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Transformation of Nitrite and Nitric Oxide Produced by Oral Bacteria to Reactive Nitrogen Oxide Species in the Oral Cavity

Umeo Takahama[1] and Sachiko Hirota[2]
[1]*Kyushu Dental College, Kitakyushu,*
[2]*Kyushu Women's University, Kitakyushu*
Japan

1. Introduction

Nitrate and nitrite are present in the human oral cavity. The main origin of these components is nitrate that is contained in leafy vegetables such as lettuce and spinach (Tamme et al., 2006). Ingested nitrate is absorbed into human body by the intestine, and part of the absorbed nitrate is secreted into the oral cavity as a component of saliva. In the oral cavity, nitrate is reduced to nitrite by nitrate-reducing bacteria (Doel et al., 2004, 2005; Zetterquist et al., 1999), and nitrite is reduced to nitric oxide (NO) by nitrite-reducing bacteria (Palmerini et al., 2003). If NO is produced from nitrite by nitrite-reducing bacteria, NO can autoxidize to dinitrogen trioxide (N_2O_3) and can react with superoxide anion radical (O_2^-) to produce a strong oxidant peroxynitrite ($ONOO^-$/ONOOH, pKa = 6.8) (ONOOH/NO_2, $E^{o\prime}$ = 2.10 V at pH 7) (Halliwell & Gutteridge, 1999). In the oral cavity, two peroxidases are present; one is salivary peroxidase that is derived from saliva and the other is myeloperoxidase that is derived from leukocytes migrated into the oral cavity. Nitrite can be oxidized by these peroxidases producing nitrogen dioxide (NO_2) (van der Vliet et al., 1997). The $E^{o\prime}$ of NO_2/NO_2^- at pH 7 is 0.99 V (Halliwell & Gutteridge, 1999). If the pH in the oral cavity decreased around 5, nitrite ion is protonated to produce nitrous acid (pKa = 3.3) that can be transformed to NO, N_2O_3, NO_2, and NO^+ (NO^+/NO, $E^{o\prime}$ = 1.21 V at pH 7) by self-decomposition (Oldrreive & Rice-Evabs, 2001). In this way, the formation of nitrite and NO by oral bacteria results in the production of reactive nitrogen oxide species (RNOS) by various reactions. This chapter deals with the mechanisms of production of RNOS in the human oral cavity under neutral and acidic conditions. Taking the mechanism into consideration, we discuss that the decrease in pH in the oral cavity results in the injury of oral tissue cells.

2. Measurements of RNOS

Production of RNOS in the oral cavity can be measured by (1) trapping of NO with Fe-*N*-(dithiocarboxy)sarcosine [$Fe(DTCS)_3$] complex, (2) transformation of 4,5-diaminofluorescein (DAF-2) and 3-amino-4-monomethylamino-2′,7′-difluorofluorescein

(DAF-FM) to their triazole forms, (3) oxidative degradation of aminophenyl fluorescein (APF) to fluorescein, (4) nitration of 4-hydroxyphenyl acetic acid (HPA), and (5) oxidation of uric acid.

Two preparations obtained from mixed whole saliva are use to study the formation RNOS in the oral cavity. One is saliva filtrate prepared by filtration of mixed whole saliva through two layers of nylon filter net, and the other is bacterial fraction prepared by centrifugation of the saliva filtrate.

2.1 NO-trapping

$Fe(DTCS)_3$ is prepared by addition of 0.03 mL of 100 mM $FeCl_3$ to 1 mL of 10 mM DTCS in 50 mM sodium phosphate (pH 7—7.6) (Fujii et al., 1996). The $Fe(DTCS)_3$ solution (0.2 mL) is added to 0.2 mL of saliva filtrate or bacterial fraction (Takahama et al., 2008a). $Fe(DTCS)_3$ is transformed to NO-$Fe(DTCS)_2$ after tapping NO. The formation of NO-$Fe(DTCS)_2$ was measured using an electron spin resonance (ESR) spectrometer with a quartz flat cell (0.05 mL). ESR spectra were recorded at room temperature under the following conditions: microwave power, 10 mW; scanning speed, 5 mT/min; line width, 0.5 mT; and amplification, 1000- or 2000-fold depending on ESR signal intensity.

2.2 Transformation of fluorescein derivatives to their triazole forms

DAF-2 and DAF-FM are used to detect the formation of NO under aerobic conditions. The fluorescent yields of these components increase significantly after the transformation to the triazole forms, namely, DAF-2T and DAF-FMT (Kojima et al., 1998, 1999). The excitation wavelengths (495 and 500 nm for DAF-2T and DAF-FMT, respectively) and the emission wavelengths (515 nm for DAF-2T and DAF-FMT) make easy to use these compounds because saliva filtrate and bacterial fraction don't absorb light around 500 nm. The formation of DAF-2T and DAF-FMT can be ascertained by HPLC (Takahama et al., 2005; 2009a).

In addition to the above fluorescent probes, APF is used to detect strong oxidants such as OH radical (OH radical/OH^-, $E^{o\prime}$ = 2.31 V at pH 7), NO_2, ONOOH, and HOCl but not O_2^- (Setsukinai et al., 2003). Because one of the oxidation products is fluorescein, the oxidation of APF can be measured fluorometically. Excitation and emission wavelengths are 490 and 515 nm, respectively. The formation of fluorescein can be ascertained by HPLC (Takahama et al., 2007a).

2.3 Nitration of HPA

HPA and tyrosine are used to detect NO_2 and ONOOH. HPA, which is produced during tyrosine metabolism by *Porphyromonas gingivalis*, is present in the mixed whole saliva (Takahama et al, 2002) and is mainly nitrated by peroxidase/H_2O_2/nitrite systems (Hirota et al., 2005; Takahama et al., 2003a, 2009b). Its nitration product is 4-hydroxy-3-nitrophenylacetic acid (O_2NHPA). This compound has absorption peaks at about 280 and 360 nm, and can be separated from HPA by HPLC (Takahama et al., 2002).

2.4 Oxidation of uric acid

Uric acid, which is present in saliva in the concentration range from 80 to 280 M (Ferguson, 1989), is an important antioxidant, and this component can be oxidized by NO_2 and

ONOOH (Halliwell & Gutteridge, 1999). The oxidation of uric acid is estimated from the absorbance decrease at 284 nm and the decrease in its concentration by HPLC (Takahama & Hirota, 2010).

3. RNOS production

Around pH 7, RNOS are generated by the autoxidation of NO that is produced by nitrite-reducing bacteria and by the peroxidase-catalyzed oxidation of nitrite. In addition to the above reactions, RNOS is generated by the self-decomposition of nitrous acid and by the reaction of nitrous acid with a salivary component SCN^- under acidic conditions (pH $\leqq$ 5.3). The followings deal with the formation of RNOS including NO in bacterial fraction and saliva filtrate.

3.1 Measurement of NO production using $Fe(DTCS)_3$

3.1.1 NO production around pH 7

The formation of NO-$Fe(DTCS)_2$ is not observed in bacterial fraction, but observed when nitrite is added to bacterial fraction (Takahama et al., 2005; Takahama et al., 2007a). The nitrite-induced NO production supports the reduction of nitrite to NO by nitrite-reducing bacteria (Palmerini et al., 2003). Nitrite concentration in the oral cavity increases after the ingestion of nitrate-containing foods (Pannala et al., 2003). The increase in concentration results in the enhanced production of NO that autoxidizes as followings,

$$4NO + O_2 \rightarrow 2N_2O_3 \quad (1)$$

Although NO can inhibit bacterial growth (Benjamin et al., 1994; Dykhuizen et al., 1996; Doel et al., 2004), the increased formation of N_2O_3 may contribute to give nitrosative stresses to tissues in the oral cavity.

3.1.2 Enhancement of NO production by decreasing pH

The pH in the oral cavity, especially dental plaque, rapidly decreases to below 5 after the ingestion of sugar-containing foods (Marsh & Martin, 1999; Lingström et al., 2000). The decrease in pH is due to the production of acid, especially lactic acid and the decreased pH returns slowly to its preingestion value. The frequency of this pH decrease depends on the frequency of ingestion of sugar-containing foods. Frequent and prolonged decrease in plaque pH results in the growth of acid-tolerant bacteria. Nitrite-induced NO production was about 5-fold faster around pH 5 than 7 in bacterial fraction, suggesting the faster production of NO by nitrite-reducing bacteria under acidic conditions (Takahama et al., 2007a, 2009a). In addition to NO production by nitrite-reducing bacteria, self-decomposition of nitrous acid is also possible for NO production under acidic conditions (Oldreive & Rice-Evans, 2001),

$$NO_2^- + H^+ \rightleftarrows HNO_2 \ (pKa = 3.3) \quad (2)$$

$$HNO_2 + H^+ \rightleftarrows H_2NO_2^+ \rightleftarrows H_2O + NO^+ \quad (3)$$

$$2HNO_2 \rightleftarrows N_2O_3 + H_2O \quad (4)$$

$$N_2O_3 \rightleftarrows NO + NO_2 \quad (5)$$

At pH 5, about 2% of nitrite is present as nitrous acid.

3.2 Measurements of RNOS production by fluorescent probes

DAF-2 and DAF-FM can be used to detect NO when pH is higher than 7 and 5, respectively (Kojima et al., 1998, 1999). Because NO is transformed to N_2O_3 under aerobic conditions (reaction 1), N_2O_3 or NO^+ donor can transform of DAF-2 and DAF-FM to DAF-2T (pKa = 6.27) and DAF-FMT (pKa = 4.38), respectively. On the other hand, radicals of DAF-2 and DAF-FM formed by NO_2- and ONOOH-dependent oxidation and peroxidase-catalyzed oxidation can react with NO to produce their triazole forms (Espey et al., 2002; Jourd'heuil, 2002). The above mechanisms of triazole formation suggest that the formation of DAF-2T and DAF-FMT increases with the increase in the concentrations of both NO and oxidants.

3.2.1 RNOS production around pH 7

DAF-2T formation in bacterial fraction is dependent on the concentration of nitrite, suggesting the contribution of NO produced by nitrite-reducing bacteria to DAF-2T formation (Takahama et al., 2005). Ascorbic acid, glutathione, uric acid, SCN^-, and phenolic compounds such as quercetin suppresses its formation (Takahama et al., 2006a). The suppression by ascorbic acid, glutathione, uric acid, and phenolic compounds can be attributed to the scavenging of NO^+, NO_2, and ONOOH, if these RNOS are contributed to the formation of DAF-2 radical and/or DAF-2T. If the DAF-2 radicals are scavenged by ascorbic acid and other antioxidants, DAF-2T formation is also suppressed. Furthermore, the above antioxidants can inhibit peroxidase-catalyzed oxidation of nitrite and DAF-2 to NO_2 and DAF-2 radicals, respectively, to suppress the formation of DAF-2T radical. SCN^- is a substrate of salivary peroxidase and myeloperoxidase around pH 7 (Pruitt et al., 1988; Tenovuo, 1989). Therefore, the inhibition of DAF-2T formation by SCN^- can be attributed to the suppression of the formation of NO_2 and DAF-2 radicals by peroxidases. Oxidation of nitrite by peroxidases proceeds as followings,

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{Compound I} + H_2O \quad (6)$$

$$\text{Compound I} + NO_2^- \rightarrow \text{Compound II} + NO_2 \quad (7)$$

$$\text{Compound II} + NO_2^- + 2H^+ \rightarrow \text{peroxidase} + NO_2 + H_2O \quad (8)$$

SCN^- can react with compound I to suppress the formation of not only NO_2 but also DAF-2 radical producing $OSCN^-$ (Tenovuo, 1989). Phenolic compounds suppress the formation of NO_2 and DAF-2 radicals by reacting with compounds I and II.

Certain bacteria in bacterial fraction produce O_2^- (Marsh & Martin, 1999; Tenovuo, 1989). Leukocytes migrated into the oral cavity also produce O_2^- (Al-Essa et al., 1994; Nakahara et al., 1998; Yamamoto et al., 1991). O_2^- produced in the oral cavity is scavenged by salivary superoxide dismutase to generate O_2 and H_2O_2 (Nagler et al., 2002). H_2O_2 generated from O_2^- is used as a substrate of peroxidases. However, O_2^- can encounter with NO produced by nitrite-reducing bacteria, resulting in the production of $ONOO^-$ in the oral cavity,

$$NO + O_2^- \rightarrow ONOO^- \quad (9)$$

$$ONOO^- + H^+ \rightleftarrows ONOOH \ (pKa = 6.8) \quad (10)$$

$$ONOOH \rightarrow NO_2 + OH \text{ radical} \quad (11)$$

$$ONOOH \rightarrow NO_3^- + H^+ \quad (11a)$$

$ONOO^-$ produced by reaction 9 is protonated to produce ONOOH around pH 7 (reaction 10). Although $ONOO^-$ is unreactive, ONOOH can transform to NO_2 + OH radical as well as nitrate (Goldstein et al., 2005; Halliwell and Gutteridge, 1999). Therefore, ONOOH can give oxidative damages to the oral tissues by itself and by producing NO_2 + OH radical. The possibility of ONOOH formation in the oral cavity is suggested by the result that superoxide dismutase enhanced and inhibited the nitrite-induced formation of DAF-2T and oxidation of APF, respectively, in bacterial fraction (Takahama et al., 2006b, 2007a,b). Superoxide dismutase can prevent the consumption of NO and the formation of $ONOO^-$/ONOOH by reaction 9, leading to the enhancement of DAF-2T formation and the inhibition of APF oxidation. The contribution of ONOOH in the nitrite-induced oxidation of APF is about 30% at pH 7 (Takahama et al., 2007a). $ONOO^-$ reacts with CO_2 producing $ONOOCO_2^-$ that decomposes to nitrate + CO_2 and NO_2 + CO_3^- radical (Goldstein et al., 2005). The concentration of CO_2/HCO_3^- in saliva (10-30 mM) suggests the reaction of $ONOO^-$ with CO_2 in the oral cavity (Ferguson, 1989).

DAF-2T formation has also been studied using saliva filtrate. The formation of DAF-2T in the filtrate is much slower than that in bacterial fraction, and is dependent on the concentration of nitrite added (Takahama et al., 2005, 2007a). This result indicates that although NO is produced by nitrite-reducing bacteria, the formation of DAF-2 radical is inhibited and/or DAF-2 radical is scavenged by certain salivary components. Nitrite-induced oxidative degradation of APF is also much slower in saliva filtrate, implying the presence of scavengers of NO_2 and ONOOH in saliva (Takahama et al., 2007a).

Saliva is collected at 0, 1, 2, 3, and 4 hours after toothbrushing, and five saliva filtrates are prepared. The rate of nitrite-induced formation of DAF-2T in each filtrate increases with the increase in time after toothbrushing (Takahama et al., 2005). The rate of nitrite-induced DAF-2T formation in bacterial fraction obtained from each saliva filtrate also increases with the increase in time after toothbrushing. These results suggest the gradual growth of nitrite-reducing bacteria in the oral cavity after toothbrushing.

3.2.2 Enhancement of RNOS production by decreasing pH

The pH of the oral cavity especially dental plaque, where peroxidase and nitrite are present, decreases to below 5 (Bayindir et al., 2005; Crossa et al., 2001; Marsh & Martin, 1999). Therefore, nitrite-induced transformation of DAF-FM to DAF-FMT has been studied using bacterial fraction in a pH range from 5.3 to 7.2. It is known that the fluorescent yield of DAF-FMT dose not change in the pH range (Kojima et al., 1999). The DAF-FMT formation was about 10 times faster at pH 5.3 than 7.2, suggesting the faster production of NO under acidic conditions as described in section 3.1.2. The faster formation of DAF-FMT also suggests the faster formation of DFA-FM radical, which can be attributed to the enhanced production of NO_2 by peroxidase/H_2O_2/nitrite systems. Contribution of peroxidase/H_2O_2/nitrite systems

to the DAF-FMT formation at pH 5.3 and 7.2 is supported by the result that SCN^- (< 0.2 mM) inhibited the DAF-FMT formation at the pH values (Takahama et al., 2009a), and the enhanced production of NO_2 under acidic conditions is supported by the results that rate of the nitrite-induced oxidation of APF in bacterial fraction is 4-fold faster around pH 5 than 7 (Takahama et al., 2007a). The faster production of NO and NO_2 around pH 5 than 7 can contribute to the much faster formation of DAF-FMT at pH 5.3 than 7.2.

The formation of DAF-FMT is suppressed by various components at pH 5.3 and 7.2. Ascorbic acid (10 M) (pKa = 4.2, 11.6) suppressed the DAF-FMT formation more than 90% at the pH values, and uric acid (100 M) (pKa = 5.4 and 10.3) suppressed the DAF-FMT formation by about 60% at pH 7.2 but not at pH 5.3 (Takahama et al., 2009a). Phenolic compounds such as quercetin and cathechin (10 M) suppressed the formation of DAF-FMT by 95 and 75% at pH 7.2 and by 40 and 10% at pH 5.3, respectively (Takahama et al., 2009a). One of the reasons for the different effects between ascorbic acid and other compounds is the degree of H^+-dissociation; more than 80% of ascorbic acid is present as mono-anion form at pH 5 and 7. On the other hand, about 40 and 83% of uric acid are present as mono-anion form at pH 5 and 7, respectively. The dissociation of phenolic OH groups may increases with the pH increase from 5 to 7; the pKa values of quercetin are 6.6 and 9.7 (Zenkevich & Guschina, 2010), and those of chatechin are 8.2 and 9.2 (El-Hady & El-Maali, 2008).

According to the above mechanism of DAF-FMT formation, ascorbic acid can suppress its formation by scavenging both NO_2 and DAF-FM radical and by inhibiting peroxidase-catalyzed production of the above components around pH 5 and 7. Oxidation rate of quercetin by bacterial fraction at pH 5.3 was about 30% of that at pH 7.2 in the absence of nitrite (Takahama et al., 2009a). This result suggests that the more efficient inhibition of DAF-FMT formation by phenolic compounds at pH 7.2 can be attributed to the more efficient suppression of peroxidase-catalyzed oxidation of nitrite and DAF-FM by quercetin. The greater inhibition of DAF-FMT formation by uric acid at pH 7.2 than 5.3 can also be attributed to more efficient inhibition of peroxidase-catalyzed reactions by uric acid at pH 7.2 than 5.3. Thus, the differences in pKa values among the above components may contribute to the different inhibitory effects between acidic and neutral conditions.

Nitrite-induced formation of DAF-FMT in bacterial fraction is enhanced by SCN^- at pH 5.3 but suppressed at pH 7.2 when SCN^- concentration is higher than 1 mM (Takahama et al., 2009a). SCN^- can react with nitrous acid (Doherty et al., 1997),

$$HNO_2 + SCN^- + H^+ \rightleftarrows ONSCN + H_2O \quad (12)$$

This reaction is possible because about 2% of nitrite is present as nitrous acid at pH 5. ONSCN may contribute to the enhanced formation of DAF-FMT, because SCN^- suppresses peroxidase-catalyzed oxidation of nitrite to NO_2 as described above. ONSCN can dissociate into SCN^- and NO^+ that is a strong oxidant (Licht et al., 1988). Therefore, SCN^--dependent enhancement of DAF-FMT formation can be attributed to the addition of NO^+ to DAF-FM or NO^+-dependent oxidation of DAF-FM to its radical to react with NO. The latter is supported by the faster NO production under acidic conditions (Takahama et al., 2007a, 2009a).

Nitrite-induced oxidative degradation of APF is about 3 and 5 times faster at pH 5.3 than 7.2 in bacterial fraction and saliva filtrate, respectively. The increased oxidation of APF with the decrease in pH may be due to the oxidation of APF by salivary peroxidase/H_2O_2 systems,

NO_2 produced by the systems, and $ONOO^-$/ONOOH. At pH 5.3, ONOOH can be produced by the following reaction in addition to reaction 9.

$$H_2O_2 + HNO_2 \rightarrow ONOOH + H_2O \quad (13)$$

As the contributions of $ONOO^-$/ONOOH are about 10 and 30% at pH 5.3 and 7.2, respectively (Takahama et al., 2007a), it is supposed that peroxidase-dependent oxidation of APF increases with the decrease in pH. Although SCN^- is a good inhibitor of salivary peroxidase at pH 5 (Pruitt et al., 1988), 2 mM SCN^- enhanced nitrite-induced oxidation of APF at pH 5.3 in bacterial fraction. The enhancement of APF oxidation by SCN^- can be supposed to be due to NO^+ produced from ONSCN.

The above results suggest that the production of NO and NO_2 increases in the oral cavity with the decrease in pH. The production of ONSCN accompanies the suppression of peroxidase-catalyzed NO_2 production by SCN^-. The production of NO, NO_2, and ONSCN is also possible in acidic dental plaque. This is supported by the presence of nitrite, SCN^-, and salivary peroxidase in dental plaque (Bayindir et al., 2005; Crossa et al., 2001; Tenovuo, 1989; Tenovuo et al., 1981). The concentration of nitrite in dental plaque is 1.2-2-fold higher than that in saliva (0.05-1 mM) (Bayindir et al., 2005; Crossa et al., 2001). The production of $ONOO^-$/ONOOH in acidic dental plaque is possible because superoxide dismutase enhanced and inhibited the formation of DAF-FMT and the oxidative of APF, respectively, in bacterial fraction at pH 5.3 in the presence of nitrite (Takahama et al., 2007a).

3.3 Nitration

Following reactions are postulated for main pathways of the nitration of HPA in the oral cavity (Hirota et al., 2005),

$$H_2O_2 + 2HPA \rightarrow 2HPA\ radical + 2H_2O\ (catalyzed\ by\ peroxidase) \quad (14)$$

$$HPA + NO_2 \rightarrow HPA\ radical + NO_2^- + H^+ \quad (15)$$

$$HPA\ radical + NO_2 \rightarrow O_2NHPA \quad (16)$$

The first step is the oxidation of HPA to its radical, and the second step is the reaction of HPA radical with NO_2. Salivary peroxidase catalyzes the nitration of HPA in the presence of both 1 mM nitrite and 0.5 mM H_2O_2 at pH 5.3 and 7.2, and rate of the nitration at pH 5.3 is similar to that at pH 7.2 (Hirota et al., 2005). During the nitration, nitrite concentration and HPA concentration decrease. The decrease in nitrite concentration has a broad peak around pH 5, whereas the decrease in HPA concentration has a peak around pH 7. The results suggest that the nitration at pH 7.2 is mainly due to the reaction of NO_2 with HPA radicals, both of which are produced by peroxidase-catalyzed oxidation of nitrite and HPA. The contribution of salivary peroxidase on the nitration is supported by SCN^--dependent inhibition of the nitration (50% inhibition, 10 M) (Takahama et al., 2003a). From the effects of pH on nitrite and HPA consumption, it is deduced that nitration at pH 5.3 mainly proceeds as followings; oxidation of HPA to its radicals by NO_2 and addition of NO_2 to the radical. In contrast to the above result, nitration of HPA is about 3 times faster at pH 5.3 than pH 7.0 when the nitration is induced by peroxidase/glucose oxidase/nitrite systems, which

produce 2 M H_2O_2/min, and the nitration is nearly completely suppressed by 1 mM SCN^- at pH 5.3 (Takahama et al., 2009b). The above two reports imply that peroxidase-dependent NO_2 production is faster in acidic than neutral dental plaque in the presence of physiological concentration of H_2O_2 in the oral cavity, which is approximately 10 M (Tenovuo, 1989).

Nitrated HPA has been detected in mixed whole saliva from patients of periodontal diseases who are older than 60 years of age (Takahama et al., 2009b). Increased concentrations of nitrite, HPA, and H_2O_2 and decreased pH in the oral cavity may contribute to the formation of nitrated HPA. In fact, the concentrations of nitrite and HPA tend to be higher in individuals with age of 60-year-old or more. Quercetin (30 M) suppressed nitrite-induced O_2NHPA formation more than 90% in bacterial fraction at pH 5 (Hirota et al., 2005) and quercetin can stay in the oral cavity for several hours after ingestion of onion soup (Hirota et al., 2001). Chlorogenic acid, which can also scavenge NO_2, stays in the oral cavity for several hours after ingestion of coffee (Takahama et al., 2007b). Therefore, quercetin and chlorogenic acid can function as scavengers of RNOS or electron donors to salivary peroxidase. Flavonoid aglycones and cinnamic acids including chlorogenic acid have been reported to be able to inhibit proliferation of oral cancer cells (Browning et al., 2005; Tanaka et al., 1993; Walle et al., 2005).

3.4 Oxidation of uric acid

Nitrite-induced production of NO_2 has been estimated by measuring the oxidation of uric acid using saliva filtrate. The rate of uric acid oxidation increases with the decrease in pH when the pH is lower than 6, suggesting that nitrous acid contributes to the oxidation of uric acid (Takahama & Hirota, 2010). To simulate dental plaque, oxidation of uric acid has been studied using bacterial fraction. H_2O_2-induced oxidation of uric acid is enhanced by nitrite, suggesting that NO_2 produced by salivary peroxidase/H_2O_2/nitrite systems contributed to the oxidation of uric acid. SCN^- (1 mM) suppresses the uric acid oxidation by about 75%, confirming that peroxidases participate in the oxidation of uric and that even if ONSCN contribute to the oxidation of uric acid, its contribution is small (Pietraforte et al., 2006; Takahama et al., 2003b; Takahama & Hirota, 2010)). The contribution of ONOOH formed by reaction 13 cannot be excluded in the oxidation of uric acid by the systems.

4. Conclusion

With the decrease in pH in the oral cavity from 7 to 5, nitrite-dependent production of not only NO, N_2O_3, NO_2, and $ONOO^-$/ONOOH but also ONSCN seems to be enhanced (Figure 1). If the concentration of nitrite in the oral cavity is increased by ingesting nitrate-rich foods, the increased nitrite concentration results in the increase in formation of NO, N_2O_3, NO_2, $ONOO^-$/ONOOH, and ONSCN under acidic conditions.

Uric acid in saliva can scavenge NO_2 and $ONOO^-$/ONOOH but not ONSCN. Even if NO_2 and $ONOO^-$/ONOOH are scavenged by uric acid, the scavenging will not be complete. Thus, if the concentrations of nitrite and SCN^- increase accompanying the decrease in pH of dental plaque, oral tissues adjacent to the plaque will be injured, because NO, N_2O_3, NO_2, $ONOO^-$/ONOOH, and NO^+ formed in acidic dental plaque can diffuse to the adjacent tissues. Diffused NO can be transformed to N_2O_3 and/or $ONOO^-$/ONOOH. Nitrite (0.05-1

mM) and SCN^- (0.1-2 mM) are always present in the oral cavity (Tenovuo, 1989), therefore it seems to be important to avoid the decrease in pH in the oral cavity to reduce RNOS-induced injuries of oral tissues.

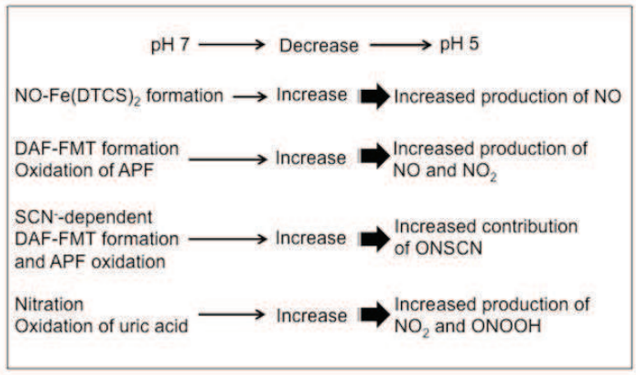


Fig. 1. Increases in RNOS production with the decrease in pH in the oral cavity

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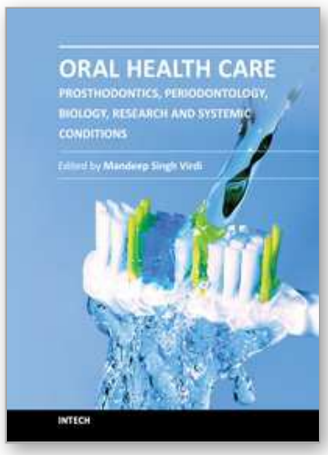
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Geriatric dentistry, or gerodontics, is the branch of dental care dealing with older adults involving the diagnosis, prevention, and treatment of problems associated with normal aging and age-related diseases as part of an interdisciplinary team with other healthcare professionals. Prosthodontics is the dental specialty pertaining to the diagnosis, treatment planning, rehabilitation, and maintenance of the oral function, comfort, appearance, and health of patients with clinical conditions associated with missing or deficient teeth and/or oral and maxillofacial tissues using biocompatible materials. Periodontology, or Periodontics, is the specialty of oral healthcare that concerns supporting structures of teeth, diseases, and conditions that affect them. The supporting tissues are known as the periodontium, which includes the gingiva (gums), alveolar bone, cementum, and the periodontal ligament. Oral biology deals with the microbiota and their interaction within the oral region. Research in oral health and systemic conditions concerns the effect of various systemic conditions on the oral cavity and conversely helps to diagnose various systemic conditions.

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Phone: +385 (51) 770 447
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Phone: +86-21-62489820
Fax: +86-21-62489821

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