance with other studies which state that administration of HBOT can reduce ICAM1 expression (Song et al, 2016). It is hoped that by decreasing ICAM1 expression, the chance of monocytes infected with *Tachyzoite T. gondii* to invade Myometrium cells decreases.

Cell death due to *Tachyzoite T. gondii* infection is caused by cell rupture due to *Tachyzoite* which multiplies in cells and immunological factors. ICAM1 is widely distributed in all cells and its expression can be regulated by cells. ICAM1 expression can be triggered by interleukin 1 (IL-1β), tumor necrosis factor (TNF-α), and interferon-gamma (IFN-γ) which increases due to Th1 which is activated by T. gondii infection. With the high ICAM1 in the myometrium it is easier for *Tachyzoite* to infect cells and multiply in them which then makes myometrial cells damaged so that it can increase the risk of abortion (El-Sayed and Ismail., 2012) However this does not occur in group A, where IFN-γ rats rose (Nurdianto et al., 2018) but abortion did not occur.

Another study found that IFN-β can reduce ICAM1, CXCL9 and CXCL10 production. Because the decrease in CXCL9 can reduce excessive cell inflammation. So further research is needed to answer this (John et al., 2019).

HBOT can reduce ICAM1 expression through eNOS induction in in vivo endothelial cells conditioned to experience hypoxia and reperfusion injury (Buras, 2010). This is in accordance with the results of group A where the administration of HBOT causes a decrease in ICAM1 expression compared to group C.

HBOT can reduce apoptosis by decreasing the expression of HIF 1α, P53 and

BNip3 besides also increasing the expression of Bcl2 in artery spiralis (Nurdianto et al., 2019) and Caspase 3. HBOT can also reduce apoptosis by suppressing apoptosis-associated speck-like protein (ASC) and Caspase 3. (Long et al., 2014)

HBOT can also reduce CD18 function which is directly related to the actin cytoskeleton and binding of actin proteins to neutrophils and decreases ICAM1 expression in endothelial cells (Uzun Gunalp et al., 2010; Lavrnja et al., 2015). In other reports besides inhibiting ICAM1 formation, HBOT can also reduce the possibility of *Tachyzoite T. gondii* to infect cells, especially myometrium through barriers in neutrophils (Uzun Gunalp et al., 2010; John et al., 2008).

The administration of HBOT can increase NO and high NO concentrations can cause an actin cytoskeleton disruption. Cytoskeletal relationships allow integrins (CD18) to mediate cell adhesion and regulate cell shape. Because polarization of the CD18 molecule is needed to form strong adhesion, NO can interfere with CD18 function through the actin disruption of the cytoskeleton. NO mediates inhibition of c-GMP PMN, inhibition of membrane guanylate cyclase, or reduction of F-actin which can contribute to cytoskeleton actin disorders and prevent CD18 polarization (Jones et al., 2010).

Thom et al., Showed that neutrophils undergoing HBOT treatment, releasing NO which causes an increase in nitrosylated actin which can eventually disrupt the intracellular F-actin assembly that regulates the function of integrins (Jones et al., 2010) and this will also disrupt the process Neutrophil *Tachyzoite T. gondii* infected with cells expressing ICAM1 so that it can reduce the possibility of myometrial cells to become infected.

Statistical results did not show significant results but from the average results it was found that administration of HBOT in pregnant *Rattus norvegicus* infected with *Tachyzoite T. gondii* could reduce ICAM1 expression.

Conclusion

The HBOT can reduce ICAM 1 expression in myometrium of mother rats infected with *Tachyzoite T.gondii* by administering a dose of 2.4 ATA 3x30 minutes for 10 sessions.

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References

Abbas, A.K ., A.H.Lichtman and J .S.Pober. 2000. Cellular and Molecular Immunology. W.B. Saunders Company, Philadelphia; 235- 338.

Abou-Bacar, A., A.W. Pfaff, S. George, V. Letscherbru, D.Filisetti, O. Villard, E . Antoni, J-P. Klein and E. Candolfi. 2004a. Role of NK cells and gamma interfere in the transplacental passage of Toxoplasma gondii in a mouse model of primary infection. Infect. Immun. ; 72 : 1397 - 1401.

Abou-Bacar, A., A.W. Pfaff, V. Letscherbru, D. Filisetti, R. Rajapakse.E.Antoni, O. Villard, J-P. Klein and E . Candolfi. 2004b. Role of gamma interferon and T cells in congenital toxoplasma transmission. Parasite Immunol.; 26 : 315 - 318.

Buras JA, Stahl GL, Svoboda KK, Reenstra WR. 2000. Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. Am J Physiol Cell Physiol. 2000 Feb;278(2):C292-302. Accessed from https://www.ncbi.nlm.nih.gov/pubmed/10666024

Channon, J .Y ., R.M. Seguin and L.H. Kasper. 2000. Differential infectivity and division of Toxoplasma gondii in human peripheral blood leukocytes. Infect. Immun.; 68 : 4822 – 4826

El-Sayed N.M., and Ismail K.A. 2012. Role of intracellular adhesion molecules-1 ICAM-1 in the pathogenesis of toxoplasmic retinochoroiditis. Journal on molecular pathophysiology.: https://www.researchgate.net/publication/272237427

Günalp Uzun, Ali Ayata, Mesut Mutluoğlu, Şenol Yıldız, Dilaver Erşanlı. 2010. Can Hyperbaric Oxygen Therapy Turn off the Angiogenic Switch in Corneal Neovascularization?.Eur J Gen Med ;7(1):118-119

John J. Hooks, Chandrasekharam N. Nagineni, Laura C. Hooper, Kozaburo Hayashi, and Barbara Detrick. 2008. IFN- Provides Immuno-Protection in the Retina by Inhibiting ICAM-1 and CXCL9 in Retinal Pigment Epithelial Cells1. J Immunol; 180:3789-3796; DOI: 10.4049/jimmunol.180.6.3789. Accessed from http://www.jimmunol.org/content/180/6/3789

Jones et al. 2010. Hyperbaric Oxygen Inhibits Ischemia-Reperfusion Induced Neutrophil CD18 Polarization by a Nitric Oxide Mechanism Plast Reconstr Surg. August ; 126(2): 403–411. doi:10.1097/PRS.0b013e3181df64a5.

Lavrnja et al. 2015. Repetitive Hyperbaric Oxygenation Attenuates Reactive Astrogliosis and Suppresses Expression of Inflammatory Mediators in the Rat Model of Brain Injury. Hindawi Publishing Corporation Mediators of Inflammation Volume 2015, Article ID 498405 Accessed from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4417949/pdf/MI2015-498405.pdf

Long, Y et al. 2014. Hyperbaric oxygen therapy reduce apoptosis after spinal cord injury in rats. Int J Clin Exp Med.; 7(11): 4073–4081.Accessed from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4276174/

McAuley, J.B.,et al. 2014. Congenital Toxoplasmosis. Journal of the Pediatric Infectious Diseases Society, Vol. 3, Suppl 1, pp. S30–S3. DOI:10.1093/jpids/piu077. Accessed from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4164182/pdf/piu077.pdf

Nurdianto, A.R., Aryati, Suryokusumo, M.G., Mufasirin. 2018. Effect of hyperbaric oxygen therapy to IFNγ and TNFα expression in pregnant Rattus norvegicusinfected with Tachyzoite of Toxoplasma gondii. Bali Medical Journal. Vol 8(1): 94-100. DOI:10.15562/bmj.v8i1.1316

Nurdianto, A.R., Aryati, Suryokusumo, M.G., Mufasirin. 2019. Elevation of Bcl2 Expression in spiralis artery of pregnant Rattus norvegicus infected with Toxoplasma gondii with Hyperbaric Oxygen Therapy. Qanun Medika Vol.3 No.2: 157-167. DOI: http://dx.doi.org/10.30651/jqm.v3i2.2706

Pastre, Maria Jose et al. 2019. Toxoplasma gondii causes increased ICAM-1 and serotonin expression in the jejunum of rats 12 h after infection. Biomedicine & Pharmacotherapy 114 (2019) 108797. Accessed from https://www.ncbi.nlm.nih.gov/pubmed/30951950

Pfaff, A.W., S. Georges, A. Abou-Bacar, V. Letscherbru, J-P. Klein, M. Mousli and E . Candolfi. 2005. Toxoplasma gondii regulates ICAM-1 mediated monocyte adhesion to trophoblasts. Immunol. Cell. Biol;

Novak, M., Madej, J.A., Dziegeil, P. 2007.The intensity of Cox 2 expression in Cell of Soft Tissue Fibrosarcomas in Dog As Related to Grade of Tumor malignation. Bull Vet Inst Pulawy 51, 275-279. 200.

Song K, Zhang M, Liu Y, Wang Y, Ma X. 2016. The effect of hyperbaric oxygen preconditioning on the expression of ICAM-1, VCAM-1, NF-κB and flap survival rate during ischemia-reperfusion injury in rat abdominal skin flap. Zhonghua Zheng Xing Wai Ke Za Zhi. 2016 May;32(3):203-7. Accessed from https://www.ncbi.nlm.nih.gov/pubmed/30044066

Ueno, Norikiyo et. al. 2014 Real-time imaging of Toxoplasma-infected human monocytes under fluidic shear stress reveals rapid translocation of intracellular parasites across endothelial barriers. Cell Microbiol. April ; 16(4): 580–595. doi:10.1111/cmi.12239. Accessed from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4141879/pdf/nihms-594544.pdf.