BMP-2 Expression of Post Tooth Extraction that Catfish Oil Application

by Theresia Indah Budhy

Submission date: 26-Oct-2018 11:43AM (UTC+0800)
Submission ID: 1027100070
File name: 1116-6367-1-PB.pdf (665.24K)
Word count: 2371
Character count: 13015

KnE Life Sciences
VMIC 2017
The Veterinary Medicine International Conference 2017
Volume 2017

Knowledge E
Engaging minds

Conference Paper

BMP-2 Expression of Post Tooth Extraction that *Catfish* Oil Application

Theresia Indah B.[1], Bambang Sumaryono[2], Ketut Suardita[3], and Amelia Putri R.[4]

[1]Lecturer Faculty of Dentistry University of Airlangga
[2]Lecturer Faculty of Dentistry University of Airlangga
[3]Lecturer Faculty of Dentistry University of Airlangga
[4]Dentist Education Students
Faculty of Dentistry, University of Airlangga, Surabaya-Indonesia

Abstract

Background: *Catfish (Clarias batrachus)* oil contain the highest amount of omega-3-PUFA among other freshwater fish. The omega-3-PUFA in fish oil produced eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is known that EPA and DHA in essentials fatty acid (EFA) could improve BMP-2 expression. Bone morphogenetic protein-2 (BMP-2) is bone stimulator which capable of inducing differentiation of mesenchymal cells into osteoblast, stimulating bone formation in wound healing process of dental extraction. **Purpose:** To prove the increasing expression of BMP-2 after *catfish (Clarias batrachus)* oil application. **Methods:** We have used the post test only design in this research. There have been 21 Rattus novergicus as research samples, and those were divided into 3 groups, group KK as control, group KP1 was given *catfish (Clarias batrachus)* oil in 5% concentration, and group KP2 was given *catfish (Clarias batrachus)* oil in 10% concentration. *Catfish (Clarias batrachus)* oil were applied into the socket of dental extraction. Rat was decapulated 7 days after fish oil application and the jaw in the treated regions and control group were cut for immunohistochemistry examination to observe BMP-2 expression. Data was analyzed using one-way ANOVA test. **Result:** There is significant difference increased of BMP-2 expression between control and treatment group. In samples given with 10% concentration of catfish oil had the most significant increase of BMP-2 expression. **Conclusion:** *Catfish (Clarias batrachus)* oil in 10% concentration could increase the expression of BMP-2 post dental extraction.

Keywords: Catfish oil, BMP-2, dental extraction.

Corresponding Author:
Theresia Indah B.
terebudhy@gmail.com

Received: 03 October 2017
Accepted: 10 October 2017
Published: 29 November 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the VMIC Conference Committee.

1. INTRODUCTION

Bone Morphogenetic Protein-2 (BMP-2), a cytokine that is part of the transforming growth factor-β (TGF-β) superfamily, was first detected in cartilage and bone.[1, 2]

Many studies have shown that BMP-2 supports improvements bone. BMP-2 to recruit stem cells to the bone healing, improve angiogenesis and cause differentiation of stem cells into osteoblasts. BMP released during bone resorption by osteoclasts, were able to induce the differentiation of mesenchymal cells towards osteoblasts (osteoinduction), stimulates bone formation in the process of remodeling and repairing.[3]

Catfish (Clarias batrachus) had higher levels of omega-3-PUFA highest when compared with fish, shrimp and other fresh fish at levels of 28% of the total fatty acid. The resulting fatty acids from fish, specifically as a source of omega-3, especially eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA).[4] Essentials Fatty Acids (EFA) and its metabolites such as γ-linolenic acid (GLA), eikosapentaenoik acid (EPA), and dokosaheksaenoik acid (DHA) is reported to increase the expression of BMP-2.[5]

Omega-3-PUFAs, such as EPA and DHA are known to have benefits in bone metabolism. Several studies have reported Bahwan omega-3-PUFAs can increase bone formation, affecting bone mass and reduce bone loss. One mechanism of omega-3-PUFAs can regulate bone metabolism, including decreases the release of prostaglandin E2 (PGE2), modulate the amount of proinflammatory cytokines, increase IGF1 production and improve the accumulation of calcium in the bones. Prostaglandins, which are generated from the precursor EFA in osteogenic cells regulate the formation and bone resorption. Several studies have shown that the experimental animals that were given omega-3-PUFA tend to show an increased rate of bone formation, possibly as the result of a stimulatory effect on the activity osteoblas.[6]

However, until now there has been no research to prove the potential of giving fish oil catfish C.batrachus in an increase in BMP-2 after extraction. Therefore, the author wants to prove C.batrachus catfish fish oil can improve the BMP-2 after extraction.

2. MATERIALS AND METHODS

Extraction of fish oil catfish (Clarias batrachus) using the method of extraction with hexane. 250g meat catfish disonifikasi with hexane at a temperature of 30 ° in 1 hour with a pause of 5 minutes every 15 minutes to avoid heat generation due to sonifikasi. The filtrate is collected in a vacuum and the residue disonifikasi back with hexane and the filtrate was taken. Both filtrate is collected and entered into a rotary vacuum evaporator to remove hexane. Added 0.1µmole BHT (butylated hydroxyl toluene) to fish oil to prevent oxidation. 250g fish meat obtained from fish oil 25ml and is made at a concentration of 5% and 10%.[7]

TABLE 1: Average Number Expression of BMP-2.

Group	Mean
KK	7.5
KP1	11
KP2	15.7

This research is an experimental research laboratory with post test only control group design. Male Wistar rats (Rattus norvegicus) 2-3 months old, weighing 200-300 grams, were divided into 3 groups: control group, (KK) treatment group 1 (KP1), and the treatment group 2 (KP2). The control group performed tooth extractions and then not given any treatment. The treatment group 1, performed tooth extractions were then given fish oil catfish (Clarias batrachus) with a concentration of 5%. The treatment group 2, performed tooth extractions were then given fish oil catfish (Clarias batrachus) with a concentration of 10%. Mice didekaputasi on the seventh day after the extraction of teeth and tooth extraction sockets former taken as preparations for immunohistochemical staining method to observe the expression of BMP-2. Calculation of BMP-2 expression is carried out under a light microscope with 400x magnification. Then do a comparison expression of BMP-2 in the control and treatment groups.

Analysis of the data using the Kolmogorov-Smirnov test to determine the normal distribution of research data. Test Levene's Test to determine homogeneity. Then analyzed using One Way Anova statistical test to determine differences in the expression of BMP-2 in the control group and the treatment group 1 and 2.

3. RESULTS

In this study, immunohistochemical staining method to look at the expression of BMP-2 on the seventh day after tooth extraction. The expression of BMP-2 are examined under a light microscope with 400x magnification

Based on research that has been done, using 21 samples of rats Wistar (Rattus norvegicus) were divided into a control group (KK), the treatment group 1 (KP1) with a concentration of fish oil 5% and the treatment group 2 (KP2) with a concentration of fish oil 10 % obtained an average expression of BMP-2 post following tooth extraction.

In this study, the expression of BMP-2 after tooth extraction has increased in the group given fish oil catfish (Clarias batrachus). The expression of BMP-2 on KP2 with catfish fish oil concentrations 10% had increased expression of BMP-2 which is the highest compared to KK and KP1.

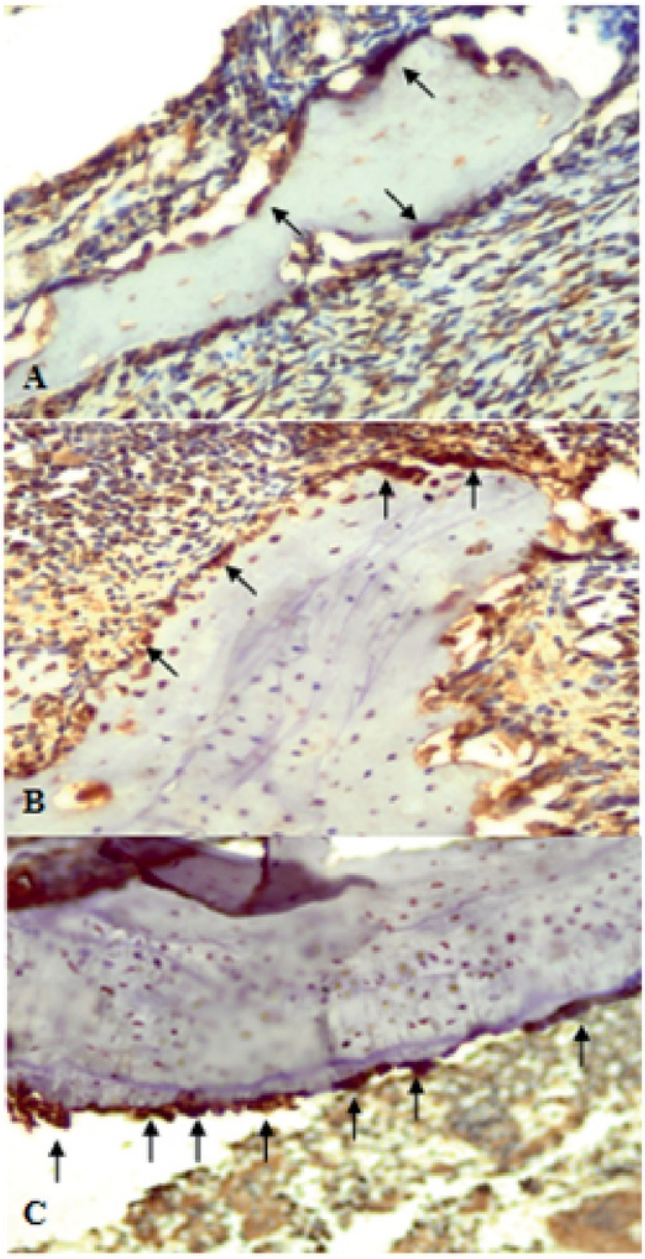

Figure 1: An overview of expression of BMP-2 (→) with immunohistochemical examination of each group designated by arrows (magnification 400x). (A) The control group; (B) The treatment group 1; (C) The treatment group 2.

Data processing amount of BMP-2 expression in rats after tooth extraction is done by using One-Way ANOVA. To perform this test before the data is to be normally

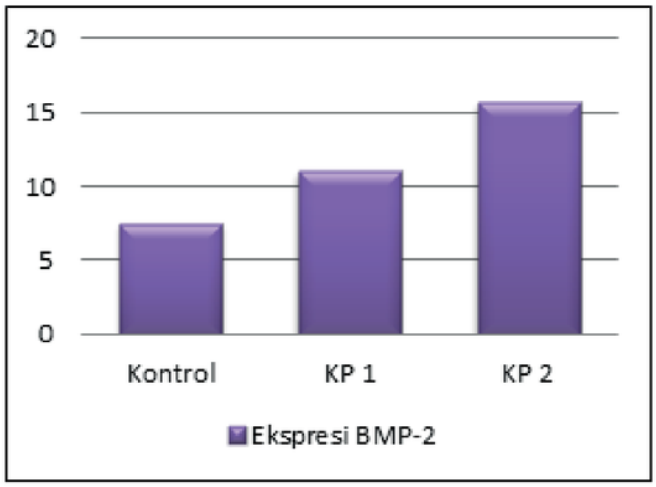

Figure 2: Graph Average Number Expression of BMP-2.

TABLE 2: Post-hoc test.

Group	KK	KP1	KP2
KK		0.041*	0.000*
KP1	0.041*		0.004*
KP2	0.000*	0.004*	

*: Indicates significant difference between groups (P < 0.05)

distributed, therefore the Kolmogorov-Smirnov test was done to see the distribution of the data. Then the variance between variables trial should be homogeneous so do Levene test. After the data were normally distributed and homogeneous, carried One-Way ANOVA test. Then, to see the best concentrations of fish oil, which means the amount of BMP-2 expression Highest Post-hoc test was used. (P <0.05)

4. DISCUSSION

One of the wounds that are often found in the field of dentistry is wound after tooth extraction. The process of wound healing in the socket former dental extractions together with the healing of wounds in soft tissues, only wound healing tooth extraction involves also the healing of bone, the process includes (1) the formation of the clot, (2) re-epithelialization, (3) the formation of granulation tissue and (4) the establishment bone.[8] In the inflammatory phase will occur inflammatory response that aims to eliminate damaged tissue and reduce the spread of infection. Inflammatory phase will occur after the proliferative phase characterized by proliferation of inflammatory

cells, such as macrophages. Macrophages will respond to such damage by releasing a variety of cytokines and mediators, including TNF-α and IL-1 at sites of inflammation. Increased production of proinflammatory cytokines can enhance PGE_2 production in the marrow tulang.[9, 10]

This research is a purely experimental to seek the benefits of fish oil catfish (Clarias batrachus) in the field of dentistry. Catfish (Clarias batrachus) had higher levels of omega-3-PUFA highest when compared with fish, shrimp and other fresh fish at levels of 28% of the total fatty acid. The resulting fatty acids from fish, specifically as a source of omega-3, especially eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA).[4] EPA and DHA which are the result of desaturation and elongation of the omega-3-PUFA (as essential fatty acids or EFAs) have an effect on bone metabolism. In his research, Viegas et al [11] explained that EPA has anti-mineralogenik, whereas DHA has the effect of pro-mineralogenik. However, when combined DHA can compensate for the effect of anti-mineralogenik of EPA. The study resulted in the increase in regulation of gene expression of BMP-2 (200%) in cells that were given DHA.

In this study showed that the expression of BMP-2 increased the concentration of fish oil KP2 10% with a mean of 15.7143 compared to the control group and KP1 concentration of 5% fish oil. It is known that fish oil contains C.batrachus Polyunsaturated Fatty Acids (PUFAs), which consists of EPA and DHA can increase the expression of BMP-2.[5] Mechanisms to connect omega-3-PUFA with increased expression of BMP-2 is through prostaglandin E_2 (PGE_2). One mechanism of omega-3-PUFAs in regulating bone metabolism by lowering the release of PGE_2 PGE_2.[6] have biphasic effects on the process of bone formation at high concentrations (10^{-6} M) PGE_2 has osteoclastic properties through the stimulation of RANK-RANKL, while at the concentration low (10-10 M to 10^{-8} M) PGE2 may modulate the effects of BMP-2 stimulation of osteoblast differentiation. PGE_2 induces the expression of BMP-2 through the activation of its receptor (EP_1, EP_2, EP_3, and EP_4) contained in osteoblasts. Activation of the receptor selective prostaglandin will affect the proliferation and differentiation of progenitor cells osteoblas.[10]

Assumed, the release of PGE_2 in the inflammatory phase activates its receptors are present in osteoblasts, thus inducing the expression of BMP-2. In addition, the application of fish oil C. batrachus also help activating receptors of PGE_2, so that the expression of BMP-2 can be increased.

In this study there was an increase of the expression of BMP-2 in mice post after administration of fish oil extraction catfish C. batrachus and showed a significant difference between the control group, KP 1 and KP 2 with a p-value of <0.05. In this study, the best concentration is a concentration of 10%, and therefore the remodeling process is expected to last well and quickly.

It is expected that this study can be the basis for further research to clarify the mechanism of increased expression of BMP-2 by fish oil catfish (Clarias batrachus).

References

[1] Csiszar, A, Smith, KE, Koller, A, Kaley, G, Edwards, JG, & Ungvari, Z 2005, "Regulation of bone morphogenetic protein-2 expression in endothelial cells: Role of nuclear factor-κB activation by tumor necrosis factor-α, H2O2, and high intravascular pressure ', Circulation.

[2] Csiszar, A, Lehoux, S, & Ungvari, Z 2009, "Hemodynamic forces, vascular oxidative stress, and regulation of BMP-2/4 expression ', Antioxidants & Redox Signaling, vol. 11, No. 7, pp. 1683-97.

[3] Sularsih & Wahjuningsih, E 2015, 'Bone morphogenetic protein-2 of Rattus norvegicus using chitosan gel with different molecular weight on wound healing of dental extraction', Dent. J. (Maj. Ked. Teeth). Vol. 48, no. 2, pp. 59-64.

[4] Chedoloh, R, Karrila, TT, and Pakdeechanuan, P 2011, 'Fatty acid composition of important aquatic animals in Southern Thailand', the International Food Research Journal, vol.18, pp. 783-790.

[5] Das, UN 2002, "Nitric oxide as the mediator of the antiosteoporotic actions of estrogen, statins, and essential fatty acids', Nitric Oxide and Osteoporosis, Pp. 88-93

[6] Kajarabille, N, Diaz-Castro, J, Hijano, S, Lopez-Frias, M, Lopez-Aliaga, I, & Ochoa, JJ, 2013, 'A new insight to bone turnover: Role of omega-3 polyunsaturated fatty acids 'The Scientific World Journal, p. 6.

[7] Kamat, SG & Roy, R 2015, 'Evaluation of antioxidant potential of Clarias batrachus oil in alloxan Induced Diabetic Mice (Mus musculus)', Diabetes & Metabolism, vol. 6, no.6.

[8] Larjava, N 2012, Oral Wound Healing: Cell Biology and Clinical Management, Canada: Wiley-Blackwell.

[9] Kumar, V., Abbas, A.K., Aster, J.C. 2013. Robbins Basic Pathology 9thEd. Elsevier Saunders: Philadelpia. Pp. 48, 53-4.

[10] Watkins, BA, Li, Y, & Seifert, MF 2001 'Nutraceutical fatty acids as biochemical and molecular modulators of skeletal biology', Journal of the American College of Nutrition, vol. 20, no. 5, pp. 410s-416S.

[11] Viegas, MN, Dias, J, Cancela, ML, & Laize, V 2012. 'Polyunsaturated fatty acids regulate cell proliferation, extracellular matrix mineralization and gene expression in a cell line gilthead skeletal seabrem' J. Appl. Ichtyol, vol. 28, Pp. 427-32.

BMP-2 Expression of Post Tooth Extraction that Catfish Oil Application

ORIGINALITY REPORT

17%	12%	14%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Leeds Student Paper	1%
2	tsukuba.repo.nii.ac.jp Internet Source	1%
3	ejournal2.undip.ac.id Internet Source	1%
4	hal.univ-brest.fr Internet Source	1%
5	scien.net Internet Source	1%
6	www.giornaleitalianodinefrologia.it Internet Source	1%
7	www.rroij.com Internet Source	1%
8	jabonline.in Internet Source	1%
9	C. Puel. "Olive oil and its main phenolic	

micronutrient (oleuropein) prevent inflammation-induced bone loss in the ovariectomised rat", British Journal Of Nutrition, 07/2004
Publication
1%

10 Kruger, M.C.. "Long-chain polyunsaturated fatty acids: Selected mechanisms of action on bone", Progress in Lipid Research, 201010
Publication
1%

11 repository.unair.ac.id
Internet Source
1%

12 Walker, Celia G., Susan A. Jebb, and Philip C. Calder. "Stearidonic acid as a supplemental source of ω-3 polyunsaturated fatty acids to enhance status for improved human health", Nutrition, 2013.
Publication
1%

13 Yun Feng Rui. "Mechanical loading increased BMP-2 expression which promoted osteogenic differentiation of tendon-derived stem cells", Journal of Orthopaedic Research, 03/2011
Publication
1%

14 Submitted to University of Bedfordshire
Student Paper
1%

15 www.bu.edu
Internet Source
1%

16	www.ebmonline.org Internet Source	1%
17	www.paramountbooks.com.pk Internet Source	<1%
18	Pratsinis, Harris, and Dimitris Kletsas. "Growth Factors in Intervertebral Disc Homeostasis", Connective Tissue Research, 2008. Publication	<1%
19	Wong, R.W.K.. "Effect of naringin collagen graft on bone formation", Biomaterials, 200603 Publication	<1%
20	Liu, Jing-Hua. "Effects of probucol on angiotensin II-induced BMP-2 expression in human umbilical vein endothelial cells", Molecular Medicine Reports, 2012. Publication	<1%
21	Soon Jung Hwang. "The Implications of the Response of Human Mesenchymal Stromal Cells in Three Dimensional Culture to Electrical Stimulation for Tissue Regeneration", Tissue Engineering Part A, 09/13/2011 Publication	<1%
22	geneticsmr.com Internet Source	<1%
23	ppjpi.unair.ac.id Internet Source	<1%

24	Ming Zhang, Sheng-hua Zhou, Sp Zhao, Xu-ping Li, Li-Ping Liu, Xiang-Qian Shen. "Pioglitazone Can Downregulate Bone Morphogenetic Protein-2 Expression Induced by High Glucose in Human Umbilical Vein Endothelial Cells", Pharmacology, 2008 Publication	<1%
25	"Bone Morphogenetic Proteins: Regeneration of Bone and Beyond", Springer Nature America, Inc, 2004 Publication	<1%
26	Bimbo, A. P.. "Sources of omega-3 fatty acids", Food enrichment with omega-3 fatty acids, 2013. Publication	<1%
27	Arounleut, Phonepasong, Peter Bialek, Li-Fang Liang, Sunil Upadhyay, Sadanand Fulzele, Maribeth Johnson, Mohammed Elsalanty, Carlos M. Isales, and Mark W. Hamrick. "A Myostatin Inhibitor (Propeptide-Fc) Increases Muscle Mass and Muscle Fiber Size in Aged Mice but Does not Increase Bone Density or Bone Strength", Experimental Gerontology, 2013. Publication	<1%
28	Raymond G Schlienger. "HMG-CoA Reductase Inhibitors in Osteoporosis", Drugs & Aging,	<1%

2003
Publication

Exclude quotes	On	Exclude matches	< 5 words
Exclude bibliography	Off		

BMP-2 Expression of Post Tooth Extraction that Catfish Oil Application

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8