The effect of casein and lactoferrin of bovine's milk on nestin expression of the exposed dental pulp

Endah Ariyati Eko Ningtyas, Widjijono Widjijono**, Siti Sunarintyas***

* *Department of Dental Nursing, Poltekkes Kemenkes Semarang*
** *Department of Dental Biomaterials Science, Gadjah Mada University*

Correspondence: endahsmg@yahoo.com

Received 12 September 2022; 1st revision 12 October 2022; 2nd revision 19 October 2022; Accepted 9 November 2022; Published online 28 December 2022

Keywords:

Nestin expressions; casein; lactoferrin; exposed dental pulp

ABSTRACT

Background: *Pulp tissue can regenerate if damage occurs. The pulp differentiates into new odontoblast-like cells in Progenitor cells. Nestin is a marker of the presence of new* dontoblast-like *and expressed in pathological conditions. Bovine's milk casein and lactoferrin contain proteins that accelerate cell growth. This study aimed to determine the effect of casein and lactoferrin on the expression of nested cells in the exposed dental pulp.*

Method: *The research method is an experimental laboratory with male 30 Spraque Dawley rats. Rats were grouped as : casein, lactoferrin, casein-lactoferrin, and Ca-$(OH)_2$. Each group consisted of 15 samples of molars which were divided into 5 samples of teeth for observation on the 7, 14, and 21 days. The first molars of rats were prepared until the pulp was exposed. The data of nestin quantity with immunohistochemical (IHC) analysis on histological preparations were analyzed using two-way ANOVA then the least significance difference (LSD) test.*

Result: *The results of the average number of nested cells on 7,14 and 21 days were highest on combination of casein and lactoferrin. Two-way ANOVA statistical proved that there was a significant difference ($p<0.05$) in each observation period and group and the number of expressions of nestin cells. LSD test showed that there was a significant difference ($p<0.05$) between groups compared to the control group.*

Conclusion: *Combination casein and lactoferrin extracts affect the expression of the number of nestin cells as a marker of* new odontoblast-like cells *in the exposed dental pulp*.

doi: http://dx.doi.org/10.30659/odj.9.2.273-278
2460-4119 / 2354-5992 ©2022 National Research and Innovation Agency
This is an open access article under the CC BY-SA license (https://creativecommons.org/licenses/by-sa/4.0/)
Odonto : Dental Journal accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3200)
How to Cite: Ningtyas *et al*. The effect of casein and lactoferrin of bovine's milk on nestin expression of the exposed dental pulp. Odonto: Dental Journal, v.9, n.2, p.273-278, December 2022

INTRODUCTION

The success of the treatment process on an exposed and vital pulp is highly dependent on the ability of the tooth to maintain pulp vitality and function. Exposed pulp tissue can be caused by a variety of injuries, either due to caries extending close to the pulp or due to accidental routine preparation procedures.[1] Treatment approaches are often carried out with indirect pulp capping in deep cavities and direct pulp capping in cases of exposed pulp. The pulp maintains its vitality and conducts a defense reaction in the form of an inflammatory state and an immune reaction by forming a dentinal bridge.[2] The defense reaction that arises against lesions that are close to or in contact with the pulp surface is the formation of tertiary dentin. Cells that act as defense cells in the dental pulp are fibroblast cells and odontoblast cells which form a row of cells at the periphery of the border between the dental pulp and the predentin layer.[3]

Damage that causes the roof of the pulp to open can cause necrosis of the underlying primary odontoblast cells.[4] If the primary odontoblast cells are necrotic due to injury, their presence will be replaced by new odontoblast like cells which will then form reparative dentin.[5] New odontoblast-like cell is a population of new odontoblast cells that arise due to severe damage resulting in the death of primary odontoblasts. The process of differentiation of new cells will occur a series of cellular development processes, in which the existing pulp cells will proliferate, migrate to necrotic areas and form new collagen dental pulp has a group of cells that are progenitor / stem cells of odontoblast cells.[10] Where these types of cells have the ability to proliferate and differentiate into odontoblasts in the event of damage to the primary odontoblast.[10] Application of dentin matrix components or growth factors into deep cavities can act as a stimulating regulator of the synthetic activity of reparative dentin formation. Reparative dentin is formed due to the differentiation of the activity of new odontoblast like cells.[6]

The marker when the differentiation process takes place in the nocrosis area is in the form of intermediate filaments.[7] which is a constituent structure of the cytoskeleton called nestin**.** Nestin is a protein molecule of an intermediate filament which has a diameter of 10 nm and has an important role for the organization and function of a cell or tissue. Nestin is one of the specific markers of new odontoblast-like cell as a pulp defense process from trauma or damage.[8] Formation of fibrous tissue matrix using mineral trioxide aggregate showed formation on day 3 with an increase in cell proliferation under the exposed pulp around the blood vessels.[9]

For a long time, it has been used as a sub-base in the treatment of capping pulp, both indirectly and directly, which has the most promising success in forming a dentinal bridge, namely calcium hydroxide ($Ca(OH)_2$). $Ca(OH)_2$ has a high pH so that it can stimulate various cell enzyme systems that cause fibroblast proliferation, migration and healing and even tissue replacement. directly with the pulp.[10] The success of a treatment on a vital pulp depends on the type and location of the injury, the age of the tooth, the material used and the quality of the filling performed.[11]

Nowadays there is an increasing effort to utilize natural ingredients as alternative medicine, one of the alternatives used is bovine's milk. Milk has an active component in the form of important protein content from bovine's milk including casein and lactoferrin. It is reported that there is 80% casein content of the total protein in bovine's milk. Casein in the 'dissolved' form has the ability to

transport calcium and phosphate in mineralization and tissue calcification.[12] Casein in bovine's milk in the form of 'dissolved' will have the ability as a transport for calcium and phosphate in mineralization and tissue calcification.[13] Lactoferrin is a protein binder that has several biological properties and specifically affects the growth of cell lines as a stimulant of DNA synthesis and accelerates growth by playing a role in cell proliferation by transporting Fe ions into cells.[14] Lactoferrin are glycoproteins that bind to iron (Fe), including transferrin and melanotransferrin. Lactoferrin in the body can activate DNA cells and act as an anabolic factor that affects osteocytes.[15] one of which is by stimulating the proliferation of osteoblasts.

This study aimed to quantitatively observe the presence of nestin expression as a marker for the formation of new odontoblast like cells in exposed dental pulp after application of casein and lactoferrin of bovine's milk.

METHODS

This study used Ethical Clearance standardized by the Health Medical Research Ethics Committee, Universitas Gadjah Mada. This research is an experimental laboratory using a post test only research design with control group design. The subjects consisted of 60 teeth of male Wistar strain rats which were divided into 3 treatment groups, namely casein, lactoferrin and a combination of casein lactoferrin and 1 control group with calcium. hydroxide ($Ca([OH])_2$).

Each group was divided into 3 groups based on the observation time period, namely days 7, 14, and 21. Observations were made on molars that had been prepared to the base of the dentin layer and were applied with casein, lactoferrin and a combination of casein and lactoferrin.

Preparation of preparations was preceded by anaesthetizing the animals using 8% chloral hydrate at a dose of 350 mg/kg body weight. The upper right and left molars of experimental animals were prepared on the occlusal surface using a round bur number 8 with a diameter of 1 mm and a depth close to the pulp then the animals were carried out with a probe until the pulp was open/perforated.

The decapitation process was adjusted to the observation days, namely days 7, 14 and 21. Animals were anesthetized with ether and cervical dislocation was performed. The tooth sample was extracted and fixed with 10% buffered formalin for 24 hours. Then decalcified with formic acid for 1 week. After that, it was planted in paraffin then cut lengthwise with a thickness of 4 mm and stained with immunohistochemistry (IHC). Quantity of nestin was calculated by immunohistochemical (IHC) observation. The number of nested cells was counted using a light microscope with 400x magnification in the pulp cavity.

RESULTS

The data obtained from the observation of the quantity of expression of the Nestinated cells are ratio scale data. The data was then calculated the mean and standard deviation of the number of positive nesting cells based on the treatment group and the period of observation made (7 days, 14 and 21), and presented in tabular form.

1. Number of pulp cells showing nesting

The average number of nestin-positive cells in the casein group and $Ca(OH)_2$ group 9control group is presented in Table 1

Table 1. The mean and standard deviation of the number of nestin cells in the casein group and $Ca(OH)_2$ group (control group) on exposed dental pulp

Observation	Total Ekxpression of Nestin Cells			
	Casein	lLactoferrin	Casein lactoferrin	Control
7 days	0,364± 0,0493	0,408±0,06834	0,666±0,02966	0,3260±0,0456
14 days	0,61±0,04183	0,578±0,04147	0,758± 0,0589	0,4980±0,0986
21 days	0,408± 0,0377	0,4540±0,3578	0,6020±0,0482	0,572± 0,0278

Table 1 was shown that in the application of casein, lactoferrin and the combination of casein and lactoferrin on the 7 to 14 day observations there was an increase the expression of the number of nestin cells but then a decrease in the 21 day observation. While in the control group there was an increase in the number of cell expression. bernestin from observation day 7 to day 21.

The results of the normality test of the data on the number of expression cells that were nested in the use of 4 ingredients, namely casein, lactoferrin, casein lactoferrin and CaOH as controls with observations at 3 different times, namely the 7, 14 and 21 days, using One simple Kolmogorov-Smirnov, showed a similar distribution of data. normal, and the homogeneity test using Levene's Test showed a $p > 0.05$ which means the data is homogeneous.

To test the data on the number of nested cells, the significance was tested using the two-way ANOVA test and the results were as shown in Table 2.

Table 2. The results of the two-way ANOVA test on the number of expression of nested cells in the application of casein extract and lactoferrin of bovine's milk as a marker of new odontoblast like cells in exposed dental pulp

	n	signification
Time period (observation)	54,049	0,000
Group	59,496	0,000
Time period x group	10,910	0,000

DISCUSSION

Analysis of variance (table 2) shows that there is an interaction effect between the observation time period and the use of casein, lactoferrin and a combination of casein milk lactoferrin cattle on the expression of bernestin cells in exposed dental pulp. This is possible because of the nature of casein and lactoferrin which tend to increase the expression of nested cells. Where both materials have biologically active molecules, namely protein groups that play a role in repair, growth and maintenance of cell structure. Due to the nature of casein and lactoferrin used as treatment materials for exposed pulp, it will affect the observation period. In this study, it is assumed that the observation time is the period of the tissue regeneration process, where it can be seen that with the observation time of days 7, 14 and 21 with different groups of materials, the number of expression of nested cells is also different.

In this study, the application of casein and lactoferrin to the exposed pulp will directly affect the underlying pulp cells, so that these materials can act as important signal transducers in the process

of tissue regeneration. The use of medicaments in pulp treatment is one of the triggers for the involvement of several growth factors to support the process of tissue regeneration.[16] With the properties of casein and lactoferrin which are classified as proteins, it will accelerate the process of 'signaling molecules' needed in the process of migration, proliferation and differentiation of damaged odontoblast cells. The application of medicaments to exposed pulp containing signaling can accelerate the deposition of reparative dentin formation. [17] The properties of lactoferrin and casein which contain bioactive properties will benefit their existence for growth factors because they can be used as triggers for triggering a 'signaling molecules''. With this 'molecular signaling ' protein changes will occur in cells including neural crest cells, namely a group of cells that have the ability to proliferate and differentiate. Changes in this protein affect the expression of cytoskeleton filaments in the cells, namely the protein nestin, as a sign that these cells have the ability to differentiate. [18]

The application of casein material can affect the expression of bernestin cells becouse casein consists of a phosphoprotein class protein that involves a phosphorylation reactin, where this reaction together with reactions that occur at the celluler level in the cytoskeleton of procursor cells will run synergistically so that Nestinated cell expression. [19]

The use of lactoferrin also had a significant difference in the amount of expression of the nested cells compared to the control. The content of lactoferrin in milk is smaller than casein but lactoferrin has the function of stimulating cell proliferation and its ability to transport iron into cells which is useful for cell growth. Lactoferrin in the absence of cytokines can stimulate the proliferation of endometrial stromal cells [20], also plays a role in cell differentiation, namely the ability to identify as a specific DNA sequence transcription factor.[21]

The number of expression of nested cells using the LSD test showed that there was no difference in the number of expression of nested cells on the use of casein observations on day 7 of the casein material observed on day 21 ($p>0,005$). This indicates that the differentiation process begins on the day 7. On day 21 to the homeostasis stage with the formation of new odontoblast like cells. However, on the use of casein material on the day 7 of observation with the combined use of lactoferrin casein material, there was a significant difference in the amount of nestin expression. This is possible because of the influence of the prominent role of the synergistically incorporated proteins in the two materials on cell proliferation and differentiation. While there was no significant difference between casein day 14, compared to lactoferrin day 14, casein lactoferrin day 7 and casein lactoferrin day 21 and control day 21, this was because at that time it was possible for the differentiation process to take place in casein and casein materials. lactoferrin on day 14, whereas on the use of casein. Lactoferrin was already on day 7 the differentiation process took place and was immediately high. While on the day 21 casein and lactoferrin began to decrease due to the differentiation process no longer taking place.

CONCLUSION

The ombination of casein and lactoferrin extract increased the expression of the number of nestin cells as a marker of the presence of new odontoblast like cells in teeth with exposed pulp.

REFERENCES

1. Yumoto H, Hirao K, Hosokawa Y, Kuramoto H, Takegawa D, Nakanishi T, et al. The roles of odontoblasts in dental pulp innate immunity. Jpn Dent Sci Rev.

2018;54(3):105–17.
2. Goldberg M, Njeh A, Uzunoglu E. Is Pulp Inflammation a Prerequisite for Pulp Healing and Regeneration ? Mediators Inflamm. 2015;2015:1–11.
3. Neves VCM, Yianni V, Sharpe PT. Macrophage modulation of dental pulp stem cell activity during tertiary dentinogenesis. Sci Rep. 2020;10(1):1–9.
4. Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith A., et al. Dental Pulp Defence and Repair Mechanisms in Dental Caries. Mediators Inflamm. 2015;2015:1–16.
5. Alexandru-Andrei I, Perlea P, Irina-Maria G, Mihai M, Loredana M, Iren M, et al. Molecular Mechanisms of Dentine-Pulp Complex Response Induced by Microbiome of Deep Caries. ARS Medica Tomitana. 2019;25(2):53–60.
6. Abdelaz P, El Zoghbi A, Shokry M, Ahmed AZ, Rasha H. Reparative dentin formation using stem cell therapy versus calcium hydroxide in direct pulp capping: An animal study. Braz Dent J. 2019;30(6):542–9.
7. Osborn M, Weber K. Intermediate filaments: Cell-type-specific markers in differentiation and pathology. Cell. 1982;31(2 PART 1):303–6.
8. Michalczyk K, Ziman M. Nestin structure and predicted function in cellular cytoskeletal organisation. Histol Histopathol. 2005;20(2):665–71.
9. Nakatomi M, Quispe-Salcedo A, Sakaguchi M, Ida-Yonemochi H, Okano H, Ohshima H. Nestin expression is differently regulated between odontoblasts and the subodontoblastic layer in mice. Histochem Cell Biol. 2018;149(4):383–91.
10. Moussa SA. Mineral Trioxide Aggregate (MTA) vs Calcium Hydroxide in Direct Pulp Capping – Literature Review. Online J Dent Oral Heal. 2018;1(2).
11. Bjørndal L, Simon S, Tomson PL, Duncan HF. Management of deep caries and the exposed pulp. Int Endod J. 2019;52(7):949–73.
12. Huang X qing, Camba J, Gu L sha, Bergeron BE, Ricucci D, Pashley DH, et al. Mechanism of bioactive molecular extraction from mineralized dentin by calcium hydroxide and tricalcium silicate cement. Dent Mater. 2018;34(2):317–30.
13. Ningtyas EAE, Santoso O, Sadhana U, Sunarintyas S. Role of Combination Casein and Lactoferrin Bovineâ€™S Collostrum As a Pulp Capping on Macrophage Expression in Male Wistar Rats. ODONTO Dent J. 2021;8(2):156.
14. Zhang JL, Han X, Shan YJ, Zhang LW, Du M, Liu M, et al. Effect of bovine lactoferrin and human lactoferrin on the proliferative activity of the osteoblast cell line MC3T3-E1 in vitro. J Dairy Sci. 2018;101(3):1827–33.
15. Van Splunter M, Perdijk O, Fick-Brinkhof H, Feitsma AL, Floris-Vollenbroek EG, Meijer B, et al. Bovine lactoferrin enhances TLR7-mediated responses in plasmacytoid dendritic cells in elderly women: Results from a nutritional intervention study with bovine lactoferrin, GOS and Vitamin D. Front Immunol. 2018;9(NOV):1–12.
16. Wang Z, Lin L, Zhang JS, Zhong X, Bellusci S, Li X. Editorial: The Fibroblast Growth Factor Signaling Pathway in Metabolic Regulation, Development, Disease, and Repair After Injury. Vol. 11, Frontiers in Pharmacology. 2020.
17. Li Z, Liu L, Wang L, Song D. The effects and potential applications of concentrated growth factor in dentin–pulp complex regeneration. Stem Cell Res Ther. 2021;12(1):1–10.
18. Mitsiadis TA, Rahiotis C. Parallels between tooth development and repair: Conserved molecular mechanisms following carious and dental injury. J Dent Res. 2004;83(12):896–902.
19. Williams KL, Topp S, Yang S, Smith B, Fifita JA, Warraich ST, et al. Casein Kinase II Phosphorylation of Cyclin F at Serine 621 Regulates The Lys48-ubiquitylation E3 Ligase Activity Of The SCF (cylyn F) Complex. Nat Commun. 2016;7.
20. Puddu P, Valenti P, Gessani S. Immunomodulatory effects of lactoferrin on antigen presenting cells. Biochimie. 2009;91(1):11–8.
21. Kumari S, Kondapi AK. Receptor-mediated targeted delivery of DNA using Lactoferrin nanoparticles. Int J Biol Macromol. 2018;108:401–7.