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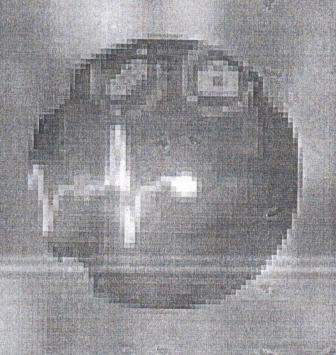
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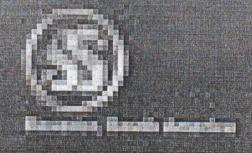
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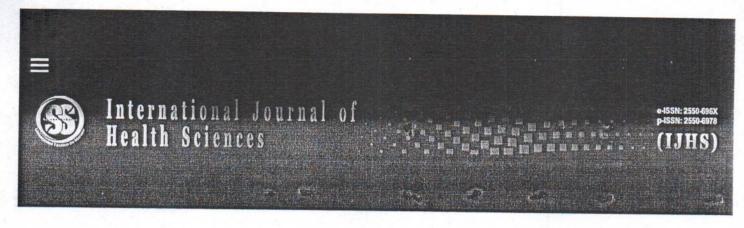
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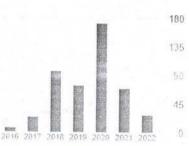
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The effects of hyperbaric oxygen therapy in improvement of Tnf- A, Hsp 70, Enos, Vegf, collagen 3, and Il 6 protein expression in wound healing

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Abstract---Wound healing is a progressive physiological process with overlapping stages. Hyperbaric oxygen therapy increases tissue oxygenation, thereby increasing tissue oxygenation and the formation of H2O2 as the second messenger of the phosphorylation of tumor necrosis factor α, NOS, VEGF, and nuclear factor β-kappa. This further study aims to determine whether hyperbaric oxygen therapy can increase collagen 3, HSP 70, IL6 expression and wound healing. This study is an animal study using "pre-test and post-test design of a randomized control group". Divided into 4 groups (HBO 2.4 ATA 3x30 minutes, 10 sessions HBO 2.4 ATA 3x30 minutes, control group without HBO), each group consisted of 7 groups with 28 male rats. Excision of the wound 1x1cm was performed. The distribution of the data was analyzed by SPSS. Hyperbaric oxygen therapy increased the expression of Collagen 3 (p=0.04), HSP 70 (p=0.03), IL 6 (p=0.02) and wound healing (p=0.002) with 5 sessions of HBO 2.4 ATA and 10 sessions of HBO 2.4 ATA increased Collage 3 (p=0.02), HSP 70 (p=0.04), L6 expression significantly (p=0.02) but did not significantly improve wound healing (p=0, 3) compared without HBO. This study was that HBO increased the expression of Collagen 3, HSP 70, IL 6 and wound healing in 5 sessions of HBO 2,4 ATA 3x30 minutes. 10 sessions of HBO 2,4 ATA 3x30 minutes increased the expression of Collagen 3, HSP 70, IL 6 without increasing wound healing.

Keywords---hyperbaric oxygen therapy, collagen 3, heat shock protein 70, interleukin 6, wound healing.

Introduction

The wound healing process is a complicated action that normally happens past a number of different mechanisms. This process occurs progressively via overlapping stages, namely the hemostasis stage, the inflammatory stage, the proliferation stage, the contraction stage, and the tissue remodeling stage (Finocchietto et al., 2009)(Schreml et al., 2010)(Velazquez, 2007). Chronic wounds are a problem for modern society, as they can be difficult to heal and can lead to long-term health problems. Chronic wounds are a major cause of morbidity in expanding country (Zhao et al., 2008)(Jiang et al., 2011)(Sander et al., 2009). There are a measurable 110 million surgical incisions made each year, but not every of them heal absolutely due to sundry reasons. These wounds then turn into chronic wounds (O'Driscoll et al., 2013). The population size and expected cost burden of chronic wounds require severe concern to early discovery, precaution, diagnosis, and treatment (Jiang et al., 2011). Hyperbaric oxygen therapy helps to advance the production by nitric oxide (NO) and hydrogen peroxide (H2O2), which are both important contributors to wound healing.

Excessive doses of RNS and ROS can harm cell survival, while therapeutic doses (2.4 ATA) of OHB help promote wound healing (Schreml et al., 2010)(Sarsour et al., 2009). When administering 100% oxygen at a pressure of 2 ATA, tissue oxygenation can increase from 30-40 mmHg in normal conditions to 250-300 mmHg. A pressure of 3 ATA allows for an increase in tissue oxygenation 10-15 times (Zhao et al., 2008). Type 1 and Type 3 collagen are formed in different proportions in normal human skin tissue. Type 1 collagen contributes primarily to skin formation as the basis of skin thickness, type 3 collagen plays a role in strengthening the reticular structure of the skin, and protein expression alternates between diffusion / diffusion (Yan et al., 2010). The heat shock protein (HSP) group is essential for normal wound healing by regulating inflammatory processes, debris removal, proliferation and migration, and collagen synthesis (Atalay et al., 2009).

IL-6 is a pleiotropic cytokine that affects both the acute-phase response and inflammation, as well as the differentiation of lymphocytes. IL-6 is produced by several cell types, including those in areas of inflammation. IL-6 is regulated by its binding to its specific receptor IL-6Ra. IL-6 plays an important role in regulating the immune response and helping wounds to heal quickly (McFarland-Mancini et al., 2010). Hyperbaric oxygen therapy help to suppress the inflammatory response, which chronic wounds not occurring (Thom, 2011). Additional oxygen flowing through the bloodstream will speed up the angiogenesis process in ischemic wounds, speeding up the healing time (Zhao et al., 2008). Many studies have demonstrated the benefits of 5 and 10 consecutive doses of OHB 2,4 ATA (Susilo et al., 2017)(Widiyanti, 2010)(Sheikh et al., 2000).

Our previous results showed that OHB significantly increased the expression of eNOS (p = 0.02), TNFa (p = 0.02), VEGF (p = 0.02), and wound healing (p = 0.002) when administered with OHB. You will need to take ATA for 3 x 30 minutes for 5 consecutive 5-day sessions. Giving OHB 2,4 ATA for 10 sessions 10 consecutive days only the expression of eNOS (p = 0.02), TNFa (p = 0.04), VEGF (p = 0.03) was significantly increased, but wound healing was not significantly improved (p = 0.3) compared with no OHB.

This follow-up study looked at whether hyperbank oxygen therapy could increase the expression of collagen 3, heat shock protein 70 (HSP 70), and interleukin 6 on wound healing. This study will look at the effects of hyperbaric oxygen therapy (HHO) on increasing the expression of INOS in immunohistochemical examination and on the wound healing process in (Rattus Norvegicus Gallus) rats. The findings of this study are expected to serve as a reference for developing fixed protocols for utilize of hyperbaric oxygen therapy (OHB) for wound healing in health care centers.

Method

This study used a randomized control group design to measure the effect of a treatment on 28 Wistar rats. The sample was randomly divided into four groups, each consisting of seven rats. Treatment group 1 (KP1) was given 5 sessions of OHB 2.4 ATA 3x30 minutes, treatment group 2 (KP2) was given 10 sessions of OHB 2.4 ATA 3x30 minutes, and each control group received no OHB (KK1 and KK2). 28 rats were given full-thickness incised wounds of 1 x 1 cm. Immunohistochemical examination for the expression of Collagen 3, HSP 70, IL 6, measurement of wound area to determine wound Immunohistochemical examination and wound area were measured on day 0 (before OHB) and day 5 after OHB in the treatment group (KP1) and the control group (KK1). Meanwhile, immunohistochemical examinations and wound areas in the KP2 group and the KK2 group were measured on day 0 and day 10 of OHB.

Normality test was performed by the Saphiro Wilks test, it was found that the data were not normally distributed, the analysis was continued by comparing the expression variables of Collagen 3, HSP 70, IL 6 and wound healing in the treatment group. with the control group using the Mann-Whitney U-test, change/delta variables using the Mann test. Withney U and comparison of Collagen 3, HSP 70, IL 6 expression variables and wound healing before and after OHB administration. Wilcoxon test was used to compare the expression levels before and after OHB administration. The correlation between increased expression of Collagen 3, HSP 70, IL 6, and wound healing was analyzed using the Spearman Rank correlation test.

Discussion

The study was conducted after the animals had been acclimated for 7 days. At first, a sample of skin tissue was taken from the experimental animal by making a full thickness excisional wound measuring 1x1 cm on the dorsal part of its body. On Day 0, skin tissue samples were taken from all of the animals (28 samples) in the treatment and control groups. The treatment group received Hyperbaric

oxygen treatment for 5 and 10 sessions. The area of wound closure and sampling of skin tissue was measured on day 5 (14 samples) and day 10 (14 samples) after Hyperbaric Oxygen treatment. Making an incision and giving immunohistochemistry to the tissue will allow us to see the results.

The expression of Collagen 3, HSP 70, IL 6 was performed under the microscope after application. This test is designed to measure the number of cells that are reactive to collagen 3, HSP70, and IL-6. The data was obtained by counting the number of cells in ten different fields of view at 40x magnification.

Condition Day 0 (before OHB) Variable Expression Of Tnfa, Enos, VEGF And Wound Area

On zero day , low-intensity TNF α , ENOS, and VEGF expression is seen in inflammatory cells. The preOHB wound area received the same treatment, that is, a 1 x 1 cm (100 mm²) full-thickness wound.

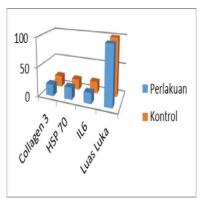


Figure 1. Mean OHB Day-0 variables of Collagen 3, HSP 70, IL6 expression and wound area

Conditions On Day 5 Of OHB Variable Expression Of Collagen 3, HSP 70, IL6 And Wound Area

In the treatment group, OHB 2.4 ATA 3 x 30 minutes was administered 5 times, and then collagen 3, HSP 70, IL6, and wound area expression were confirmed in the control and treatment groups. Figure 2 shows the average results of examining the collagen 3 expression variables HSP 70, IL6, and the wound area. On day 5, the expression of collagen 3, HSP 70, and IL6 was increased with high intensity in the treatment and control groups, but in the treatment group the increase in the expression of Collagen 3, HSP 70, IL6 was higher than the control group. It mean wound healing was higher in the OHB-treated group than in the control group (smaller wound area and better healing).

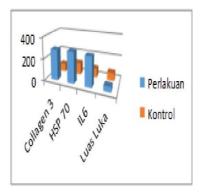


Figure 2. Diagram of mean OHB on day 5 of variable Collagen 3, HSP 70, IL6 expression and wound area

OHB Condition Day 10 Variable Collagen 3 Expression, HSP 70, IL6 and Wound Area

In the treatment group, OHB 2.4 ATA 3 x 30 minutes was administered 10 times, and then collagen 3, HSP 70, IL6, and wound area were confirmed in the treatment and control groups. OHB day 10 of administration. In Figure 3, it can be seen that the expression intensities of collagen 3, HSP 70, and IL6 still high, but lower than on day 5.

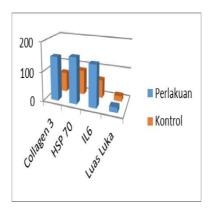


Figure 3. The diagram of the mean OHB on day-10 of the variable Collagen 3, HSP 70, IL6 expression and wound area

On the other hand, the wound healing variables showed no significant difference between the treatment and control groups.

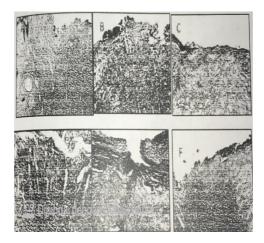


Figure 4. An overview of Collagen 3 expression using an Olympus CX31 light microscope with objective magnification of 40x. A: day-0 control group, B: day-5 control group, C: day-10 control group, D: day-0 treatment group, E: day-5 treatment group, F: treatment group 10th day. In the control group on day 0, the intensity of Collagen 3 expression was still low, on day 5 the intensity of Collagen 3 expression decreased again



Figure 5. An overview of HSP 70 expression using an Olympus CX31 light microscope with an objective magnification of 40x. A: day-0 control group, B: day-5 control group, C: day-10 control group, D: day-0 treatment group, E: day-5 treatment group, F: treatment group 10th day. In the control group on day 0, the intensity of Collagen 3 expression was still low, on day 5 the intensity of HSP 70 expression increased, then on day 10 the intensity of HSP 70 expression decreased again

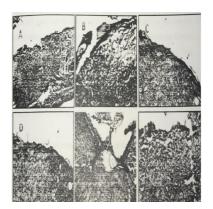


Figure 6. An overview of IL 6 expression using an Olympus CX31 light microscope with objective magnification of 40x. A: day-0 control group, B: day-5 control group, C: day-10 control group, D: day-0 treatment group, E: day-5 treatment group, F: treatment group 10th day. In the control group on day 0 the intensity of IL 6 expression was still low, on day 5 the intensity of IL 6 expression increased, then on day 10 the intensity of IL 6 expression decreased again

The results of examining the expression of Collagen 3, HSP 70 and IL 6 in the treatment group and control group on day 0 found the expression of Collagen 3, HSP 70 and IL 6 with low intensity (1 - 1). After 5 sessions of OHB 2,4 ATA, on day 5 there was an increase in the expression of Collagen 3, HSP 70 and IL 6 increased (2 - 2) with an increased number of inflammatory cells, where an increase in the expression of Collagen 3, HSP 70 and IL 6. On the group treatment it is higher. On day 10, it can be seen that the intensity is significant compared to the group control. The expression of Collagen 3, HSP 70 and IL 6 was still high, but the number of cells expressing it was reduced, while number of inflammatory cells remained large (2 - 1).

Collagen is a protein that is mostly produced primarily by mammals and plays a fundamental role in the formation of stromal tissue in the epidermis. Collagen type 1 and type 3 are formed in normal human skin tissue in different proportions. Collagen type 1 is the main contributor to skin formation as the basis for skin thickness, while collagen type 3 plays a role in strengthening the reticular structure of the skin, where protein expression alternates and spreads (Yan et al., 2010).

The Heat shock protein (HSP) group is important for normal wound healing by regulating the inflammatory process, cleaning debris, proliferation and migration, and collagen synthesis. The HSP group is important in maintaining cellular homeostasis and protecting cells from damage. HSPs are important in normal wound healing through their ability to modulate inflammation, cell proliferation, migration and collagen synthesis (Atalay et al., 2009). IL6 regulates the immune response and helps speed up wound healing (McFarland-Mancini et al., 2010). For complete wound closure in larger wound areas, cell migration often leads to proliferation. Cell proliferation is dependent on the availability of oxygen. In this state, cytokines and chemokines (EGF, TGFa, KGF, NGF, IGF1, IL1, IL6) released by keratinocyte stem cells stimulate keratinocyte proliferation in a process called

"proliferative training". The highly metabolically active epithelialization process is highly reliant on oxygen and ROS (Schreml et al., 2010).

The administration of OHB can affect the release number of cytokines and growth factors that are important in wound healing. OHB regulates the production of fibroblast growth factor (FGF) and collagen. In patients with Crown disease, OHB regulates the level of IL-1, IL-6, and TNF- α (Al-Waili & Butler, 2006). In the presence of hypoxia and hyperoxia / OHB, several biological processes and growth factors are stimulated and increased. These processes include angiogenesis, collagen synthesis, osteoclast activity, VEGF release and TNF α .

Oxygen can stimulate biological processes under hypoxic and high oxygen conditions. This is called the oxygen paradox. One of the mechanisms was to stimulate fibroblasts to form collagen via the peroxide pathway. This can occur during hypoxic wounds and OHB treatment. Peroxides formed during OHB therapy are similar to the stimuli encountered during hypoxia. Another mechanism is the stimulation of cytokines that begins with hypoxia, after which further regulation of cytokine increase is supported by the presence of hyperoxia that occurs during OHB therapy. VEGF, TNFa TGFB, and PDGFB are released in hypoxic wounds as an initial stimulus, but their activity is increased under high oxygen conditions, especially in the presence of lactic acid (Susilo et al., 2017).

This evidence suggests that OHB administration has the effect of promoting overt healing through the mechanism of collagen 3, HSP 70, and IL 6 expression during the inflammatory and proliferative phase. Administering OHB after surgery may reduce the length of stay in the hospital. Therefore, it is expected that postoperative patients will have reduced costs and healing times, which will allow them to resume their normal activities more quickly. This needs further research into the effects of hyperbaric oxygen therapy on different types of wounds, including difficult-to-heal wounds, and post-traumatic and post-surgery wounds. This evidence suggests that long-term administration of OHB does not have significant benefits for wound healing. After five sessions of OHB, it may be necessary to take a break before proceeding to the next session. Further investigation is needed to determine the effect of the amount of time needed for wound healing on the rate and range of intervals between OHK treatments. Further studies should be conducted with a wider range of dose variations (among 1-5 sessions and among 5-10 sessions) to find the prime dose of hyperbaric oxygen therapy for uninfected acute wounds.

Conclusion

The conclusions of this find out about had been OHB 2,4 ATA 3x30 minutes for 5 periods elevated the expression of Collagen 3, HSP 70 and IL 6 and accelerated wound healing; whereas OHB 2,4 ATA 3x30 minutes for 10 periods elevated the expression of Collagen 3, HSP 70 and IL 6 however did not speed up wound healing. This evidence shows that the administration of OHB has the effect of accelerating wound recovery through the expression mechanism of Collagen 3, HSP 70 and IL 6. Giving OHB has the viable to be used for adjuvant therapy after surgical treatment and certain cases, so that it is predicted to abridge the length of hospital stay and minimalize costs and recuperation period for postoperative

patients and patients can revert to their ordinary activities. For the more research is necessary to completed establish the interval period for repetition of hyperbaric oxygen therapy after 5 periods (cure period) and utilize of OHB as servant treatment for various extraordinary types of wounds.

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