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AN OVERVIEW OF POTENTIAL ANTIOXIDANT MECHANISM OF ACTION FOR METRONIDAZOLE

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Abstract:
Metronidazole has been used topically and systemically for over 50 years, however research on its antioxidant capabilities is still insufficient, ambiguous, and conflicting. Because its antioxidant qualities are mostly hypothesized based on in vivo data, research to test if metronidazole has antioxidant activity in vitro have been conducted. Narrative review conducted through the electronic databases, for all relevant articles published up to the beginning of 2022. Metronidazole has limited iron-reducing and hydrogen donor action under the conditions employed. The ability to scavenge hydroxyl radicals cannot be established. In the H2O2/.OH-microperoxidase-luminol system, it serves as a pro-oxidant, yet it can reduce induced lipid peroxidation.

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INTRODUCTION:

Metronidazole (MTZ, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is a bactericidal drug that is administered systemically and topically to various protozoa (Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis) against a wide variety of gram-negative (Bacteroides and Fusobacterium spp.) and gram-positive anaerobic bacteria (Peptostreptococcus and Clostridia spp.). It is beneficial in Helicobacter pylori infections, Crohn's disease (CD), postoperative wound infection prevention, and external application, such as rosacea and acne skin [1,2]. Since its release in 1959, clinical use has progressively increased to the point where it is currently one of the most commonly used pharmaceuticals, and it is on the World Health Organization's core list of medicines [3,4]. Metronidazole absorption, use, and metabolism differ depending on its application. Under aerobic conditions, it decomposes into two primary metabolites in humans: 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 1-acetic acid-2-methyl-5-nitroimidazole [5].

Metronidazole is an anaerobic organism inhibitor that transforms to acetamide and N-(2-hydroxyethyl) oxamic acid. Its nitro group is converted to hydroxylamine within the cell. numerous enzymes are involved in the reduction to create active metronidazole metabolites, and numerous illnesses, such as protein disorder, DNA damage, and oxidative stress leading to cell death, have been seen in sensitive Giardia [6,7]. Metronidazole and its hydroxy metabolite are both mutagenic to bacteria due to base-pair substitutions, and they are all effective against Trichomoniasis [8]. Aside from the use of metronidazole, no decreased metabolites have been discovered in human plasma, and no DNA damage has been observed. Although metronidazole is not thought to be carcinogenic, several studies have revealed that it may raise the risk of lung cancer [9,10].

In vivo research suggests that it has antioxidant and free radical scavenging capabilities. MTZ's putative antioxidant activity is mediated via the inactivation of neutrophil-released inflammatory mediators (e.g., reactive oxygen species, IL-8) and the lowering of oxidative stress [10]. It reduces the generation of hydrogen peroxide and hydroxyl radicals in a dose-dependent manner in the presence of neutrophils but has no impact in the absence of neutrophils [11]. When administered as an antibiotic, periodontitis has shown a reduction in oxidative stress. Metronidazole reduces damaged proteins in the colon, which helps with inflammatory bowel disease (IBD) [12]. Metronidazole, alone or in combination with ciprofloxacin, has a moderately good effect in the treatment of Crohn's disease (CD) [13]. Because recent evidence suggests that microbiota in the stomach may play a role in the etiology of CD and colitis ulcerosa (CU), the use of metronidazole in perianal fistula may be beneficial. It considerably reduced the amounts of malondialdehyde (MDA) in burn sufferers' blood [13].

DISCUSSION:

The pathomechanisms of many diseases, including oral problems, are influenced by oxidative stress. Periodontitis, osteitis, and oral cancer may be exacerbated by an oxidoreductive state imbalance. Furthermore, several xenobiotics' harmful effects, including medications like metronidazole (MTZ), may be linked to these illnesses [14]. Metronidazole is a chemotherapy that comes in a variety of forms and is used both locally (as a gel, ointment, or liquid) and systemically (as pills or infusion fluids of varying strengths). It has mostly been used to treat protozoan and anaerobic infections of numerous organs, including the oral cavity. The extensive use of MTZ in dentistry, periodontal and endodontic treatment, and dental surgery has brought the attention of researchers to the detrimental consequences found after its application in the oral cavity [15]. Oxidative stress is defined as an increase in the number of reactive oxygen species (ROS) produced by their excessive production or impairment of the body's defense mechanisms. It causes several molecular alterations in the physiology of cells and tissues, resulting in changes in the shape and size of biological macromolecules [16].

The antioxidant barrier is a defense system created by living organisms against the damaging action of ROS. They are made up of enzymatic and nonenzymatic antioxidants, both of which are found in saliva. As a result, saliva plays a crucial role in the fight against oxidative stress [17].

The salivary peroxidase system, which includes peroxidase secreted by the salivary glands and myeloperoxidase released from neutrophilic granules, accounts for the majority of the salivary antioxidant potential [18].

Salivary peroxidase prevents hydrogen peroxide (H_2O_2) from accumulating in the oral cavity and catalyzes the oxidation of thiocyanates, bromides, and iodides. The anions and acids generated in this process (OSCN, HOSCN) inhibit bacterial, viral, and yeast

cells. Myeloperoxidase catalyzes the oxidation of chlorides, resulting in hypochlorous acid, a potent germicidal agent [19]. However, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in saliva are moderate in comparison to their activities in blood [20].

Researchers have recently focused on the role of oxidative stress in the pathomechanisms of a variety of disorders, including those affecting the mouth and masticatory system. Numerous studies have proven that oxidoreductive imbalances are the root cause of periodontal disease, bone problems, and oral cancer [21]. Furthermore, the hazardous action mechanisms of many xenobiotics, including medications like metronidazole, are linked to these illnesses. The mutagenic potential of this medicine has been proven in experimental research, which may be connected to the reduction in the nitro group of MTZ and the generation of reactive oxygen species [22]. Numerous investigations have focused on metronidazole's mutagenesis potential, which their authors attribute to a reduction in the nitro group and the formation of reactive oxygen forms [21,22].

Previously, the authors of this work used a similar research model on rats' salivary glands and alterations in oxidative stress potential. However, the study was concerned about the effect of increased zinc supply on oxidative damage of the sublingual gland in chronic cadmium exposure [22]. Cadmium exposure at 5 mg and 50 mg Cd/dm3 caused oxidative stress in the rats' sublingual glands. It decreased TAS and GSH levels while increasing LPO and TOS. In the presence of cadmium, increasing the supply of zinc by 79% or 151% over the typical dietary intake of this microelement totally avoided the reduction of TAS and GSH levels, as well as the buildup of LPO, H2O2, and TOS in the investigated glands at both exposure levels. The results showed that higher zinc intake protected sublingual gland tissue from chronic cadmium exposure [23].

Metronidazole absorption occurs without the need of particular systems such as transporters and is dependent on metabolic activity to keep the membrane charged. As such, it is a prodrug that is poorly, if at all, reactive. However, by reducing the nitro group (Fig. 1), metronidazole is changed into a reactive intermediate that reacts with various targets in the cell. To this day, it is unclear which intermediate, as defined by the number of electrons transferred to the nitro group, is the real dangerous form. Several hypotheses were advanced, including the nitroradical anion stage (one electron transferred), the nitroso stage (two electrons transferred), and the hydroxylamine stage (four electrons transferred). Importantly, metronidazole has a relatively low midpoint redox potential (486 mV), which is significantly lower than the midpoint redox potentials of NADPH and NADH (about 320 mV each), resulting in only trace amounts of metronidazole being reduced in aerobes. Furthermore, in a redox cycling reaction, oxygen can re-oxidize the metronidazole nitroradical anion, resulting in the formation of superoxide anions and the re-established prodrug. However, intracellular oxygen concentrations are low in microaerophiles and anaerobes, and substances capable of reducing metronidazole and activating it to its lethal form are abundant. Several such parameters have been discovered in various microaerophilic or anaerobic organisms over the last three to four decades. The first enzyme proposed to be involved in metronidazole reduction was pyruvate:ferredoxin oxidoreductase (PFOR), which transfers electrons from pyruvate to the electron transporter protein ferredoxin, which also contains iron-sulphur clusters. Ferredoxin, on the other hand, has a very low midpoint redox potential (430 mV) and can transfer electrons to the metronidazole nitro group, resulting in metronidazole nitroradical anions that can be easily detected by electron paramagnetic resonance spectroscopy [24,25]. With the possible exception of bifidobacteria, the PFOR pathway is found in almost all anaerobes susceptible to metronidazole, making it an obvious choice for metronidazole activation in live organisms. However, it was discovered at the same time that rat liver microsomes or specific flavin enzymes, such as xanthine oxidase, can decrease metronidazole under anaerobic circumstances. Indeed, multiple flavin enzymes implicated in metronidazole reduction have been identified in microaerophiles and anaerobes, including thioredoxin reductase (TrxR) in T. vaginalis, E. histolytica, and G. lamblia and nitroreductase RdxA in H. pylori. Many investigations have been carried out in order to determine the primary activation routes in anaerobic and microaerophilic pathogens. Surprisingly, downregulation or deactivation of PFOR in T. vaginalis, Tritrichomoas fetus, or B. fragilis showed only a minor, if any, effect on metronidazole susceptibility [26]. However, when PFOR was downregulated in G. lamblia, it had a significant detrimental influence on metronidazole susceptibility. In turn, TrxR overexpression made G. lamblia more sensitive to metronidazole. However, thus far, it has been unable to attribute metronidazole decrease to a single enzymatic route. Even non-enzymatic reduction of metronidazole by cysteine and ferrous iron under anaerobic circumstances has been described. As a result, it is safe to conclude that metronidazole

reduction in microaerophiles and anaerobes is accomplished by a variety of mechanisms, some of which may be non-enzymatic. Most organisms are less likely to develop metronidazole resistance as a result of this scenario. The sole possible exception is RdxA in H. pylori, which has been identified as the primary metronidazole activating enzyme in multiple independent studies [27].

Trecy and Webster observed hydroxyl radical scavenging ability when neutrophils were present in the system, but no radical scavenging action when neutrophils were not present. There was no dose-dependence or antioxidant effects found in an in vivo trial without neutrophils [28]. For 30 hours, Andrioli et al. investigated the toxicity of metronidazole on the roots of Allium cepa. They discovered that metronidazole-induced oxidative stress resulted in a considerable increase in antioxidant defense parameters [29]. Metronidazole activated hydrogen peroxide, superoxide, and nitrogen-free radicals when used to treat Trichomoniasis. These findings are consistent with our findings that metronidazole has a prooxidant activity. Metronidazole metabolites, on the other hand, are prooxidant in vivo, because metronidazole enters microorganisms to generate hazardous intermediates, free radicals (e.g. MTZ-NO2-, MTZ-NO2H, MTZ-NO, ONOO, MTZ-NHOH), which induce DNA damage and trigger oxidative stress [30]. NADH oxidase, alcohol dehydrogenase, thioredoxin peroxidase, nitroreductase 1, nitroreductase 2, ferredoxin, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidoreductase are all necessary for the synthesis of active metronidazole metabolites. Following activation, MTZ-NO can react with superoxide to create more radical peroxynitrite (ONOO), which is then transformed to nitrate [29,30]. In wastewater treatment, metronidazole is degraded by an H2O2-photocatalytic reaction in the presence of zinc. The organic molecules are decomposed by hydroxyl radicals generated in the aqueous media. The procedure conditions are identical to those used to measure hydroxyl radical scavenging capabilities, except that in our method, iron microperoxidase serves as the catalyst. As a result, in these conditions, metronidazole does not scavenge hydroxyl radicals but instead oxidizes to form free radicals before decomposing. Metronidazole is destroyed by NO2 release during high-energy photocatalysis, whereas in vivo at low energy exposure it dissociates by NOOH release to generate multiple intermediates [31].

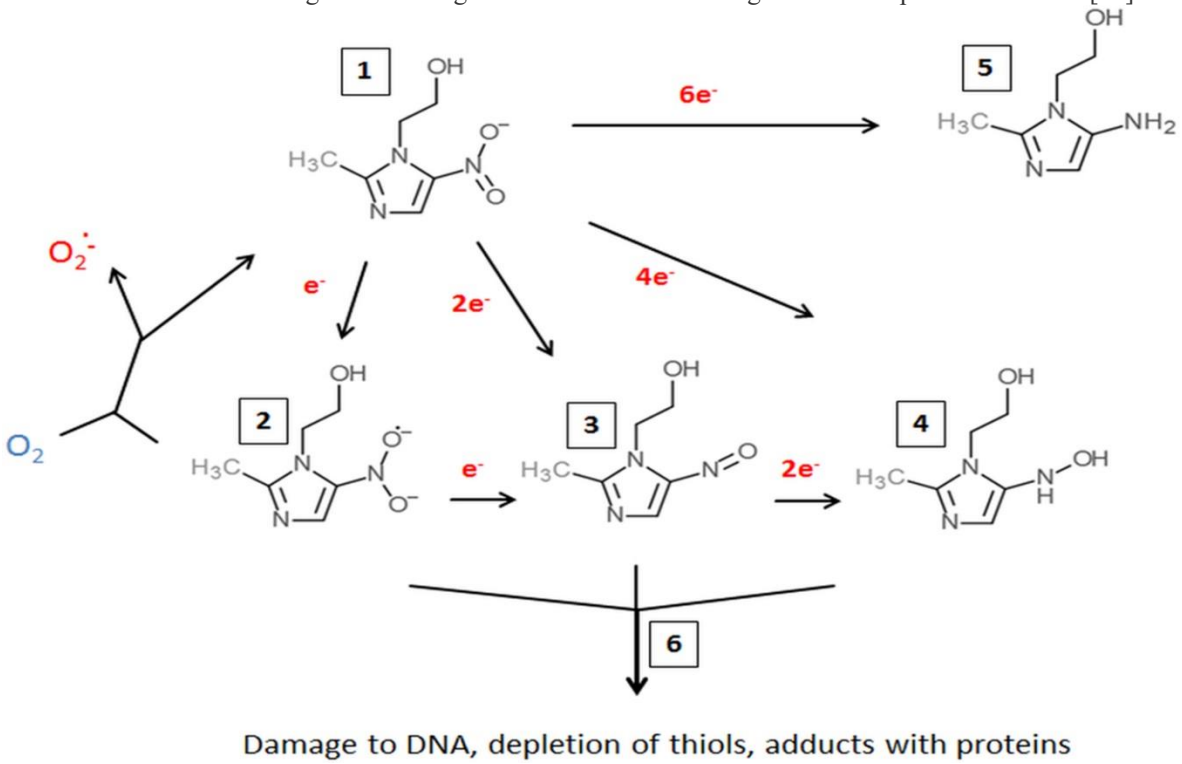

Fig. 1. Metronidazole reduction and toxicity in microaerophiles and anaerobes. Metronidazole enters the cell (**1**). Depending on the number of electrons transferred to the nitro group, a nitroimidazole radical anion (**2**), a nitrosoimidazole (**3**) or a hydroxylaminimidazole (**4**) is formed. Reduction can be either sequential, (**2→3→4**) or catalysed in one step.

The conclusion of lipid peroxidation inhibition is consistent with the findings of Narayanan et al. [32] in a simple skin lipid model, where MDA concentrations were reduced by 25, 36, and 49% in the presence of 10, 100, and 500 g metronidazole/ml, respectively. Based on previous research, they concluded that metronidazole's antioxidant effect can be mediated through two mechanisms: reducing free radical production via neutrophil activity and reducing free radical concentration via its free radical scavenging property [32]. In vitro free radical scavenging activity was not confirmed in our experiments. However, we discovered that lipid peroxidation was moderately inhibited. As a result, issues arise regarding why the results are so inconsistent, why it suppresses lipid peroxidation, and why it lacks radical scavenging properties. Rodrigez and Caseli recently showed that metronidazole interacts with phospholipids, and the interaction varies depending on the chemical composition of the lipid; additionally, metronidazole interacts with the surface of DNA [33].

Photoaging (extrinsic aging) was once thought to be an accelerated form of chronologic (intrinsic) aging, but it is now recognized as statistically and qualitatively distinct. ROS caused by UV light—both oxygen free radicals and products of skin lipid peroxidation—are thought to contribute to photoaging skin damage. ROS have also been linked to intrinsic skin aging [34, 35]. In experimental tests, the antioxidant vitamins E and C applied topically reduced UV-induced skin damage. Photoaging has been described as a significant backdrop to rosacea, emphasizing its clinical characteristics. Rosacea does appear to be linked to solar elastosis and maybe heliodermatosis. Both types of aging have been linked to capillary damage in stage II rosacea [35].

Protein and lipid oxidative degradation can both be harmful. Because protein carbonyls are frequently catalysts, they may be the most direct vehicle for imparting oxidative damage on cells [23,35]. Because the creation of protein-bound carbonyl groups appears to be a widespread process during protein oxidation, as well as the early development and relative stability of oxidized protein, the use of protein carbonyl groups as a marker may offer some advantages over lipid peroxidation products. Furthermore, cells breakdown oxidized proteins in hours and days, whereas lipid peroxidation products are detoxified in far shorter durations [35]. Protein carbonyls' chemical stability makes them attractive targets for laboratory measurement and also beneficial for storage: their storage stability has been established for 3 months at 80°C. GSH levels, which are critical in cellular antioxidant defense, were similar throughout the bowel [33,35].

In their study, Kohanski et al. [36] discovered that aminoglycoside antibiotics cause the generation of hydroxyl radicals. Furthermore, literature data indicate that ROS produced during antibiotic use play an important part in their bactericidal activity, however they also show a mutagenic effect not just in relation to bacterial cells [36]. According to Vatansever et al. [37], free radicals are an appealing weapon against infections. Strategies for employing ROS in antimicrobial therapy are promise, but more research is needed before they may be used as an effective therapeutic in the future. Cornejo-Garcia et al. [38], on the other hand, evaluated the oxidoreductive potential of the blood in individuals who had an adverse reaction to medications such as amoxicillin, cefaclor, and metamizole. The scientists discovered that in the research group with immune response symptoms, SOD activity increased whereas GPx decreased. Furthermore, carbonyl protein concentrations and lipid peroxidation were shown to rise.

As previously stated, metronidazole's toxicity is associated with its metabolism in the human body to two main metabolites: 2-methyl-5-nitroimidazole-1 acetic acid (AAM) and 1-(2-hydroxyethyl)-2-hydroxy-5-nitroimidazole (HM). The HM metabolite has been demonstrated to be more genotoxic than the parent drug [37]. This could be related to the nucleophilic substitution process of these metabolites, which occurs when the imidazole ring in DNA is disrupted, or to the reduction of the MTZ nitro group and the generation of ROS [38]. According to the literature, DNA damage occurs as a direct result of ROS, resulting in single or double DNA strand breaks and nitrogenous base change. Considering MTZ's ability to cause ROS and DNA damage, we chose to explore its influence on the oxidoreductive state of the rat salivary glands [37].

Pélissier et al. [39] studied the effects of metronidazole on the tissues of the small and large intestines, as well as the liver, in patients with inflammatory bowel disease (IBD). The condition could be caused by both antibiotic medication and oxidative stress. As a result, the authors measured the concentrations of oxidative damage markers of proteins (carbonyl proteins (PC)) and lipids (MDA), as well as glutathione as a nonenzymatic antioxidant, in metronidazole-treated rats' intestinal tissues and liver. They discovered that taking this medicine at a dose of 80 mg/kg b.w. for one week reduced PC levels in the large intestine by 31% when compared to the control group. The level of GSH

in the colon also reduced, indicating an antioxidant balance disturbance. However, metronidazole had no effect on PC or GSH levels in the small intestine or liver. According to the scientists, GSH is present in large concentrations in most tissues, and diet is the primary driver of its homeostasis; thus, decreased levels of this antioxidant thiol were detected in IBD patients but not in healthy people.

Spolidorio et al. [40] investigated SOD, GPx, and CAT activities, as well as lipid peroxidation, in the rat submandibular and parotid glands after treatment with calcineurin inhibitors such as cyclosporin (10 mg/kg mc) and tacrolimus (1 mg/kg mcg). Long-term, 60-day administration of immunosuppressive medications resulted in a decrease in SOD, CAT, and GPx activity in both salivary glands and an increase in LPO, indicating that these drugs induce oxidative stress. Similarly, Kdzierska et al. [41] found that immunosuppressants have an effect on the development of oxidative stress in rats.

The assessment of total oxidant potential (TOS) and total antioxidant potential (TAS) in various bodily tissues, including salivary glands, is one of the available approaches for assessing the oxidant and antioxidant balance. TOS measures the intensity of stress in the body and the total amount of peroxides in all cellular macromolecules, whereas TAS measures antioxidants' ability to eliminate free radicals and prevent their creation [41].

CONCLUSION:

Metronidazole's general antioxidant capabilities were not established since, even at high doses, it displayed only minor iron-reducing and hydrogen-donor action. Its ability to scavenge hydroxyl radicals in the H2O2/.OH-microperoxidase-luminol system was also not established since the molecule acted as a prooxidant. Based on in vitro experiments, it was able to reduce induced lipid peroxidation. Despite its lengthy history of use, metronidazole has remained a trustworthy medicine for the treatment of most anaerobic/microaerophilic infections, distinguishing it from most other antimicrobials that acquire resistance considerably more quickly. This is certainly due to its pleiotropic mechanism of action, which targets a vast number of molecules in the cell rather than just a few or even just one, as most antimicrobials do. In truth, metronidazole's mode of action is deceptively simple: it penetrates the cell without the assistance of any transportation machinery and unleashes its deadly power after being reduced to its nitro group, a reaction that occurs only at extremely low oxygen concentrations. Nonetheless, metronidazole resistance occurs more frequently in some pathogens than others, and despite its overall tolerance, metronidazole can induce unpleasant side effects. Furthermore, metronidazole and other 5-nitroimidazoles are still being investigated as potentially carcinogenic. The current study summarizes the most relevant components of metronidazole and provides a thorough discussion of resistance and safety concerns.

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