KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS HASANUDDIN
FAKULTAS KEDOKTERAN
Jl. Perintis Kemerdekaan Km. 10 Makassar 90245 Telp.(0411)586010, 586297

SURAT KETERANGAN JURNAL

Yang bertanda tangan dibawah ini menerangkan bahwa :

Nama	:	Mendy Junia Hatibie
No Pokok	:	P0200314040
Program Pendidikan	:	Doktor (S3)
Program Studi	:	Ilmu Kedokteran
Judul Jurnal	:	

Hyperbaric Oxygen Therapy for Second Degree Burn Injury Wound Healing : an Experimental Study in Rabbit.

Naskah tersebut telah terindeks SCOPUS, Wolters Kluwer International Journal, SKU Description : Gold Hybrid OA – Advances in Skin and Wound Care (NSW 1527-7941) wwwsubmissions.

Demikian surat keterangan ini dibuat untuk dipergunakan sebagaimana mestinya.

Makassar , 1 Februari 2019
an. Dekan
Wakil Dekan Bidang Akademik dan Pengembangan,

Dr. dr. Irfan Idris, M.Kes
NIP.19671103 199802 1 001

----- Forwarded Message -----
From: Order Notification <notification@pti.com>
To: leo rendy <rendy_mercury@yahoo.com>
Sent: Tuesday, November 6, 2018, 7:56:44 AM GMT+7
Subject: Order Accepted - Order Number: WK0024357

Thank you for doing business with Wolters Kluwer.
Your Order Number WK0024357 has been accepted.

Order Placed By: leo rendy
Purchaser's Email: rendy_mercury@yahoo.com

Ship to:

Jl.julang 3 no.6
tanah sareal
085342358574
Kota Bogor, Jawa Barat 16161
ID

Order Number: WK0024357

Ordered Items:

Quantity: 1
SKU Name:OA-NSW_ONL
SKU Description: Gold-Hybrid OA - Advances in Skin and Wound Care (NSW)(1527-7941)_lwwesubmissions-D-18-00373R1_Hyperbaric oxygen therapy for second degree burn injury wound healing: an experimental study in rabbit_9 october 2018_None of the Above/Other_2700_Mendy J. Hatibie_____
Product Name: Gold-Hybrid OA

Hyperbaric oxygen therapy for second degree burn injury wound healing: an experimental study in rabbit

Mendy J. Hatibie*, Andi A. Islam**, Mochammad Hatta***, Yefta Moenadjat****, Rudy H. Susilo*, Leo Rendy*
*Department of Surgery, University of Sam Ratulangi, Faculty of Medicine, Manado, Indonesia
**Department of Surgery, University of Hasanuddin, Faculty of Medicine, Makassar, Indonesia
***Molecular Biology and Immunology Laboratory, University of Hasanuddin, Faculty of Medicine, Makassar, Indonesia
****Department of Surgery, University of Indonesia, Faculty of Medicine, Jakarta, Indonesia

Abstract
Background: Wound healing process consists of inflammation, proliferation, and remodelling phase, which main features are inflammation cells, neo-angiogenesis, and epithelization. Mechanism of hyperbaric oxygen therapy is O_2 pressure over one atmosphere will increase oxygen pressure in tissue. The main outcome measure is wound healing.
Methods: An experimental research with subject 36 rabbits, given deep second degree burn wound, which separate into two groups, 18 rabbits had been given hyperbaric oxygen therapy 2,4 ATA for 6 days, other group as control.
Result: Compare with control group, hyperbaric oxygen therapy group showed more robust inflammatory cells (p=0,025) and epithelization (p=0,024) but no significantly difference in angiogenesis (p=0,442)
Conclusion: hyperbaric oxygen therapy may improved second degree wound healing by increasing inflammatory cells migratin and epithelization process.
Keyword: wound healing, hyperbaric oxygen therapy, second degree burn wound

BACKGROUND

Burn is one of the most devastating injury who need complex management with high morbidit and mortality rate. Acute thermal injuries requiring medical treatment affect nearly half a million Americans each year, with approximately 40,000 hospitalizations and 3,400 deaths annually.[1] The treatment of burn wounds with hyperbaric oxygen therapy (HBOT) was first investigated in the mid-1960s and gained attention in the decades following, but controversy remains over potential risks and costs.[2] Recent work in rat models has shown that hyperbaric oxygen reduces healing time and improves scar appearance of burn injuries.[3] Advancements in hyperbaric chambers have reduced the overall cost associated with treatment, and controlled clinical trials in humans are beginning to produce data supporting the conclusion that hyperbaric oxygen is safe and effective for improving burn wound healing.[4,5]

The postulated mechanisms of a beneficial effect of hyperbaric oxygen on burn wounds are decreased edema formation due to hyperoxic vasoconstriction, increased collagen formation, and improved phagocytic killing of bacteria, therefore may decrease morbidity, lessen the need of surgery sespecially for second degree burn), and reduced hospitalization lenght of stay.[6] However, this modality of burn wound treatment is still not uniformly accepted. We decided to work animal study to streghtening the role of hyperbaric oxygen therapy especially in the treatment of second degree burn wounds. The purpose of the study is to assess hiperbaric oxygen therapy on second degree burn injury at rabbit in terms of inflamation cell, angiogenesis, and epithelisation.

MATERIAL AND METHODS

This experimental study is approved by the ethical committee for biomedical researches of faculty of medicine Universitas Sam Ratulangi, Indonesia. This study involved 36 New Zealand male rabbit (with mean weight of 1,200-1,400 grams and contained in a quarantee area.

A partial skin thickness burn injury 2x1 cm in size was made at the back of subjects under general anesthesia with intramuscular administration of ketamine (100 mg/kg body weight). The

anesthetized animals were exposed to direct flame for 5-7 seconds through 1.5x1.5 cm window in an asbestos network.

Subjects were assigned randomly to two groups, 18 rabbit in each. The first group (group A) was given HBOT therapy as 90 minutes session of 2.4 ATA for 6 consecutive days, while group B received no treatment except vaseline album ointment on the surface of the wound (act as control group). At day 14, all subjects were terminated and burn area was excised and send to the pathology laboratory for histological examination. Standard tissue slices were prepared and painted with hematoxylin-eosin.

Histological outcome measures included number of inflammatory cells, angiogenesis, epithelization. Angiogenesis was determined by counting the number of blood vessels. All microscopic measurements were carried out by one independent blind pathologists.

Kolmogorov-Smirnov test was done to determine whether two datasets is normally distributed or not. Mann Whitney U test is used to compare differences between two group.

Result

During the quarantee process all subjects survived and complete 14 days of observation process and terminated. Wound healing progress which was assessed by histological examination as inflammatory, angiogenesis, and epithelization cells number showed that HBOT group had less inflammatory cells and angiogenesis compare with contorl group and had more epithelization (see Table 1).

Table 1. Number of inflammatory cells, angiogenesis, and epithelization of burn wound healing area between hyperbaric therapy and control group

Group	Statistic number	Inflammatory cells	Angiogenesis	Epithelization
A (HBOT) n=18	Range	70-1200	8-50	10-80
	Mean	340.00	25.78	28.61
	Standard deviation	317.953	18.985	22.739
B (control) n=18	Range	70-950	8-50	5-60
	Mean	516.67	27.33	14.17
	Standard deviation	273.195	18.156	13.201

Kolmogorov-Smirnov test resulted that data was not normally distributed, therefore the differences between two groups were analysized with Mann Whitneyy U test. There is significant differences between group A and B in terms of inflammation cells (p=0.025) and epithelization (p = 0.024) (*see* Figure 1), while no significant difference for angiogenesis (p=0.442, significant if <0.05).

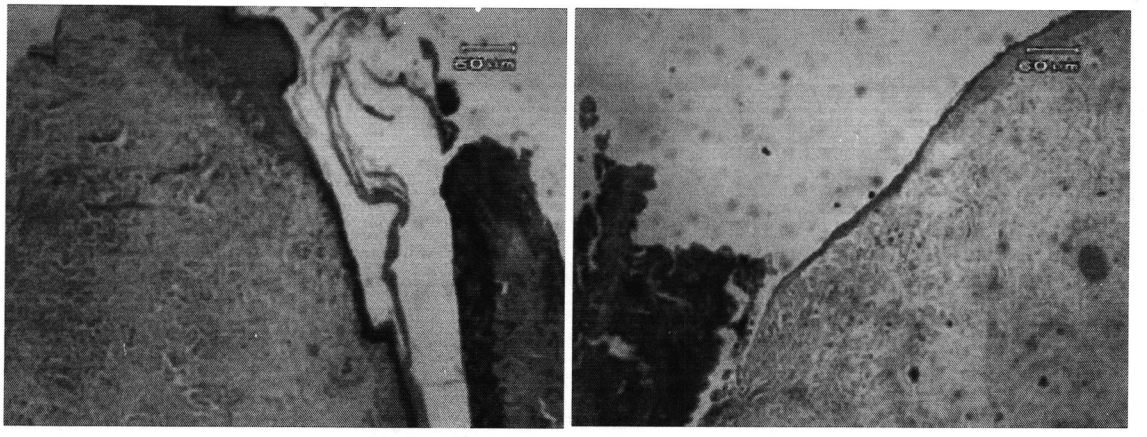

Figure 1. Histological view of epithelization, seen as dark purple line (yellow arrow) on the edge of wound that received hyperbaric oxygen therapy (left) compare with control group (right). Epithelization lining on HBOT group is more robust and thick while in control group is very thin and absent in certain area (orange arrow).

Discussion

The depth of burn wound may be classified into superficial (epidermal), partial thickness, and full thickness burn.[7] The tissue damage is not just caused by hot or cold overexposure, chemical agents, and radiation exposure alone but along with it thera are multitude mechanism of burn wound progression. Prolonged inflammation results in an accumulation of cytotoxic cytokines and free radicals, along with neutrophil plugging of dermal venules. Increased vascular permeability and augmentations of interstitial hydrostatic pressure lead to edema with vascular congestion. Hypercoagulability with thrombosis further impairs blood flow, while oxidative stress damages endothelial cells and compromises vascular patency. The main burden in these condition is failure of oxygen and nutrient supply to injured cells.[8] A number of studies have investigated the utility of various agents in modulating these mechanisms of burn wound progression. During hyperbaric oxygen treatment, oygen pressure higher than 1 atm will increase O_2 gradient pressure which promote difussion process of oxygen into tissue. Oxygen may also dissolved in plasma and may reach tissue demanding of oxygen thorugh macro- and microcirculation.[9,10] Oxygen supply which enter to cells through transport from circulation into interstitial.

Inflamatory cells reduction

Thermal injury causing local microcirculation destrucion followed by ischemia and further necrosis of surounding tissue if no treatment is given. Edema and swelling is prominent as result of increased capillary permeabillity, decreased of intravascular oncotic pressure, and damaged lymphatic system. Further damage is developed by failure of oxygen and nutrient supply to tissue, especially tissue in ischemia area who needs adequate nutrient to recover and survive. Thrombus

and neutrophil pluging the capillaries are the main source of oxidant and induce cell dextruction and reperfusion injury.[11]

HBOT may increase capillary oxygen plasma level therefore may increased soluble oxygen plasma level which is beneficial in hypoperfusion tissue and edematous environement at wound area. In HBOT, reduction of edematous envirorenment may induce microcirculation flow therefore perfusion rate are maintaned at adequate level and neutrophil as main actor of cell injury

The number of infalmmatory cells decreased after HBOT. Previous study by Thom et al showed that 2.8-3 ATA HBOT mediated inhibition of neutrophil β2 integrin adhesion.[12] The dose of oxygen pressure in this study is at 2.4 ATA, however this lower dose showed the same effect in terms of number of infalmmatory cells within wound cavity. Theorically, HBOTdoes not inhibit neutrophil antibacterial functions because the G-protein coupled 'inside-out' pathway for activation (such as that triggered by endotoxin) remains intact, and actin S-nitrosylation is reversed as a component of this activation process.[13,14] Probably the most compelling evidence that HBOT does not cause immunocompromise comes from studies in sepsis models, where HBOT has a beneficial effect.[15,16]

Decreased angiogenesis

Regional angiogenic stimuli influence the efficiency of new blood vessel growth by local endothelial cells (termed angiogenesis) and they stimulate the recruitment and differentiation of circulating stem/progenitor cells (SPCs) to form new vessels as a process termed vasculogenesis.[17] HBOT has benefit effects on this processes.[12] During wound healing, angiogenesis started during proliferation phase few days after inflammation phase up until three weeks. There was no significant difference between HBOT group and control group. The sampels of this study were terminated in 14 days the time in which the proliferation phase still in process and maybe the reason of the insignificant difference in terms of angiogenesis.

Increased epithelization

HBOT has influence in epithelization of second degree burn injury by minimizing destructive effect of hypoxia condition and have faster rate of mitosis of epithelial cells and epithleization migration to wound cavity. Previous studies showed different result whether HBOT increases rate of epithelization in second degree burn.[19-21] From Korn study, it was stated that for a while epithel cell may survive without oxygen but cannot divide and migrate. HBOT increasing oxygenation of hypoxic tissue who may not survive without additional supply of oxygen during ischemic condition.[22] This study showed faster epithelization in experimental group vs control which support earlier conclusions that adequate oxygen level is mandatory for wound healing.

Conclusion

HBOT have beneficial effect on burn wound healing. The reason for this is caused by reducing edema and preserve adequate oxygen in microcirculation therefore maintaining the rest of epithel elements in dermal layer who may speed up epithelization as new coverage of burn wound and supress unecessary inflamation process who may give negative effect on normal wond healing process.

References

1. Gibran NS, Wiechman S, Meyer W, Edelman L, Fauerbach J, Gibbons L, et al. American Burn Association consensus statements. J Burn Care Res. 2013;34:361–5
2. Cianci P, Sato R. Adjunctive hyperbaric oxygen therapy in the treatment of thermal burns: a review. Burns. 1994;20:5–14.
3. Selcuk CT, Ozalp B, Durgun M, Tekin A, Akkoc MF, Alabalik U, et al. The effect of hyperbaric oxygen treatment on the healing of burn wounds in nicotinized and nonnicotinized rats. J Burn Care Res. 2013;34:e237–43.
4. Cianci P, Slade JB, Jr, Sato RM, Faulkner J. Adjunctive hyperbaric oxygen therapy in the treatment of thermal burns. Undersea Hyperb Med. 2013;40:89–108.

5. Eskes A, Vermeulen H, Lucas C, Ubbink DT. Hyperbaric oxygen therapy for treating acute surgical and traumatic wounds. Cochrane Database Syst Rev. 2013;12
6. Bilic I, Petri NM, Bezic J, Alfirevic D, Modun D, Capkun V, et al. Effects of hyperbaric oxygen therapy on experimental burn wound healing in rats: a randomized controlled study. UHM 2005, Vol. 32, No. 1
7. Friedstat J, et.al. Burns In : Schwartz's Principle of surgery. 10th ed. Mc.Graw-Hill Education. New York. 2015.p.227-229
8. Shupp JW, Nasabzadeh TJ, Rosenthal DS, Jordan MH, Fidler P, Jeng JC. A review of the local pathophysiologic bases of burn wound progression. J Burn Care Res. 2010 Nov-Dec; 31(6):849-73
9. Sahni T, Singh P. John MJ. Hyperbaric oxygen therapy: current trends and applications. JAPI.vol.51. March 2003
10. Delaney JS, Montgomery DL. How can hyperbaric oxygen contribute to treatment? The physician and sportsmedicine.March 2001 (29) no.3: 77-84
11. Kalogeris T, Baines CP, Krenz M, Krthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol. 2012; 298: 229–317.
12. Thom SR. Hyperbaric oxygen-its mechanism and efficacy. Plast Reconstr Surg. 2011 Jan; 127(suppl1): 131S-41S.
13. Actin S-nitrosylation inhibits neutrophil beta2 integrin function. Thom SR, Bhopale VM, Mancini DJ, Milovanova TN. J Biol Chem. 2008 Apr 18; 283(16):10822-34.
14. Thom SR, Bhopale VM, Milovanova TN. Neutrophil beta-2 integrin inhibition by hyperoxia is due to actin S-nitrosylation and delocalized thioredoxin reductase. Undersea and Hyperbaric Med. 2010 Suppl in press
15. Hyperbaric oxygen protects from sepsis mortality via an interleukin-10-dependent mechanism. Buras JA, Holt D, Orlow D, Belikoff B, Pavlides S, Reenstra WR. Crit Care Med. 2006 Oct; 34(10):2624-9.
16. Intermittent hyperbaric oxygen therapy for reduction of mortality in experimental polymicrobial sepsis.Thom SR, Lauermann MW, Hart GB. J Infect Dis. 1986 Sep; 154(3):504-10.
17. Tepper OM, Capla JM, Galiano RD, et al. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. Blood. 2005;105:1068–1077.
18. Germonpre P. Burns. In: Mathieu D, editor. Handbook on Hyperbaric Medicine. Netherlands: Springer; 2006. pp.479–94.
19. Korn HN, Wheeler ES, Miller TA. Effect of hyperbaric oxygen on second degree burn wound healing. Arch Surg. 1977;112(6):732-737
20. Shoshani O, Shupak A, Barak A, Ullman Y, Ramon Y, Linderbaum E, et al. Hyperbaric oxygen therapy for deep second degree burns: an experimental study in the guinea pig. Br J Plast Surg. 1998 Jan;51(1):67-73.
21. Perrins D. Failed attempt to limit tissue destruction in scalds of pig skin with HBO2. In: Wada J and Iwa T, editors. Book of proceedings of the fourth international congres on hyperbaric medicine. Baltimore (MD): Williams&Wilkins; 1970. p. 381-385.
22. Niezgoda JA, Cianci P, Folden BW, Ortega RL, Slade JB, Storrow AB. The effect of hyperbaric oxygen therapy on a burn wound model in human volunteers. Plast Reconstr Surg 1997; 99:1620-1625