A central role for reactive oxygen species (ROS) in the pathogenesis of temporomandibular joint disorders: All roads lead to ROS

Adam Kevin Wade-Vallance, Maxwell Ng, Katherine Kuo, Candice Luo, Seana Adams, Gurleen Dhaliwal & Caberry Yu

*Authors contributed to equally to this work.

Abstract
Temporomandibular joint disease (TMD) is a musculoskeletal pain disorder occurring at the temporomandibular joint (TMJ), the interface of the skull's temporal bone and the mandible. This literature review examines the anatomy, epidemiology, biochemistry and cellular biology of TMD in order to frame underlying biochemical and cellular events within the human context of the disease. This review identifies several key elements pertaining to TMD pathogenesis, including mechanical stress-induced hypoxia-reperfusion, disruption of mitochondrial function, arachidonic acid catabolism (through prostaglandins and leukotrienes), cartilage degradation, bone resorption, and breakdown of joint lubrication. Based on a thorough analysis of established correlations and causations we propose an overarching mechanism that provides a holistic representation of TMD, something noticeably absent in the literature to-date. This mechanism clearly highlights the central role of reactive oxygen species (ROS) in the pathogenesis of TMD, a conclusion holding significant implications for both treatment and our understanding of the disease.

Introduction
The TMJ is a synovial joint located bilaterally at the jaw. It is directly involved in the opening and closing of the mouth, mastication, lateral excursions, and protrusion. Primary joint structures include the mandibular fossa, articular tubercle, and mandible head between which an articular disc is situated. The articular disc, composed of fibrous connective tissue, mitigates friction by separating surrounding bones. TMD typically arises from chronic compression of the TMJ, which induces a number of detrimental effects including the production of ROS and anterior disc displacement, a painful TMD symptom clinically known to limit jaw mobility.

ROS, such as superoxide anion (O$_2^-$) and hydroxyl radicals (OH$^-$), serve diverse cellular functions in signal transmission, cellular activation, and regulation. Despite these essential roles, ROS levels must be regulated by antioxidants to prevent deleterious off-target reactivity. If this balance is compromised and ROS accumulate, serious consequences including DNA damage, protein denaturation and lipid peroxidation can occur. This phenomenon, termed oxidative stress, acts to propagate TMD.

Currently, few review articles discuss the effects of oxidative stress on TMD pathology. Milam et al. propose a pathway for oxidative stress-induced TMJ damage. They describe mechanical stress as the primary instigator of ROS synthesis with subsequent regulatory pathways involving ROS. Although recent studies continue to investigate the effects of mechanical stress on the TMJ, they fail to properly discuss the full implications of mechanical stress on the downstream ROS pathways. Several primary research studies have investigated specific cellular mechanisms disrupted by ROS in TMD, such as the lysing of hyaluronic acid, but rarely do they reference TMD’s primary cause - mechanical stress. Inadequate contextual framing is also observed in a study exploring the role of iron in OH$^-$ formation via the Fenton reaction, which does not consider potential sources of iron. By synthesizing the limited scopes of individual papers such as these, we have created a more complete view of the role of ROS in TMD (Figure 1). Temporomandibular joint disease (TMD) is a musculoskeletal pain disorder occurring at the temporomandibular joint (TMJ), the interface of the skull's temporal bone and mandible. This literature review examines the anatomy, epidemiology, biochemistry and cellular biology of TMD in order to frame underlying biochemical and cellular events within the human context of the disease. This review identifies several key elements pertaining to TMD pathogenesis, including mechanical stress-induced hypoxia-reperfusion, disruption of mitochondrial function, arachidonic acid catabolism (through prostaglandins and leukotrienes), cartilage degradation, bone resorption, and breakdown of joint lubrication. Based on a thorough analysis of established correlations and causations we propose an overarching mechanism that provides a holistic representation of TMD, something noticeably absent in the literature to-date. This mechanism clearly highlights the central role of reactive oxygen species (ROS) in pathogenesis of TMD, a conclusion holding significant implications for both treatment and our understanding of the disease.
Figure 1: Overview of central pathways involved in the pathogenesis of temporomandibular joint disorder highlighting the central role of reactive oxygen species. Illustrated here are the hypoxia reperfusion, mitochondrial dysfunction, arachidonic acid catabolism, disc displacement via impaired lubrication, bone resorption, and cartilage degradation pathways. Although emphasis is placed on the left side of the image, these processes would in reality be mirrored across the fibrous articular disc. This figure was developed using Adobe Illustrator, please see the legend for all abbreviations.

Xanthine Oxidase-Mediated ROS generation

ROS have expansive involvement in the pathogenesis of TMD\textsuperscript{8-18}. One ROS-producing mechanism is the xanthine oxidase pathway (Figure 1)\textsuperscript{9-10}. First, some form of mechanical stress at the TMJ, such as tight clenching or prolonged maximal mouth opening during a dental procedure, increases intra-articular pressure\textsuperscript{9}. Once joint pressure exceeds 40 mmHg, peripheral arteriolar blood pressure is overpowered, resulting in transient hypoxia of TMJ tissues\textsuperscript{9}. Hypoxia induces a metabolic shift in affected tissues that produces ROS following TMJ relaxation and tissue reperfusion\textsuperscript{14-15}. This shift is mediated by xanthine oxidase, which generates O$_2^-$ during reoxygenation by reacting in the presence of oxygen with hypoxanthine that accumulates in the hypoxic state\textsuperscript{8, 14-18}.

Mitochondria

Mitochondria represent another source of ROS\textsuperscript{10}. Under physiological conditions, mitochondria release O$_2^-$; this release is exacerbated under hypoxic conditions due to mitochondrial inhibition, as follows and shown in Figure 1\textsuperscript{10-11}. Generally, mitochondria attempt to pass off electrons through the mitochondrial respiratory chain to synthesize water\textsuperscript{10, 12}. Oxygen depletion stymies this pathway, causing electron leakage\textsuperscript{12}. This leakage engenders O$_2^-$, which leads to the oxidative decarboxylation of $\alpha$-Ketoglutarate and the subsequent inhibition of its cofactor, prolyl hydroxylase domain-2 (PHD2)\textsuperscript{11}. The role of PHD2 is to hydroxylate hypoxia-inducible factor 1$\alpha$ (HIF-1$\alpha$) in order to target it for proteasomal degradation. Therefore its inhibition causes HIF-1$\alpha$ accumulation\textsuperscript{10, 11, 19}. Amassed HIF-1$\alpha$ acts as a subunit to complete the HIF-1 transcription factor\textsuperscript{10, 11, 19}. Increased HIF-1 leads to certain detrimental effects implicated in TMD, as discussed in ‘Cartilage degradation and bone resorption’.

Arachidonic Acid Catabolism: Prostaglandins & Leukotrienes

Prostaglandins (PGs) are first synthesized from arachidonic acid (AA) by most human cells\textsuperscript{20}. In the context of TMD, PGs increase ROS synthesis\textsuperscript{21}. Mechanical stress, cytokines, and growth factors can release AA from the phospholipid membrane\textsuperscript{20}. ROS stimulate cytokine production, causing upregulation of PG synthesis\textsuperscript{22}. PG synthesis occurs via the enzyme cyclooxygenase-2 (COX-2), which produces ROS as a byproduct (Figure 1). This results in greater levels of ROS in the joint\textsuperscript{22}.

Once released, AA is metabolized by COX-1 and COX-2 to form PGH2\textsuperscript{20}. PGH2 is subsequently converted into PGE2, a process known to generate ROS as a byproduct\textsuperscript{23}. In fact, Kerins et al. demonstrated a reduction in orofacial pain in a rat TMD model following COX-2 inhibition by measuring
mealtimes (indicating more pain) than treated rats, suggesting COX-2 is integral to TMJ inflammation and pain. Further studies showed COX-2 inhibition downregulates NADPH activity, thus reducing ROS levels. This is potentially because AA can synthesize ROS through NADPH independent of COX-1 and COX-2. As an alternative to conversion into PGs, Coccolo et al. demonstrated that AA produces ROS by interfering with the mitochondrial electron transport chain, as AA addition to resiping cell increased hydrogen peroxide ($H_2O_2$) levels compared to basal conditions.

Leukotrienes (LTs) may also stimulate inflammation and subsequent ROS production in the TMJ. LT synthesis begins when 5-lipoxygenase-activating protein translocates AA to 5-lipoxygenase, which converts the AA to LTA4. LTA4 then undergoes one of several conversion pathways, the most pertinent of which forms LTB4. LTB4 produces ROS through a rac-dependent pathway which mediates AA-induced ROS generation through a PLA2 linked cascade. Woo et al. explored LTB4 as a crucial mediator in tumour necrosis factor-α (TNF-α) induced ROS generation via NADPH. Results revealed dose-dependent ROS upregulation by LTB4, the effects of which were inhibited by an LTB4 receptor (BLT) antagonist. Furthermore, blocking of the specific G-protein coupled to BLT receptors decreased ROS production in a dose-dependent manner. Hence, these studies thus delineate the vital role of PGs and LTs on the production of ROS through arachidonic acid catabolism.

Once ROS accumulate via the aforementioned pathways, including the xanthine oxidase, mitochondria pathway, and arachidonic acid pathways, they damage collagen and the lubricative layer by reacting with key molecules in the joint and compromising their functional integrity through processes which will forthwith be explored.

Cartilage degradation and bone resorption

The TMJ is composed of bone, a fibrous articular disc, and fibrocartilage (Figure 1), which contains fibroblasts, chondrocytes, and type I and II collagen. Cartilage plays an essential role in TMJ function by reducing joint loading and providing a surface for the articulation of the disc, and fibrocartilage (Figure 1). As previously described in this article, mechanical stress can produce ROS in the TMJ. Regarding condylar bone resorption, Tanaka et al. observed increased VEGF immunopositive chondrocytes following mechanical stress in rat TMJs. Furthermore, the area subjacent to the deep hypertrophic cartilage demonstrated signs of significant increase in osteoclast presence. VEGF chemo-attracts osteoclasts; inhibition of VEGF function via antagonist chimeric proteins or anti-VEGF antibodies impairs osteoclast invasion. Thus, the stimulation, differentiation, and migration of osteoclasts into cartilage are all induced by VEGF production. This may result in cartilage destruction through vascular invasion, which converts cartilage into bone. Therefore, the production of ROS that leads to VEGF accumulation causes physiological degradation of the TMJ.

TMJ lubrication, hyaluronic acid, and oxidative stress

The lubricative layer expedites the articulation of the various bony components of the TMJ and is thus essential to normal function. Surface-active phospholipids (SAPLs) are the central molecules responsible for this phenomenon. Their polar heads bind the articular surfaces while their dual hydrophobic moieties extend into the joint space forming hydrogen bonds. The resulting hydrophobic interface enables low friction coefficients even under high loads. Hills observed that washing away SAPLs and lamellar body depositions caused a 150% friction increase, confirming the importance of SAPLs in TMJ lubrication.

Hyaluronic acid (HA) is widely accepted to be protective in joint lubrication. HA was initially presumed to directly lubricate the TMJ, but this hypothesis was overturned when hyaluronidases were observed to negligibly affect joint lubrication. It was later established that polymeric HA fulfills a protective, body-guarding function for SAPLs through
dose-dependent inhibition of phospholipase A2 (PLA2)1,49. PLA2 degrades SAPLs and is constitutively secreted into the TMJ synovial fluid, making HA’s bodyguarding function critical1,49.

Several nuances can be applied to this general understanding of HA function. Firstly, HA can be subdivided into high-molecular weight (HMW) and low-molecular weight (LMW) varieties which are produced by three separate HA synthases (HAS)52-67. HAS-1 and HAS-2 polymerize HMW-HA at ~2000 kilodaltons (kDa) and are upregulated by pro-inflammatory cytokines and transforming growth factor β-1 (TGFβ-1)56,57. HAS-3 polymerizes LMW-HA at only 200 kDa and is upregulated by pro-inflammatory cytokines but downregulated by TGFβ-153-57. HMW-HA scavenges ROS at the expense of molecular integrity more effectively and broadly than LMW-HA58. HMW-HA also inhibits PLA2 activity, whereas LMW-HA exacerbates it53,58-60. Wang et al. examined the effects of HMW-HA on the gene expression of 16 osteoarthritis-associated factors in fibroblast-like synoviocytes (FLS) from human osteoarthritis knees61. They found HMW-HA significantly downregulates interleukin-8, inducible nitric-oxide synthase, aggrecanase-2 and TNF-α mRNA61. Down-regulatory effects against all 16 tested factors were observed, yet insufficient sample size (15) hindered the significance of these results61. Wang et al. further demonstrated that inhibition of CD44 (HA’s major receptor) with monoclonal antibodies impeded these down-regulatory effects, invoking CD44 as an intermediate in HMW-HA’s gene regulatory effects (Figure 1)61.

HA filaments aggregate non-covalently with the proteoglycans aggrecan and lubricin, this interaction is facilitated by the glycoprotein link protein (LP)1,46,48,60,62-63. Aggrecan is a macromolecule (~1-4MDa) which contributes to joint loading capacity, binding both HA and LP63. Lubricin acts as a water-soluble carrier of SAPLs to enable their deposition on articular surfaces1,46. Finally, LP associates with HA and both proteoglycans through separate binding domains to facilitate aggregation60,63.

ROS interrupt this tightly-knit system in diverse ways1,3,58,60,63,64. For example, Roberts et al. found OH- mediates LP peptide bond cleavage60. They postulate OH- radicals are produced from H2O2 by a Fenton reaction localized at a free iron ion chelated by histidine residues within LP itself60. This hypothesis represents a useful microcosm illustrating how free transition metal ions (like iron) can exacerbate the negative effects of ROS. Importantly, HA suffers limited cleavage by H2O2, but more vigorous depolymerisation via OH- attack58,60,63. These destructive effects compromise protein integrity and cumulatively abolish all aforementioned intermolecular associations (save for that between HA and LP), thereby exposing the SAPLs to PLA2-mediated lysis1,3,49. Loss of SAPLs results in a thinner lubricative layer, increasing joint friction63.

Increased friction can stress joint tendons, culminating in progressive anterior disc displacement1,3,60,63. Cumulatively, these studies demonstrate a significant protective, anti-inflammatory role for HMW-HA in the TMJ. This is supported by in-vivo animal studies, such as Lemos et al.’s study of osteoarthritic rat RMJs that found a decrease in adverse morphological changes and MMP activity following HMW-HA administration65. Manfredini et al. reviewed HMW-HA injection in humans and found a consistent and lasting reduction in pain66. Despite these promising results and a clear biochemical basis, HMW-HA therapy must be thoroughly compared to corticosteroids in future trials to elucidate the superior treatment66.

Conclusion

ROS are central to the pathogenesis of TMD, yet very few articles holistically discuss the effects of oxidativeative stress on the TMJ1,3,18. To our knowledge, no review article has aimed to create a single picture broadly summarizing all molecular pathways involved in TMD-pathogenesis. Following a comprehensive review of the literature, we found that mechanical stress-induced hypoxia-reperfusion disrupts the respiratory chain reaction of mitochondria, contributing to ROS generation10,12. AA also plays a critical role in ROS production through the metabolism of AA into PGE2 by COX enzymes20-21,23. Furthermore, AA is an important leukotriene precursor30. Leukotrienes such as LTB4 are crucial mediators in TNF-α-induced, AA-induced, and rac-dependent ROS generation pathways3,55. The upregulated ROS production can increase HIF-1 and VEGF. VEGF’s subsequent upregulation of vascular invasion, and MMP and osteoclast activity can degrade the cartilaginous components of the TMJ, thus compromising its structural integrity. As ROS accumulate in the TMJ they degrade HA, exposing SAPLs to PLA2-mediated lysis. This thins the lubricative layer, increasing joint friction and thereby engendering anterior disc displacement1,3,60,62-64. Based on information presented in this review it is not surprising that HMW-HA has shown therapeutic potential in both animals and humans65-66. Indeed, this review supports a central role for ROS in TMD pathogenesis as observed in Figure 1, revealing its importance as a target for TMD treatments.

References:

61. Wang, C. T., Lin, Y. T., Chiang, B. L., Lin, Y. H., Hou, S. M. High molecular weight hyaluronic acid down-regulates the gene expression of osteoarthritis-associated cytokines and enzymes in fibroblast-like synoviocytes from patients with early...