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MINI REVIEW

Oxidative stress and temporomandibular joint disorders

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Summary Etiology of temporomandibular joint disorders (TMD) was estimated as excessive mechanical stresses inflicted on the temporomandibular joint (TMJ). The stresses including bruxism, clenching and oral parafunctional habits in daily life cause irreversible damage in the joint tissue. As the stress loading to the TMJ, it has been shown that increase of generation of free radicals, biosyntheses of arachidonic acid catabolite, release of neuropeptide and cytokines, and activation of matrix degrading enzymes from various TMJ tissues were observed. Few studies of reactive oxygen species (ROS) in TMD were reported. The authors postulate mechanisms that provably involved in the production of ROS in the TMJ and the subsequent induction of molecular events that may amplify damage to the joint tissues.

1. Introduction

It is well known that reactive oxygen species (ROS), including free radicals such as superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($HO^{\bullet}$), are highly reactive molecules usually generated during cellular respiration and normal metabolism. Under normal physiological condition, free radicals may also function as activating molecules involved in cellular function, such as signal transmission of various gene expressions. Accumulation of excess free radicals in a tissue contributes to a pathologic condition by damaging extracellular and intracellular molecules as well as excessive activation of cellular processes, such as extracellular matrix turnover, DNA damage, denaturation of protein, or lipid oxidation [1].

To control free radical mediated reaction, antioxidant enzymes [e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] existed in the body that function to convert highly reactive free radicals to less active molecular species [1—4]. In addition, low-molecular-weight antioxidant, such as ascorbate, tocopherol, glutathione, flavonoid, tannins, uric acid and bilirubin, scavenge free radicals as they are produced in metabolically active tissues. Free radical reactions and oxidative damage are in most cases held in check by aforementioned antioxidant defense mechanisms, but where an excessive amount of ROS are produced or defense mechanisms are impaired, oxidative damage such as lipid peroxidation may occur. Such kind of unbalance between production of ROS and antioxidant defense system was made definite as oxidative stress. It has also been suggested that mechanical stresses can lead to ROS-induced oxidative stress of the temporomandibular joint (TMJ) [5]. Excessive production of oxidative stress in the

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TMJ thus results in tissue damage, which further propagates to TMD.

Electron spin resonance (ESR) has been recognized as one of the most powerful techniques for the detection of free radicals in biological tissues and cells. We have developed an ESR-based technique to detect free radical reactions in biological system *in vitro* or *in vivo*. Using the ESR-spin trapping technique, we have previously demonstrated that hydroxyl radical ($HO^{\bullet}$) could be generated by catalytic reaction of H_2O_2 with free iron ions (Fenton reaction) in the IL-1α-induced TMJ rat arthritis model or in synovial fluid (SF) of the TMD patients [6,7]. In the present review, we discussed the oxidative stress related to the pathogenesis of the TMD, and demonstrate that the improvement of the oxidative stress would raise the possibility of constructing novel treatment strategies for TMD.

2. ROS generation and TMD

ROS can be generated in the TMJ by several pathways: they include (1) direct mechanical injury [8], (2) hypoxia-reperfusion [9], and (3) arachidonic acid catabolism to the articular tissues [1]. We know very little about the characteristics of mechanical stresses generated in human TMJ with the jaw movement (Fig. 1).

Intra-articular pressures have been recorded in some human TMJs during jaw movements: voluntary clenching has produced much higher intra-articular pressures in the TMJ, reaching the range of 8—200 mmHg (mean, 63.90 $\pm$ 52.25 mmHg). When it goes up above 40 mmHg, the intra-articular pressure surpasses the peripheral arteriolar pressure can cause temporary hypoxia followed by reoxygenation on cessation of clenching [9]. It is certainly reasonable to speculate that physical stresses impacting vascular tissues of the TMJ under abnormal conditions (i.e., articular disc displacement) could greatly exceed in magnitude the end capillary perfusion pressures of these tissues. Therefore, the hypoxia-reperfusion model could be feasible for ROS production on the basis of inadequate intra-articular pressures. With increasing hypoxia, local cell populations undergo a shift in metabolism that can lead to the production of ROS when perfusion is reestablished. When joint is relaxed and articular tissues are reperfused, xanthin oxidase may react with accumulated hypoxanthine or xanthine in the presence of oxygen to form $O_2^{\bullet -}$ (Fig. 2).

Arachidonic acid catabolism leading to the production of PGE_2 and LTB_4 in the articular tissues stimulates mast cells and leukocytes producing a variety of proinflammatory cytokines and free radicals. The hydroperoxidase-catalyzed conversion of PGG_2 to PGH_2 (cyclooxygenese pathway) result in transformation of 5-HPETE (5-hydroperoxygenease eicosatetraenoic acid) to 5-HETE (5-hydroxy-genease eicosatetraenoic acid) both yielding free radical, including fatty acid radical and HO (Fig. 3) [1].

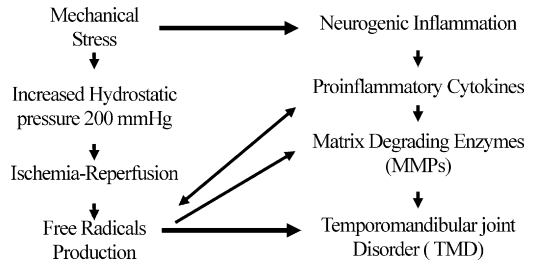

Figure 1 Proposed mechanisms of the degenerative TMJ disease.

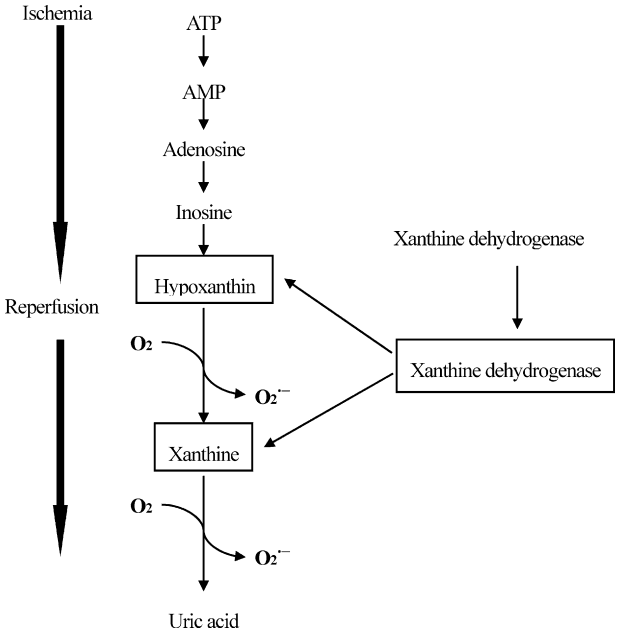

Figure 2 Mechanisms of generation of superoxide anion radical by ischemia-reperfusion with xanthine dehydrogenase.

3. Molecular deterioration of TMJ tissues caused by ROS

ROS affect various molecular species of the TMJ and deteriorate the TMJ function: they include (1) reduction of SF viscosity by depolymerization and/or molecular configuration of hyaluronic acid (HA) [10,11]; (2) reduction of lubrication of the articular surface by deterioration of the surface-active phospholipid (SAPL) layer, which acts as an extremely efficient boundary lubricant and protector of articular surfaces [12]; (3) breakdown of collagen proteoglycans [13]; (4) activation of cartilage degrading enzymes such as matrix metalloproteinases. The ROS, especially $HO^{\bullet}$, strongly reacts with the membrane lipids to begin a chain reaction of autoxidation which can subsequently result in the production of carbon-centered radical ($R^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), and peroxyl radicals ($ROO^{\bullet}$), all of these are markers of lipid peroxidation and of the disruption of cellular homeostasis. Nitzan reported that lysis of SAPL layer by phospholipase A_2 ($sPLA_2$) together with the depolymerization of HA caused by free radicals may result in a deteriorated lubrication of the articular surface, thus further proceeding to the internal derangement (ID) of the TMJ [9]. The $HO^{\bullet}$ also degrades collagen and proteoglycans (Pgs) into low molecular masses, which act as immunogens for synoviocytes or chondrocytes [14]. These denatured Pgs may induce proinflammatory cytokines from various cells in the TMJ compartment. Also interesting was our evidence that $HO^{\bullet}$ can dose dependently

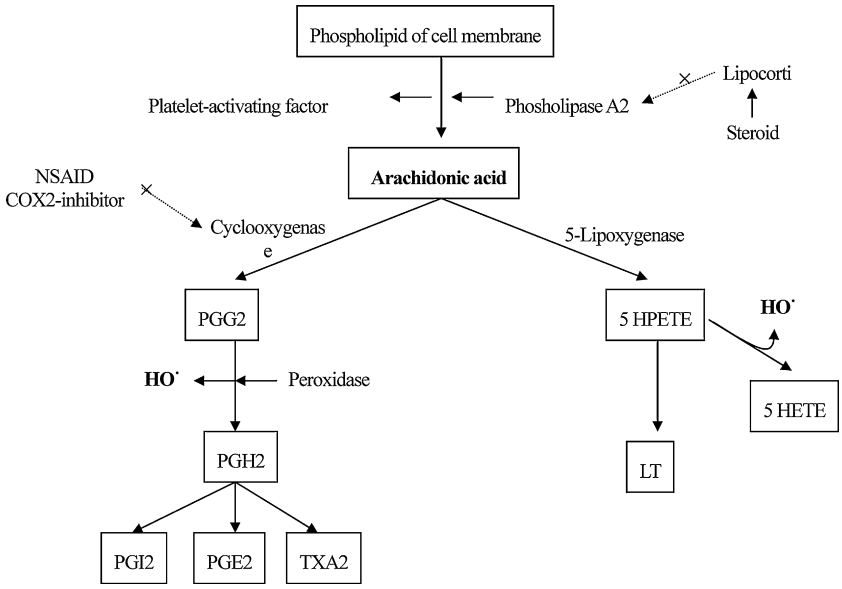

Figure 3 Mechanisms of generation of ROS from arachidonic acid catabolism.

activate pro MMP9 to the activated one *in vitro* possibly by affecting its molecular configuration. These data suggest that free radicals may cause molecular deterioration, which further proceeds to degenerative changes in the TMJ.

4. ROS production in synovial fluid from TMD patients

Cytokines, IL-1, and TNF-alpha have been shown to increase the release of $O_2^{\bullet -}$ from cultured chondrocytes and synovial cells in a dose-dependent fashion thereby causing DNA damage. Both inhibition of hyaluronic acid synthesis and activation of proteases that degrade extracellular matrix of the articular cartilage have also shown to be caused by $O_2^{\bullet -}$. We could successfully detected three different ESR spectra of DMPO spin adducts in SF from IL-1-induced TMJ arthritis of rats [6]. These were closely corresponded to the spin adducts of DMPO-OH, DMPO-H$^{\bullet}$, and DMPO-R$^{\bullet}$, respectively [15,16], indicating that production of three different free radical species were, HO$^{\bullet}$, hydrogen radical (H$^{\bullet}$), and a carbon-center radical (R$^{\bullet}$). Interestingly, these results are consistent with the ESR spectra obtained from SF in the TMD patients (Fig. 4) [7].

We examined the effects of SOD, an $O_2^{\bullet -}$ scavenger, and DFO, an iron chelator, on generation of free radicals in the SF samples of TMD patients. We observed quite high signal intensities of DMPO-OH in SF from 3 osteoarthritis (OA) patients (Fig. 5) and 3 internal derangement (ID) patients (Fig. 6). The high signal intensities of DMPO-OH in both OA and ID patients were diminished by SOD as well as DFO. On the contrary, the low-intensity signals for DMPO-OH in SF from other 3 OA patients were conversely enhanced by addition of SOD, suggesting existence of excess amount of $O_2^{\bullet -}$ in the SF. Iron chelating agent, DFO, strongly depressed the DMPO-OH signal intensity in SF from both OA and ID patients [7].

ROS generated by synoviocytes, chondrocytes and inflammatory cells have been implicated in the initiation and progression of TMD [9,17]. In the present study, we have provided the evidence of ROS generation in the SF from TMD, and we could identify that HO$^{\bullet}$ may be critical in the diseased TMJ. HO$^{\bullet}$ has strong reactivity to lipid membrane and initiate a chain reaction of autoxidation of the lipid that can subsequently result in production of carbon-centered (R$^{\bullet}$), alkoxyl (RO$^{\bullet}$), and peroxyl radicals (ROO$^{\bullet}$). These lipid peroxidations associated with disruption of cellular function [18]. Under normal physiological conditions, $O_2^{\bullet -}$ is rapidly dismutated to H_2O_2 through catalytic reaction with SOD (Eq. (1)). HO$^{\bullet}$ can be formed by the Haber—Weiss reaction from $O_2^{\bullet -}$ and H_2O_2

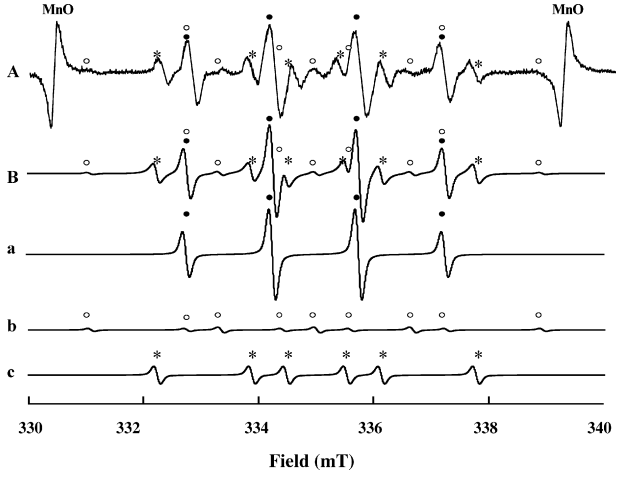

Figure 4 ESR spectra of DMPO spin adducts of synovial fluid (SF) from TMD patients. (A) We confirmed three components in the ESR spectrum in SF with DMPO (B, a) by computer simulation, they were resoled as DMPO-OH (B, b), DMPO-H$^{\bullet}$ (B, c) and DMPO-R$^{\bullet}$ (B, d). ESR spectra indicate the hydroxyl radical (HO$^{\bullet}$; filed circles) hydrogen radical (H$^{\bullet}$; open circles) and carbon-center radical (R$^{\bullet}$; *). The signal intensity of peaks marked with a dagger of the spectrum (A) was normalized as a relative height for HO$^{\bullet}$, against the standard signal intensity of the MnO marker.

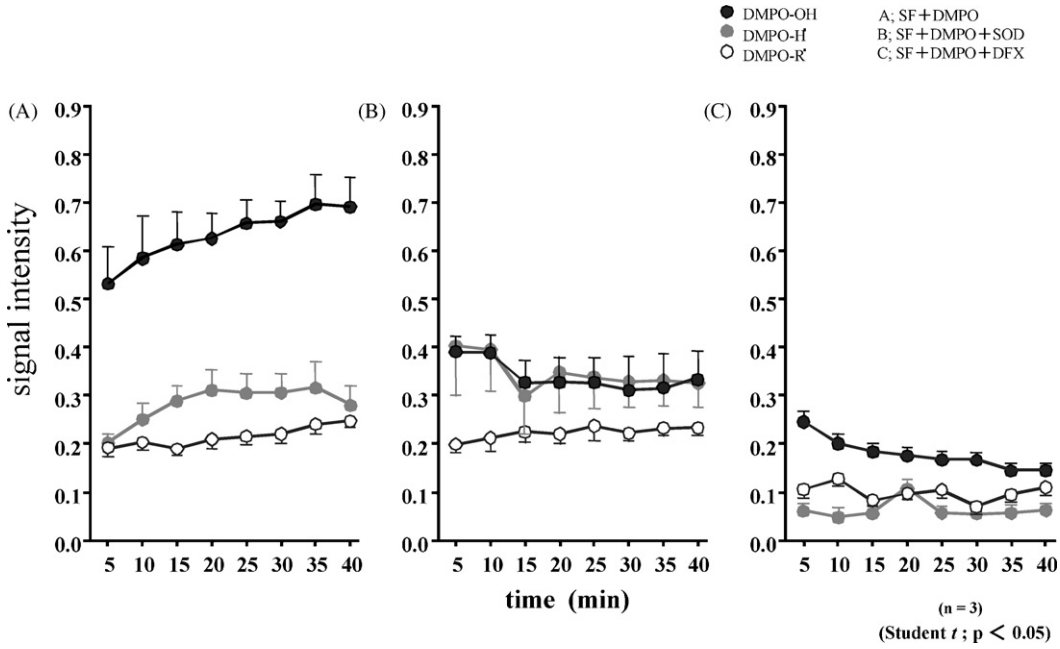

Figure 5 Effect of superoxide dismutase (SOD) and desferrioxamine (DFO) on the signal intensity of DMPO-OH, DMPO-H• and DMPO-R• spin adduct in SF from TMJ with internal derangement (ID) patients by ESR-DMPO spin trap technique. The signal intensity of three kinds of radical was detected in the SF, in panel (A) the ESR spectra of the SF from ID were shown as control. In panel (B), the ESR spectra of the SF after addition of SOD were depicted. Also shown were ESR spectra of the SF after addition of DFO (C). All of these signals were diminished by the addition of SOD or DFO.

(Eq. (2)). However, this reaction is considered to be too slow to compete with the dismutation reaction indicated in Eq. (1).

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1)$$

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + HO^{\bullet} + OH^- \quad (2)$$

HO• can be generated more efficiently via the Haber–Weiss reaction if iron ion is being existed, a reaction known as the biological Fenton reaction (Eqs. (3) and (4)).

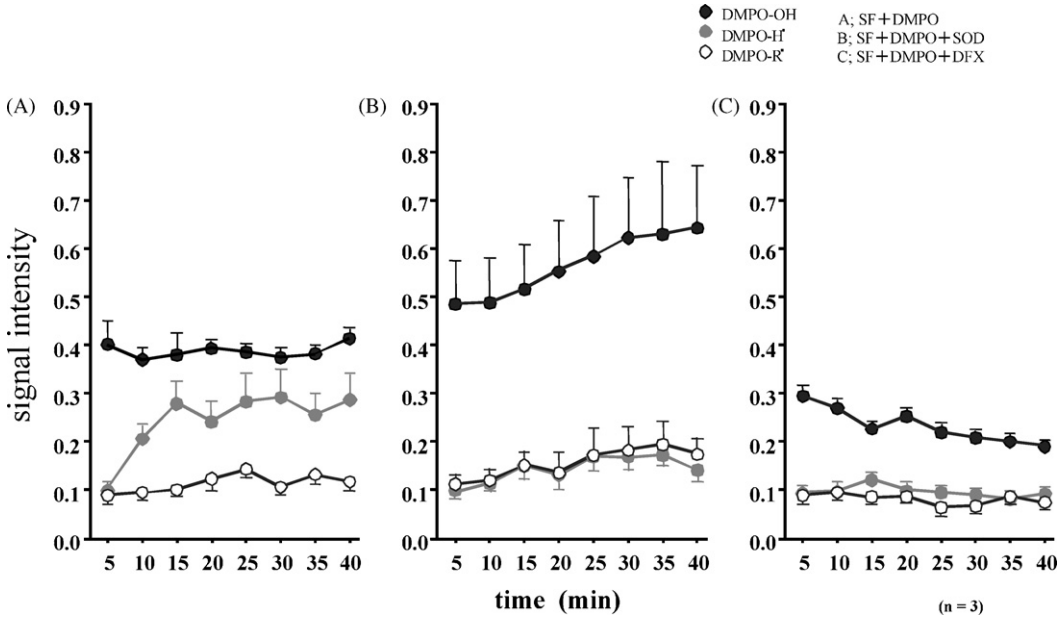

Figure 6 Effect of superoxide dismutase (SOD) and desferrioxamine (DFO) on the signal intensity of DMPO-OH, DMPO-H• and DMPO-R• spin adduct in SF from TMD with osteoarthritis (OA) patients. The signal intensity of three kinds of radical was detected in the SF, in panel (A) the ESR spectra of the SF from ID were shown as control. In panel (B), the ESR spectra of the SF after addition of SOD were depicted. Also shown were ESR spectra of the SF after addition of DFO (C). Hydroxyl radical signal in SF from OA patients was enhanced by SOD (B). However, these three kinds of radicals in SF from OA patients were all scavenged by DFO (C).

$$O_2^{\bullet -} + Fe^{3+} \rightarrow O_2^{\bullet -} + Fe^{2+} \quad (3)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad (4)$$

Existence of this type of reaction in the diseased TMJ was suggested by the ability of DFO to reduce the high-intensity signal of the DMPO-OH spin adduct on ESR [6,7]. Other researchers already reported that SF form rheumatoid arthritis (RA) patients contains sufficient micromolar amounts of free iron to allow hydroxyl radical formation, and that the capacity of iron-binding proteins is not enough in the RA patients, even if a large amount of iron-binding proteins (lactoferrin, transferrin and ferritin) are present in the normal joint [19]. Thus, the generation of $HO^{\bullet}$ via Fenton reaction was considered utmost important in the pathophysiology of TMD. These results suggest that $HO^{\bullet}$ generated in the inflamed TMJ may develop TMD. It has been reported that deposition of redox-active heme-iron derived from denatured hemoglobin was found in the TMJ-SF as a result of microbleeding into the joint space [20]. These data further suggest that non-protein-bound iron ion in the SF may lead to HO(production and subsequent lipid peroxidation via a Fenton-type reaction (Eq. (4)).

We have shown that in three cases of OA patients the addition of SOD increased the intensity of the DMPO-OH [7]. An increased generation of $HO^{\bullet}$ in the arthritic TMJ thus implies abundant presence of H_2O_2 due to dismutation of $O_2^{\bullet -}$ by exogenously added SOD in the SF. This may due to the enzymatic ability of SOD to catalyze the formation of H_2O_2 from $O_2^{\bullet -}$, thereby increasing the availability of substrate H_2O_2 for the iron-dependent generation of $HO^{\bullet}$ (Fenton reaction). The time-dependent increase of $HO^{\bullet}$ generation *in vitro* may suggest that the dismutation still continuing in the SF taken from the inflamed TMJ. This evidence suggests that SOD synthesis might be compromised against overwhelming $O_2^{\bullet -}$ in some patients with OA [7]. We could detect the DMPO-OH spin adduct, indicating $HO^{\bullet}$ generation, while it could not be observed DMPO-H spin adduct (Fig. 6) [7]. It would be possible that more rapid reaction of DMPO with HO(existed compared to the reaction of DMPO with $O_2^{\bullet -}$ or depolymerization of HA by $HO^{\bullet}$ in the TMD SF sample. Furthermore, the impaired TMJ SF may include more iron ion derived from denatured hemoglobin compare to that of the normal [5,20]. We could provide evidence that $HO^{\bullet}$ in TMD SF generated via biological Fenton's reaction, and it would be one of the most harmful ROS in the TMJ.

5. Conclusion

The $HO^{\bullet}$, which is the most probable oxygen intermediate involved in tissue damage, has been directly proven to be generated in the diseased TMJ. Free irons and endogenous irons, possibly derived from hemoglobin in the arthritic TMJ, might play a role in driving the catalytic reaction to generate $HO^{\bullet}$. We could accumulate the ESR data for detecting ROS production in SF from TMD patients and to correlate ROS production to the TMD. We could postulate new treatment strategies such as antioxidant therapy for TMD in the near future.

Acknowledgment

The data of Figs. 4–6 in this paper are cited from MANEY Publishing (Ref. [7]).

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