The Therapeutic Potentials of Intermittent Hypoxia on Bone Healing: A Systematic Review

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Abstract

The use of a low dose intermittent hypoxia is a potential strategy for the implementation of the nonpharmacological method to cure the bone disease. However, nowadays there havent reached a strong scientific consensus on the bone metabolism process against the hypoxia. Though, there are roles of HIF-1α, VEGF and mRNA which can accelerate the bone healing.

This research aims to analyze the IH's potential therapeutic of the healing process against the bone fracture.PubMed, Google Scholar, Dentistry and Oral Sciences, CINAHL are online databases to search the experimental study data in English which inquiry on the intermittent hypoxia effectiveness in the bone fracture healing. Two studies on male animals with bone injury using continuous therapy method of IH. The increase of HIF-1α, VEGF, and mRNA expressions is inversely proportional to the bone fracture healing.

Based on this systemic review, intermittent hypoxia may promote bone healing. Evidenced by increased bone formation, bone mass, and bone strength. However, the additional research is still needed for the optimal duration for bone regeneration and maintance of anatomically shaped bone grafts in the bone regeneration process.

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Introduction

The bone fracture is a kind of trauma which commonly occurs. This condition will be followed by the bone healing process which is a complex process and consists of mechanical and biological phases.[1,2] The delayed bone healing process has a bad impact against the patient's life quality, other than a quite substantial impact against the community.

In a literature review reported by Roberts and Drissi (2020), it is identified that 5-10% of the individuals suffered the difficulties in the bone healing in which such condition further needs a subsequent treatment in form of an additional invasive action that can be associated with the degradation of the life quality.[1,3]

Besides that, based on a literature study reported by Scottel et.al (2015), it is mentioned that the delayed healing against the long bone, regardless its anatomy location, is an injury which weakens patients. A patient with a delayed healing against the fracture in his lower arm has the lowest score of life quality, and it is only about 32% of them that can do activities perfectly normal.[4]

Since the injury impact is still high due to the bone healing, further research on the bone fracture healing becomes a main topic that is required to be developed.[5,6,7]

Two therapies which are the most researched to increase the bone healing biologically namely a therapy using bone morphogenetic protein and fibroblast growth factor which works locally and a systemic therapy in which parathyroid hormone and NEL-like

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protein 1 (NELL-1) combined with polyethylene glycol (PEG). In addition to that, the tissue engineering with hypoxia inducible factor (HIF)-1 which is proven influential against the bone fracture healing is also a kind of therapy that keeps to be developed to be used in the bone healing process. It is because HIF-1α is proven to regulate the genes expression related to angiogenesis and osteogenesis.[8]

Both processes play an important role in the process of bone growth, development and healing since the blood vessel formation is very required to supply nutrition, oxygen, growth factor, sitokin, osteoblast, and osteoclast precursor. Osteogenesis is always preceeded with the existence of angiogenesis, and the bone formation process with vascularization is an integrated process called as the ”angiogenic–osteogenic coupling”. [9,10]

The HIF-1α is a main transcriptional regulator of the cellular response against the hypoxia.[10] In the hypoxia condition, the HIF complex will be accumulated in the cell core and will then be bound with aryl hydrocarbon receptor nuclear translocator (ARNT). This kind of bound will then activate genes responding against hypoxia, such as vascular endothelial growth factor (VEGF)[11].

The VEGF is an essential mediator of the process of angiogenesis, which has the potency to increase the bone morphogenetic protein (BMP) which induces the bone formation.[10] If the hypoxia condition occurs in a mild phase, lapses, and takes place in a certain period of time, and interrupted with normoxia condition, then such condition is called intermittent hypoxia (IH).

The Intermittent Hypoxia is identified to be able to provide many benefits, including increasing the bone mineral density particularly in the case of bone injury. The use of a low dose intermittent hypoxia is categorized into a nonpharmacological therapy using a simple and safe method, including in the bone healing.[12]

This research is intended to analyze the IH’s potential therapeutic against the healing process of bone fracture, while in the previous research results it can be assessed on the effectiveness of the intermittent hypoxia in the bone healing process.

Materials and methods

In this literature review, it is conducted the analysis in reference to the Preferred Reporting Items for System Review and Meta-Analysis (PRISMA). The assessment in this systematic review is carried out by 3 (three) assessors in an independent manner. Those three authors (KM, KS, TM) formulated the research plan, determined the inclusion and exclusion criteria and the data taking method. Two assessors independently conducted the critical assessment process and done the data analysis.

The searching strategy in this research is made by using some combined keywords using the Boolean Operator for the “AND/OR” word. Keywords which are used among other things are (“intermittent hypoxia”) AND (“bone healing” OR “bone” OR “healing” OR “therapeutic”). The literature search from online databases covered PubMed, Google Scholar, Dentistry and Oral Sciences, CINAHL from 2011 to 2021. The search results were then filtered by title and abstract then continued with overall filtering of the article substance based on the selection criteria carried out by two assessors and an adviser.

Assessment Criteria

The assessment is conducted against complete articles in English with the inclusion criteria: (1) experimental animals are rabbit or rat; (2) male; (3) having an injury bone; (4) intermittent hypoxia; (5) experimental research. Meanwhile its exclusion criteria are as follows: (1) unhealthy experimental animal; (2) chronic intermittent hypoxia (the IH exposure is more than 2 months); (3) the environment with the oxygen level less than 17%; (4) fake experimental research.

Bias Risks

For the internal quality of each study, it is carried out the assessment by using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE). This assessment tool consists of ten (10) assessment items, starting from the low bias risk with the quality criteria assessment of >75%, moderate bias risk with the quality criteria assessment of 50%-74% and the high bias risk with the quality criteria assessment of <49%[13].

Data Extraction

An author (KM) carried out the data taking and another author (KS) verified the data taken.

The data details were taken comprises: (1) animal type and sex type of the experimental animal; (2) type of bone injury; (3) form, frequency, and the length of intermittent hypoxia. The data taking results are presented in form of the table.

Results

Article selection

Based on the identification results, it is found 1,436 articles which consist of PubMed (917), Google Scholar (80), CINAHL (428), and Dentistry and Oral Sciences (11). After being made the duplication check, it was found 1,434 articles. Further, it was selected with PICOS and found the incompatible data of 1,432 articles. In the final result, it was obtained 2 articles which satisfied the inclusion criteria that is determined. The PRISMA graphic about the article selection is presented in the figure1.

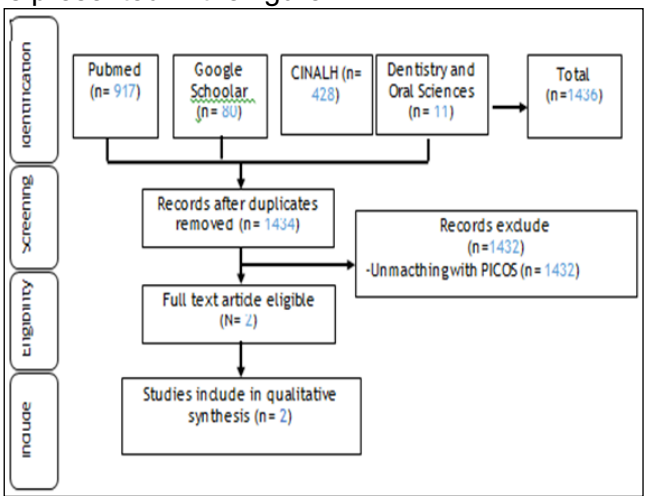

Figure 1. PRISMA flow diagram bias risks.

Bias Risks

The assessment of bias risks against the selected articles is presented in the table 2. Two selected cases satisfied the criteria of low bias risk or having a high quality of the article.

Data Extraction

It was found two articles on the research about the effects of Intermittent Hypoxia (IH) against the bone healing with male rats as the experimental animal. The exposure of intermittent hypoxia in moderate level (the oxygen level of 10-12%) to trigger the healing process of the bone injury in this research is created through the hypobaric hypoxia chamber.[14,15]

The fracture healing is quicker in the hypoxia exposure compared to the control in the 2nd, 4th and 8th weeks after the fracture. Compared to the control, the bone healing scores against the IH exposure in the 2nd and 4th weeks are significantly larger. The fracture healing scores against the IH exposure: 3.2±0.8, the control: 1.3 ± 1.0 (P<0.01) and in the 8th week, though its IH exposure is larger, there is no significant difference, and the fracture remodeling against the IH exposure almost perfectly occurs.[14,15]

Item	Type of bias	Domain	Li Zhang 2021	Junjie Qiao 2018
1	Selection bias	Sequence generation	1	1
2	Selection bias	Baseline characteristic	1	1
3	Selection bias	Allocation concealment	1	1
4	Performance bias	Random housing	1	1.
5	Performance bias	Blinding	1	1
6	Detection bias	Random outcome assessmen	1	1
7	Detection bias	Blinding	1	1
8	Attrition bias	Incomplete outcome data	UC	UC
9	Reporting bias	Selective outcome reporting	1.	1
10	Other	Other sources of bias		

Item	Type of bias	Domain	Li Zhang 2021	Junjie Qiao 2018
1	Selection bias	Sequence generation		
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9	Reporting bias	Selective outcome reporting		
10	Other	Other sources of bias		

: Low Risk : Unclear : high 89% 89%

Table 2. Bias Risks Assesment.

The examination result of the callus microstructure in the fracture area showed that based on total bone volume (TV) there is no significant difference between the IH exposure (38.59 ± 6.71) and the control (36.20 ± 3.86; P>0.05), meanwhile based on bone volume examination (BV), comparison between BV and TV and Bone Mineral Density (BMD) entirely showed the significant difference between the IH treatment and the control.

In the BV examination (against the IH exposure: 24.85 ± 3.13, the control: 18.29 ± 1.13; P<0.001), BV/TV (against the IH exposure: 65.77 ± 12.46, the control: 50.08 ± 3.82; P< 0.05) and in the BMD (against the IH exposure : 0.99 ± 0.01, the control: 0.95 ± 0.03; P< 0.05).[14]

The bone growth in the 4th and 8th weeks against the IH exposure is larger more than the control, it showed the endochondral ossification

process which is better in the exposure compared to the control. [14,15]

The cell response mechanism is against the hypoxia: expressions of HIF-1a and VEGF increased in the treatment compared to the control in the 2nd and 4th weeks and equal to the 8th week. The expression of HIF-1a mRNA is larger two times (2x) compared to the control. The expression of VEGF mRNA in the 2nd week > 83.0% and in the 4th week > 62.6% more. The level of CD31 increased against the IH exposure in the 2nd, 4th, and 8th weeks.[14]

Levels of protein and mRNA in SDF-1 and CXCR4 against the IH exposure are higher in the 2nd, 4th, and 8th weeks compared to the control.[14] It showed that SDF-1 and CXCR4 is influenced in the intermittent hypoxia exposure which caused the improvement in the bone healing.[14]

The osteoblast activity increased significantly against the IH exposure compared to the control in the 2nd week. The expression of mRNA in the runt-related transcription factor (RUNX2) increased two times (2x) larger, mRNA in the osterix increased three times (3x) higher and the mRNA in the collagen type 1 is two times (2x) larger. Besides that there was the increase of alkali phosphatase and osteocalcin (OCN).[14,15]

The exposure of intermittent hypoxia training (IHT) increased the biomechanics against the tibia fracture significantly compared to either the control or the remote ischemic preconditioning (RIPC) ($P<0.05$).[15]

Discussion

The research on the benefit of the intermittent hypoxia (IH) against the bone healing is still very rare to be conducted. In the latest 10 years, we only found 2 (two) articles related to the research carried out in Asian region namely China, where it was found a mild dose hypoxia provided in the interval within a certain period of time which is interrupted with the normoxia condition known as the intermittent hypoxia (IH) which can give a lot of benefits.[12] The IH condition in this research uses the chronic intermittent hypobaric hypoxia (CIHH) and the intermittent hypoxia training (IHT) which share the same principles with the ischemic preconditioning (IPC) and the hypoxic adaptation in the height.[16]

In this research, IH with the oxygen level of 10-11% for 6 hours and 12% for 25 minutes once (1) in a day.[14,15] The moderate hypoxia (9-16% of the O_2 inspiration) with the frequency of 3-15 episodes a day gives a lot of benefits and without incurring the disruption. The accumulation of low dose giving with some periods is simple, safe and effective and easy to be applied so that it is considered as a potential therapy means to be applied in some clinical abnormalities.[12]

The IH is used as the nonpharmacological therapy to improve the health, oxygen usage and prevention and treatment of various diseases.[16] The IH can provide benefits for the prevention of cardiovascular disease, reduce the ischemic injury against the brain and can provide the protection against kidneys from the oxidative damage.[17,18,19] However, in this literature review, total articles found were limited, which describe the IH's benefit in the improvement of the bone healing process.[14,15]

The advancement in molecular sector and genetic engineering in using small animals for the research such as rats and mice is increasingly popular. In the fracture model against the rat, then genetic change is easily happened so that it is easy to be learned with the antibody.[20] Rats which are used are male rats since there is the effect of hormonal factor and the bone remodeling in the condition of lack estrogen. In the hypoxia condition, the micro-environment of the bone can be changed so that it can damage the differentiation of osteoblast and increase the osteoclast formation which therefore reduce the bone formation and increase the bone resorption.[21]

In the initial phase of healing process in the bone fracture is the inflammation occurs 24 hours after the injury occurrence. The inflammation phase ends in the third day after the injury, which in this phase micro blood vessels around the fracture area. In the 14th day after the fracture entered the endocondral.[6,22] This bone healing process approximately takes place for 35 days.[23] In order to conduct the analysis in the process of bone healing, the examination uses micro-radiograph, micro-CT, histomorphometry and biomechanics torsion testing.[20]

The expressions of HIF-1α and VEGF increased in the treatment compared to the control in the 2nd and 4th weeks and equal in the 8th week.[14] In the hypoxia condition, the

concentration of HIF-1α will increase because the HIF-1α gene is a transcript factor which is very much active in taking a role as the regulator of the gene expression in the hypoxia condition. The HIF complex will be immediately formed after the stimulus of hypoxia in several minutes appears. The increasing concentration of this HIF-1α can trigger the establishment of the angiogenic factor, such as VEGF.[24,25] The HIF governs genes and VEGF is a gene which becomes a main target.[26] The HIF-1 line is the master of the angiogenesis regulator.[27]

Osteogenesis and angiogenesis are processes which are very much related during the bone growth, development and remodeling. Meanwhile the IH triggers the formation of VEGF which can function as the ossification trigger through the establishment of neovascularization or directly influenced the bone cells. It can provide the beneficial effect, such as in the process of growth, development and healing of the bone fracture.[11,14,15,28] The appearance of this HIF-1a and VEGF showed the increase of neovascularization in the initial phase.[14]

Hypoxia can increase the BMD in the femoral and lumbar bones of the rat, through the increase of osteogenic factor, such as alkaline phosphatase, bone morphogenetic protein-2 is as good as the VEGF in the periodontal tissue.[29] The IHT increases the differentiation of osteoblast and mineralization, viewed from the osteoblast markers (VEGF, Runx2, ALP and OCN).[15]

The remodeling process can be identified through the bone formation marker and bone resorption marker. The bone formation marker is the result of the osteoblast activity, which consists of propeptide of pro-collagen type 1, alkaline phosphatase and osteocalsin.[14,15,30] The increase of this bone formation marker shows that the IH increases the osteoblastic differentiation and the bone formation through the regulation increase of RUNX2, osterix and collagen type I.[14,15]

In order to find out the progress of the bone healing process, it can be further viewed from the bone microstructure in the callus formation I the fracture area through the parameter of total volume (TV), bone volume (BV), ratio of BV against BT and bone mineral density.[14]

It appeared that the increase of the mRNA expression in the collagen type 1 is two times (2x) larger against the IH exposure, which showed the increasing activity of osteoblast against the IH exposure in the fracture healing process.[14] Bone consists of 4 cell types: osteoblast, osteoid, extracellular matrix, and osteoclast.[31]

Osteocyte and osteoblast are dissolved in the bone matrix and becomes the very important feature of the bone.[32] Collagen type 1 is a large bone matrix component which is started to be established on the14th day after the fracture, on the 17th-35th the remodelling starts to occur in the phase of primary bone and it becomes strong. [23] Osteocyte plays a main role in governing the bone formation and resorption.[31]

Conclusions

The IH exposoure can increase the bone healing process. It is proven by the increase of bone formation, bone mass, and bone power. IH increase the bone healing process through the increse of HIF-α which plays a regulator role of the genes expression in the hypoxia condition. In order to fill in the gap with the same research done in China, in Indonesia it is especially should be developed further researches related to the IH method development for an optimum duration which induces the process of the bone regeneration.

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Declaration of interest

The authors declare that there are no conflicts of interest.

No	Country	Reference	Sample						Time Observe	Intervention	Exposure Impact
			Type	Age	Size	Amount	Random	Traumatized			
1	China	Li Zhang 2021 (1)	Sprague-Dawley rats male	Adult	-	6 tail/ group	Control vs Exposed	femur	2,4,8 week	Exposure Type: HHC Length/ Pressure: 50kPa O_2 Level: 10%-11% Frequenscy : 1/day Exposure Time: 6 hour	- There is a faster healing in CIHH (week: 2,4,8) - Significant increase in the number of callus fracture healing - TV is no different - BV is higher than control - BMD increases - CIHH enhances bone mineralization - Week 4 and 8 the woven bone is bigger than control - Osseointegration process is better than control - There is an increase in VEGF and HIF-1a compared to control (week 2,4) almost the same at week 8 - SDF-1/CXCR4 higher than control (mg 2,4,8) - RUNX2, Osteric and Collagen type 1 increased in treatment - CIHH promotes bone healing through regulation of angiogenesis via HIF-1a/VEGF
2		Junjie Qiao 2018 (2)	Sprague -Dawley rats male	18-20 months	485+ /- 60 gr	96 32:IHT 32 :RIPC 32:control	Control vs Exposed	tibia	7,14, 28 day	Exposure Type: RIPC: 3x/ 10 second O_2 Level: 12% Frequenscy : 5x Exposure Time: 5 minute	- Callus formed was significantly larger in the treatment than the control - BV on IHT is more than RIPC even though on the 7th day, it is not significant - BV, BMD, BV/TV and Tb.N's value on IHT are greater than RIPC in each observation - On day 28 the BV value decreased in IHT, but in others increased BMD.TV and TbN - In the callus bridge treatment more than the control day on 14, day on 28 the fracture line has disappeared - In RIPC, callus density is seen around the fracture - In the experiment, it showed greater bone strength than control and significantly greater IHT than RIPC - VEGF, Runx2, ALP and OCN. Greater in IHT than RIPC IHT has a differentiating effect on osteoblasts, which plays a role in the early stages of fracture healing

Table 1. Extraction table.

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