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Literature Review

The Role of Nitrite Producing Bacteria and Its Mechanism to Prevent The Dental Caries

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ABSTRACT

Background: *Dental caries is the most common disease and often occurs in the oral cavity that can be found in adults nor children and until now many studies about dental caries have been established to reduce the caries level. In our daily life, nitrate (NO_3^-) can be found in the green vegetables which can be reduced to NO_2^- by some oral bacteria. NO_2^- can inhibit the growth and acid production of pathogenic bacteria such as Streptococcus mutans to prevent the dental caries. There have been many studies that explain the effect of NO_2^- as an antibacterial agent, but there are limited studies that explain about the role of NO_2^- on maintaining the oral health and its relation to the NO_3^- reduction mechanism involved the environment in the oral cavity.* ***Purpose:*** *To analyze the role of NO_2^- producing bacteria and its mechanism to prevent the dental caries.* ***Results:*** *The amount of NO_2^- in the oral cavity produced by reduction of NO_3^- depends on diet and the number of NO_3^- reducing bacteria, especially obligate and facultative anaerobes. NO_2^- can inhibit the growth and metabolism of cariogenic bacteria.* ***Conclusion:*** *NO_3^- reducing bacteria in the oral cavity, a diet consisting of NO_3^- plays an important role in the prevention of dental caries through the NO_3^-- NO_2^- mechanism to reduce the number and metabolism of cariogenic bacteria.*

Keywords: *nitrate; nitrite; dental caries; nitrate reduction; nitrite production; health care quality; human and health*

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INTRODUCTION

The oral cavity is a very important part of the body because it acts as the entrance of all substrate into the body. The oral cavity also has an important role in masticatory, aesthetic and speech development. Therefore, maintain the healthy of oral cavity can improve the systemic health and increase the quality of life.[1]

Dental caries is the most common disease in the oral cavity and occurs in adults nor children.[2] Moreover the prevalence of dental caries is still high not only in Indonesia, but also around the world, especially in the developed countries.[1] The results of *Riset Kesehatan Dasar* (RISKESDAS) in 2018 showed the prevalence of dental caries in Indonesia was 45.3%. Futhermore, other research of PDGI (*Persatuan Dokter Gigi Indonesia*) showed 89% of patients with dental caries are children.[3] Dental caries starts with the the acid produced by acid producing bacteria through carbohydrate metabolism such as *Streptococcus mutans* and *Lactobacillus* spp resulting in demineralization enamel.[1]

Some literature showed high frequency of green vegetables intake reduce the risk of dental caries.[4] In our daily life, nitrate (NO_3^-) can be found in green vegetables such as spinach, lettuce and cabbage. The level its intake strongly related to the NO_3^- - NO_2^- - NO pathway.[5] This NO_3^- - NO_2^- - NO pathway can support the oral and systemic health.[6] Furthermore, NO_3^- will be absorbed through the gastrointestinal tract, and 25% will be secreted to the oral cavity as saliva.[1] Hence, NO_3^- is always available in the oral cavity.

In the oral cavity, there are various facultative anaerobic bacteria such as *Veilonella, Neisseria, Rothia, Actinomyces, Corynebacterium, Haemophilus* and *Kingella* which can reduce NO_3^- to NO_2^-.[5] These bacteria can regulate the level of NO_2^-, through the mechanism of reducing NO_3^- to NO_2^-.[8] Then, NO_2^- can inhibit the growth of pathogenic bacteria such as *Streptococcus mutans*, *Poryphormonas gingivalis*[1] and inhibits the acid production in dental plaque[6] hence it plays a role in preventing the oral disease especially dental caries.[1]

Recently, there have been many studies that explain the effect of NO_2^- as an antibacterial agent, however, there is limited study that explain about the role of NO_2^- on maintaining the oral health especially dental caries and it

relations to the NO_3^- reduction mechanism involved the bacteria and environment in the oral cavity. Then, the aim of this study was to analyze the role of NO_2^- producing bacteria and its mechanism to prevent the dental caries.

REVIEW(S)

NO_2^- production

The NO_2^- can be produced through endogenous and exogenous. Endogenous NO_2^- can be obtained through NO_3^- - NO_2^- - NO pathway. Exogenous NO_2^- obtained through diet intakes containing high concentration of NO_3^- like from green vegetables such as lettuce, spinach, cabbage and beets.[9] In addition, low concentration of NO_3^- can be found in diet such as bread, cereals and dairy products.[11] Exogenous NO_2^- is also obtained from intakes NO_2^-. Main source of NO_2^- consumptions comes from drinking water with levels of NO_2^- below 0.1 mg/l and preserved foods as preservative component to prevent the oxidation of fatty acids, including prevent the rancidity.

In the oral cavity, food containing NO_3^- such as green vegetables such as spinach, lettuce and cabbage will be reduced to NO_2^- by a specific enzyme reductase of facultative anaerobic and obligate anaerob bacteria. These bacteria use NO_3^- as an alternative terminal electron acceptor to generate ATP under anaerobic conditions. Due to increasing of NO_3^- diet and the enzymatic activity of bacteria, level of NO_2^- in the saliva become very high.[12] Next, NO_2^- can be reduced through non-enzymatically and enzymatically way to produce NO.[8]

NO_2^- from saliva will be swallon to the stomach, and turns to NO through non-enzymatically pathway at low pH in gastric. Nitric acid (HNO_2) is formed through the protonation of NO_2^-, the decomposed HNO_2 will form NO. As in the following equation :[13]

$H^+ + NO_2^- \leftrightarrow HNO_2$

$HNO_2 \leftrightarrow NOOH$

$NOOH + NO_2^- \leftrightarrow N_2O_3 + OH^-$

$N_2O_3 \rightarrow NO_2 + NO$

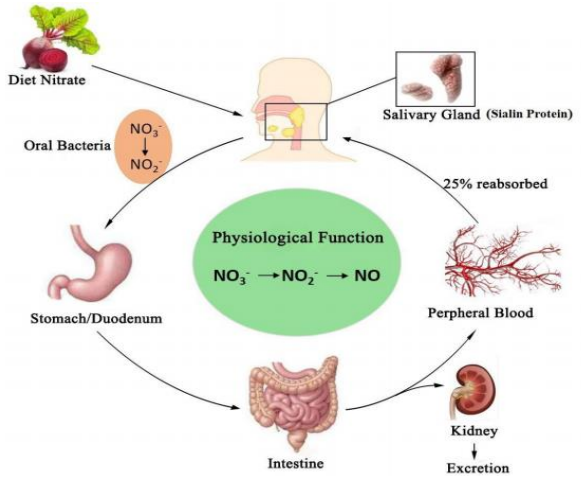

Figure 1. NO_3^- - NO_2^- - NO pathway[10]

This NO acts as a defense mechanism in the body against ingested pathogenical microorganisms.[14] Furthermore, NO give effect to improve the integrity of the gastric mucosa, including increasing mucosal blood flow and mucus formation.[10]

Residual formation of NO_3^- and NO_2^- are absorbed through the small intestine, some of NO_3^- and NO_2^- will be passed to the blood circulation.[10] In the blood vessels, there is an enzymatic reduction of NO_2^- to NO through mammalian nitrite reductase, NO_2^- will be converted to NO which is mediated by deoxyhaemoglobin (Deoxy-Hb).[12] In addition, NO can also be produced by endothelial nitric oxide synthase (eNOS) mediated by L-arginine and oxygen (O_2) as a substrate, a cofactor that plays an important role in the binding of L-arginine. NO is then oxidized to NO_2^- and NO_3^- by oxy hemoglobin.[8] NO plays a role in the occurrence of vasodilation so that it can reduce blood pressure.[10] Furthermore, NO which has been oxidized to NO_3^-, as much as 70% of NO_3^- is excreted through the kidneys[6] and 25% will be absorbed and concentrated from the blood circulation to the salivary glands, this process will be mediated by sialine protein which is an electrogenic NO_3^-/H^+ transporter in the plasma membrane of salivary gland acinar cells, which plays an important role in the balance regulation of NO_3^- - NO_2^- - NO pathway.[10]

Function of NO_2^-

NO_2^- is an essential compound that has been used as an antibacterial agent. Then, NO_2^-can be found in canned food as a preservative component.[7] Furthermore, NO_2^- is used as an antibacterial agent because it can inhibit the metabolism and growth of pathogenic bacteria such as *Streptococcus mutans* which is responsible as a main bacterium in dental caries by inhibiting acid production and its growth. NO_2^- which has been reduced to NO can have a positive effect on the integrity of the gastric mucosa.[8] In addition, NO_2^- which is enzymatically metabolized can maintain systemic stability in the body, including lowering blood pressure, restoring vascular dysfunction.[18]

The Role of NO_2^- Producing Bacteria In The Oral Cavity

In the oral cavity, NO_3^- will be reduced to NO_2^- by some oral bacteria as part of bacterial metabolism.[17] *Veilonella* is the one of main NO_3^- reducing bacteria that can produce NO_2^- from NO_3^- under aerobic and anaerobic conditions and its requires lactate in the oral cavity. The optimum NO_2^- level of *Veillonella* is produced under acid and anaerobic conditions, and turn lactate into propionate, acetate, formate, and pyruvate.[1] Besides *Veillonella*, the other main NO_2^- producing bacteria are *Actinomyces*, *Schaalia* dan *Neisseria.*

Factors Affect The Levels of NO_2^- In The Oral Cavity

There are several factors that affect NO_2^- levels in the oral cavity, such as diet contained, NO_3^-, anaerobic bacteria, efficiency of NO_3^- transport via sialine in salivary glands

and bacterial metabolic activity.[17,18] The high amount of NO_3^- reducing bacteria in the oral cavity is associated with an increasing of NO_2^- concentration in saliva, after consuming diet containing NO_3^-, hence NO_3^- diet and NO_2^- producing bacteria affect to the NO_2^- levels in the oral cavity.[17] Furthermore, mainly NO_3^- reducing bacteria are obligate and facultative anaerobic bacteria, then anaerobic condition also one of the factor that affect the level of NO_2^- in the oral cavity.[18]

DISCUSSION

NO_2^- is an essential compound that has been used as an antibacterial agent for many years because it can inhibit the growth of pathogenic bacteria. It also can inhibit the metabolism and growth of pathogenic bacteria in the oral cavity that causes caries and periodontal disease[1] by inhibiting acid production and the growth of pathogenic bacteria such as *Streptococcus mutans* and then NO_2^- play an important role in caries prevention. NO_2^- can act as an antibacterial agent through cellular mechanisms, including NO_2^- can interfere with energy metabolism, cause proton gradient collapse, inhibit enzyme metabolism, damage cell membranes and bacterial DNA.[20] NO_2^- interferes to energy metabolism by inhibiting oxygen uptake, oxidative phosphorylation and proton-dependent active transport. Under aerobic conditions, NO_2^- is a strong inhibitor of glucose transport and can interfere to energy conservation by inhibiting oxygen uptake.[15]

NO_2^- will be ionized into nitrite anion, which cannot pass through the bacterial cell membrane. Under the acidic conditions, wherein the nitrite anion is converted to nitric acid, it is inherently unstable to dismutation into NO which can easily penetrate cell membranes and inhibit bacterial glucose metabolism.[6] NO can react with iron-sulfur protein from bacteria where iron-sulfur protein is important in energy metabolism of both aerobic and anaerobic bacteria. NO can inactivate iron-sulfur proteins, ferrochetalase and aconitase are iron-sulfur proteins that are sensitive to NO.[16] Other than that, NO_2^- can act as an antibacterial agent through the destruction of bacterial DNA.

In the oral cavity, NO_3^- derived from foods such as green vegetables such as spinach, lettuce and cabbage will be reduced to NO_2^- by the specific enzyme nitrate reductase from some facultative and or obligate anaerobic commensal bacteria[12] such as *Veilonella, Neisseria, Rothia, Actinomyces, Corynebacterium, Haemophilus* and *Kingella*.[5] Research conducted by Doel (2005)[21] showed the production of NO_2^- was greatest on the dorsal surface of the tongue. The dorsal surface of the human tongue is a surface with many papillae and a fairly large surface. Therefore, the number of anaerobic bacteria at this site is higher than other mucosal surfaces.[23]

Furthermore, NO_3^- in saliva will contact to the bacteria that able to reduce NO_3^- to NO_2^- as part of bacterial metabolism. Generally, the main of NO_3^- reducing bacteria found in the oral cavity can be grouped into two categories, namely obligate anaerobes such as *Veillonella atypica*, *Veillonella dispar* and facultative anaerobes such as *Actinomyces odontolyticus*, *Rothia mucilaginosa.*[17] This is in line with the research of Suzuki *et al.,* (2020)[22], which showed the number of NO_3^- reducing bacteria was higher under anaerobic than aerobic conditions, so facultative anaerobes and obligate anaerobes were the main bacteria involved in the process of reducing NO_3^- to NO_2^- in the oral cavity. The capacity to produce NO_2^- was related to the number of NO_3^- reducing bacteria in the oral cavity.[22] This is in line with the research of Burleigh *et al.,* (2018)[18] that the number of NO_3^- reducing bacteria in the oral cavity is associated with an increasing of the in the NO_2^- concentration in saliva, after consuming foods containing NO_3^-. However, there are other factors, which affect NO_2^- concentration, including the efficiency of NO_3^- transport via sialine in the salivary glands, and the metabolic activity of bacteria.[25]

In dentistry, caries is the most common disease in the oral cavity and can be found in children, caused by acids produced by the fermentation of dietary carbohydrates by oral pathogenic bacteria[7] such as *Streptococcus mutans* and *Lactobacillus spp*. This species of cariogenic bacteria produces strong acids such as lactic acid, which causes a decrease in salivary pH below 5.5 leading to demineralization of enamel.[18]

Veilonella is obligate anaerobic bacterium and one of the main NO_3^- reducing bacteria in the oral cavity.[17] *Veillonella* utilizes lactic acid as an essential carbon for energy source, converting it into weaker acids such as acetic, propionic and formic acids. Dental caries is caused by acids produced by acidogenic bacteria, such as *Streptococcus mutans*, while acid neutralization, such as the conversion of lactic acid to weaker acids, can contribute to preventing tooth surface demineralization and promoting remineralization. Therefore, *Veillonella* species are considered as one of the beneficial bacterial species to prevent dental caries.[1]

Research conducted by Wicaksono *et al.,* (2020)[1] *Veillonella* can produce NO_2^- from NO_3^- under aerobic and anaerobic conditions and requires lactate in its production. The level of NO_2^- production is higher under anaerobic conditions, indicating that its metabolic activity is sensitive to oxygen. Under aerobic conditions, *Veillonella atypica* and *Veillonella parvula* require lactate to produce NO_2^-, and the production of NO_2^- in these bacteria increases under acidic conditions (pH 5). Meanwhile, under anaerobic conditions, *Veillonella* produces propionate, acetate, formate, and pyruvate from lactate oxidation and produces NO_2^- from NO_3^-. NO_3^- is reduced to NO_2^-, while lactate oxidation will be converted to pyruvate which is further metabolized into formate, acetate, and propionate via the formate-acetate and propionate pathways. Under anaerobic conditions, in *Veilonella parvula* there is a relationship between lactate metabolism and NO_2^- production because with the addition of NO_3^- there is an increase in metabolic end products and an increase in NO_2^- production.[1]

NO_2^- can increase the pH level in dental plaque before and after glucose intake, this indicates that NO_2^- can

inhibit acid production in dental plaque by reducing the acidogenicity and aciduric of dental plaque, which are considered important factors in the initiation and promotion of caries.[6] Dental plaque bacteria take external sugars into cells and metabolize them via glycolysis, resulting in acid production. Excess glucose will be stored in cells as glycogen like intracellular polysaccharides and used as a source of energy, resulting in acid production via glycolysis, when no external glucose is available.[26] Therefore, these results suggest that NO_2^- inhibits acid production from external glucose and intracellular polysaccharides.[6] The inhibitory effect of NO_2^- on acid production requires bacteria-mediated acid conditions. NO_2^- is a weak acid with a pKa of 3.38 and thus under neutral pH conditions, most of the NO_2^- ionizes to the nitrite anion, which cannot penetrate through the bacterial cell membrane. Under acidic conditions, nitrite anion is converted into nitric acid, which can easily penetrate cell membranes and inhibit bacterial glucose metabolism.[6] Nitric acid which is, inherently unstable, dismutation to NO. Thus, when NO_2^- in saliva is in contact with the acidic environment around the teeth caused by acid-producing bacteria such as *Streptococcus mutans*, NO produces bacteriostatic and bactericidal effects.[19] This is supported by the research of Hohesin *et al*., (2016)[25] that NO_2^- significantly inhibits acid-producing bacteria by exerting a bactericidal effect through the formation of antimicrobial NO from NO_2^- under acidic conditions due to carbohydrate fermentation, which can increase pH. Thus, with the higher number of NO_2^- in the oral cavity, the growth and survival of acidogenic bacteria will be limited.

Consumption of beet juice containing 400 mg of NO_3^- for 15 days produces about 0.2 mM NO_2^-, which is a concentration known to kill the acidogenic bacterium *Streptococcus mutans.*[25] This is supported by research by Doel (2004)[24] which shows that NO_2^- with a concentration of 0.2 mM can kill *Streptococcus mutans*. Meanwhile, in a study conducted by Wicaksono *et al*., (2020)[1] the growth of Streptococcus mutans was inhibited by NO_2^- at a concentration of 0.5 mM. The concentration of NO_2^- in the oral cavity is influenced by several factors, such as NO_3^- intake, the activity of NO_3^- reducing bacteria, salivary flow rate, and endogenous NO_3^- production.

Wicaksono *et al.,* (2020)[1] showed in their research that NO_3^- had no effect on the growth of *Streptococcus mutans* or *Veillonella* species, except at high concentrations (100 mM). Furthermore, concentration of NO_3^- in the oral cavity ranges from 0.8 mM (unstimulated saliva) to 4 mM (stimulated saliva). Then, NO_3^- itself can not inhibit the growth of *Streptococcus mutans*, however NO_2^- through reduction NO_3^- into NO_2^- or directly intake NO_2^- can inhibit the growth of *Streptococcus mutans* as cariogenic bacteria.

Hence, there is a high possibility that diet, oral bacteria and their metabolic products affect the oral health, where NO_3^-, which is easily obtained through green vegetables (diet), will be reduced by various oral bacteria (NO_3^- reducing) to produce NO_2^-, then the NO_2^- can be used to inhibit the growth and metabolism of pathogenic bacteria that cause caries, and also NO_2^- can be used to lower blood pressure, therefore the mechanism of reducing NO_3^- to NO_2^-, the NO_2^- producing bacteria play an very important role in maintaining the oral and systemic health.

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