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Review

Chlorine dioxide and chlorite as treatments for diabetic foot ulcers

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The global prevalence of diabetes mellitus is rising and is predicted to exceed 10% by 2030. Foot ulcers are a frequent complication of diabetes mellitus. Existing treatments for diabetic foot ulcers are only partially effective and when these ulcers do not heal, amputation of the affected limb may result. It is estimated that an amputation due to diabetic foot infection occurs every 30 seconds somewhere in the world. Thus, more effective treatments are needed. One possible treatment is chlorine dioxide, a chemical compound that shows great promise as a treatment for diabetic foot ulcers. A review of the literature finds multiple mechanisms by which chlorine dioxide and the related compound chlorite may assist in the treatment of diabetic foot ulcers. These include reducing hyperglycemia, decreasing oxidative stress, improving vasculopathy, slowing the progression of neuropathy, decreasing inflammation, treating infection and improving wound healing. Chlorine dioxide and chlorite are found to be safe and effective when used in low doses. Additional research into the potential benefits of chlorine dioxide and chlorite as treatments for diabetic foot ulcers is recommended.

Key words: Diabetes mellitus, oxidative stress, vasculopathy, neuropathy, infection.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic and inflammatory disease that affects millions of people worldwide. DM is characterized by hyperglycemia, which triggers metabolic signaling pathways responsible for diabetic complications (Volpe et al., 2018). One frequent complication is foot infections (Fard et al., 007).

Diabetic foot ulcers (DFUs) are defined as foot lesions that may affect the skin, soft tissue and bone in lower limbs causing an aggravating infection in diabetic patients (Ramirez-Acuña et al., 2019). Factors contributing to the development of DFUs include hyperglycemia, vasculopathy, peripheral neuropathy, infection, chronic

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inflammation and impaired wound healing (Davis et al., 2018; Marston, 2006; Ramirez-Acuña et al., 2019; Singh et al., 2005).

DFUs are an increasingly severe global public health problem due to the growing number of people who are developing DM (Yingsakmongkol et al., 2011). The estimated global prevalence of DM was 9.3% (463 million people) in 2019. By 2030 cases are expected to rise to 10.2% (578 million) and by 2045 to 10.9% (700 million) (Saeedi et al., 2019).

The prevalence of foot ulcers among individuals with DM is 4 to 10% and the lifetime risk of developing an infection is 25% (Singh et al., 2005; Richard and Schuldiner, 2008). Foot complications are the single most common cause of morbidity among individuals with diabetes and the most common reason for hospitalization (Kruse and Edelman, 2006; Lim et al., 2017). Many individuals with DFUs undergo lower extremity amputation, which leads to a very poor quality of life and a 5-year mortality rate similar to or worse than colon cancer, breast cancer and prostate cancer (Armstrong et al., 2007).

The Infectious Diseases Society of America (IDSA) recommendations for treatment of DFUs include antibiotic therapy for infection, proper dressing and off-loading of pressure for the wound, and revascularization if ischemia is present (Lipsky et al., 2012). Lim et al. (2017) also recommend reducing blood glucose levels. While helpful, these strategies are only partially effective and non-healing DFUs may result in amputation of the affected limb. Limb amputation occurs 10 to 30 times more often in diabetic patients than in the general population and it is estimated that an amputation is performed every 30 seconds for an individual with DM somewhere in the world (Richard and Schuldiner, 2008; Singh et al., 2005). Mortality rates following amputation are 20% at 1 year, 41% at 3 years, and 63-73% at 5 years (Apelqvist et al., 1993; Jupiter et al., 2016), highlighting the importance of finding more effective treatments for DFUs.

Chlorine dioxide (Al-Bayaty et al., 2012; Mawas et al., 2022) and chlorite (Maraprygsavan et al., 2016; Yingsakmongkol, 2013) have been suggested to help with the treatment of diabetic wounds).

METHODS

This is a narrative review exploring the use of chlorine dioxide and chlorite as treatments for diabetic foot ulcers. A literature search looking for studies examining the pathophysiology or treatment of diabetic foot ulcers was conducted as well as the use of chlorine dioxide or chlorite as a treatment for diabetic foot ulcers or other types of wounds. Pubmed, Cochrane and Google Scholar databases were searched using the following terms: "chlorine dioxide," "chlorite," "WF10," "NP001" "diabetic foot ulcer," "diabetic foot infection," "wound healing," "safety," and "pathophysiology." These terms were

searched individually and in combination. In addition, reference sections of articles were reviewed for manuscripts that contained information relevant to our topic. We included in vitro and in vivo studies that involved both animals and humans. Information from these sources that was relevant to the aforementioned objectives was included in the narrative review.

PATHOPHYSIOLOGY OF DFUS

In individuals with DM, a number of factors predispose or contribute to the development of DFUs (Figure 1). In the context of hyperglycemia, oxidative stress contributes to vasculopathy, neuropathy and impaired immune functioning (Marston, 2006; Markuson et al., 2009). When a skin ulceration develops either as a result of peripheral neuropathy or a trauma-induced wound, microorganisms colonize and proliferate in the wound causing damage to tissue and triggering a host response accompanied by inflammation. This clinical infection can then spread to surrounding tissues (Lipsky et al., 2012).

Hyperglycemia, oxidative stress, and vasculopathy

Prolonged exposure to hyperglycemia is the primary causal factor in the pathogenesis of DFUs (Aronson and Rayfield, 2002). Hyperglycemia induces a number of changes in vascular tissue that combine to accelerate atherosclerosis, which is one of the primary pathophysiological mechanisms contributing to vasculopathy in individuals with DM (Aronson, 2008).

In vascular tissues, oxidative stress accelerates atherosclerosis. Oxidative stress can result from either the overproduction of reactive oxygen species (ROS) or a reduction in antioxidant defense mechanisms (Wei et al., 2009).

In diabetes, hyperglycemia triggers metabolic signaling pathways that increase oxidative stress (King and Loeken, 2004; Pang et al., 2020; Volpe et al., 2018). One of these pathways is *autoxidation* in which glucose enolizes, thereby reducing molecular oxygen and yielding superoxide anion (O^{2-}), the hydroxyl radical ($\cdot OH$), and hydrogen peroxide (H_2O_2) (Wolff and Dean, 1987; Nishikawa et al., 2000). A second pathway involves the production of advanced glycation end products (AGEs). AGEs are a family of compounds that are the products of nonenzymatic reactions between reducing sugars and proteins, lipids or nucleic acids (Prasad et al., 2019). AGEs bind to receptors for advanced glycation end products (RAGEs), triggering an overproduction of ROS (Goldin et al, 2006). A third pathway involves the hyperglycemia-induced increase in production of intracellular ROS via the mitochondrial electron transport chain (Nishikawa et al., 2000). A fourth pathway involves the increased creation of superoxide by activated monocytes. Following the extravasation of monocytes

into the vessel wall, a burst of superoxide occurs via activation of NADPH oxidase (NOX). This increase in superoxide contributes to the development of atherosclerosis by participating in oxidation of low-density lipoproteins (LDLs), which leads to the formation of foam cells, and the development of atherosclerosis (Cathcart, 2004). Hyperglycemia can also increase oxidative stress by decreasing glutathione scavenging activity and reducing NADPH content in vascular endothelial tissues (Kashiwagi et al., 1996).

Increased oxidative stress triggers the activation of several signaling pathways involved in the pathogenesis of DFUs. These include polyol pathway activation (Pang, 2020), increased production of AGEs and RAGEs, activation of the protein kinase C (PKC) system (Aronson and Rayfield, 2002), overactivity of the hexosamine pathway (Aronson, 2008), release of pro-inflammatory cytokines (Rask-Madsen and King, 2013; Thiruvoipati et al., 2015), and inactivation of antiatherosclerotic enzymes such as eNOS and prostacyclin synthase. Through these pathways, oxidative stress reduces nitric oxide levels, damages cellular proteins, promotes leukocyte adhesion to the vascular endothelium and increases inflammation (Sheetz and King, 2002). These changes promote the development of atherosclerosis which leads to peripheral artery disease (PAD), ischemia and ultimately the development of DFUs.

Inflammation and the immune system also play key roles in the development of atherosclerosis with both the innate and adaptive immune systems participating. Atherogenesis is characterized by the chronic accumulation of monocytes/macrophages that produce ROS and release proinflammatory cytokines within the arterial wall. Monocytes differentiate *in situ* into macrophages that ingest oxidized LDLs, thereby becoming foam cells that form a plaque, which leads to atherosclerosis (Li et al., 2017).

The adaptive immune response contributes to the development of atherosclerosis when lymphocytes transmigrate into the arterial wall where macrophages present the protein component of the LDL particle to T-lymphocytes, triggering the production of proinflammatory cytokines. Also B2 cells produce IgG antibodies, which promote atherosclerosis by participating in CD4 T-cell activation and stimulating effector T-cell proliferation (Li et al., 2017).

Neuropathy

The most common microvascular complication of DM is neuropathy. Approximately 50% of individuals with DM develop symptomatic peripheral neuropathy within 25 years of symptom onset, and neuropathy is responsible for 50% of DFUs (Vincent et al., 2004; Volmer-Thole and Lobmann, 2016). The majority of DFUs result from relatively minor trauma in the presence of sensory neuropathy, which impairs the sensation of pain. As a

result, injuries may not be noticed for weeks (McNeely et al., 1995; Volmer-Thole and Lobmann, 2016).

Oxidative stress plays a major role in the pathophysiology of diabetic neuropathy (Feldman, 2003). Oxidative stress causes abnormal axon morphology, altered neuronal membrane permeability, and modifications of various cellular proteins (Negi et al., 2011). Oxidative stress is also associated with the development of apoptosis in neurons and supporting glial cells (Vincent et al., 2004). Lowering blood sugar levels, which reduces oxidative stress, decreases the risk of neuropathy (Rask-Madsen and King, 2013).

Impaired immune functioning and increased inflammation

Hyperglycemia and oxidative stress also contribute to DFUs by impairing immune functioning and increasing inflammation. Serum glucose levels >150 mg/dl are associated with impaired immune system functioning (Syafril, 2018). Mechanisms by which hyperglycemia impairs immune functioning include impaired polymorphonuclear (PMN) granulocyte functions such as chemotaxis, migration, adherence, phagocytosis and intracellular killing, impaired monocyte and macrophage functions, and reduced number of CD4 T lymphocytes with a subsequent reduction in CD4/CD8 ratio. Hyperglycemia also triggers an increase in proinflammatory cytokines (McMahon and Bistrian, 1995).

Infection

In individuals with DM, elevated blood sugar levels are associated with an increased risk of infection. Factors contributing to this increased risk include tissue hypoxia and impaired host defenses (Pozilli and Leslie, 1994). The latter results from impaired interleukin-1 (IL-1) release from macrophages and compromised PMN function including decreased mobilization, impaired chemotaxis and reduced phagocytic activity (Butler et al., 2005). The presence of infection results in increased wound healing time (Marston, 2006).

WOUND HEALING AND ROS

Wound healing is generally conceptualized as occurring in four phases: Coagulation, inflammation, proliferation and maturation. ROS play an important role in each of these phases. With regard to DFUs, the mechanisms by which ROS influence healing have been well studied.

ROS are produced by all vascular cell types but the two main sources in the vasculature are the mitochondrial electron transport chain and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX). NOX
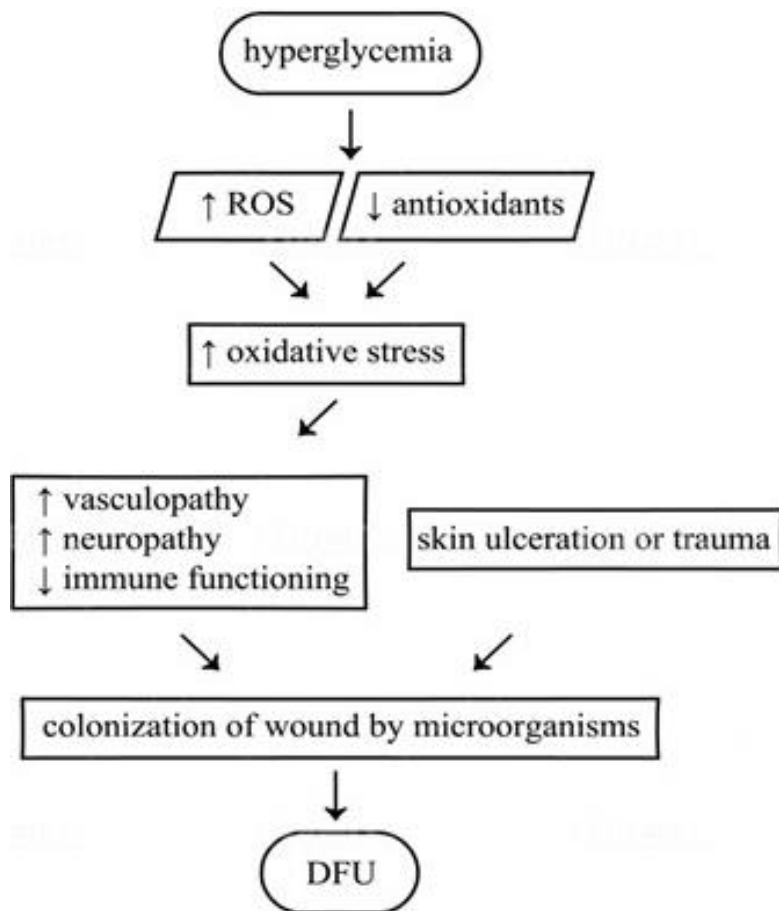
Figure 1. Pathophysiology of diabetic foot ulcers.
Source: Yosuf/fiverr

generates superoxide by transferring electrons from NADPH to oxygen. Mitochondria produce superoxide via the electron-transport chain (ETC) and superoxide is subsequently converted to hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD2) (Figure 2).

Reducing oxidative stress either by decreasing the overproduction of superoxide or increasing antioxidant activity has been suggested as a strategy to reduce diabetic complications. However, conventional antioxidants have not fared well in this regard. It has been suggested this could be due to the fact that these agents scavenge ROS in a stoichiometric manner, or alternatively because exogenous antioxidants may be inactivated before they reach the vasculature. Inducing endogenous antioxidant systems or using SOD or catalase mimetics has been suggested as potentially more effective than administering exogenous antioxidants (Brownlee, 2001; Wassmann et al., 2004).

In DM, elevated serum glucose levels increase mitochondrial production of superoxide (Brownlee, 2001). Increasing superoxide impairs wound healing whereas reducing superoxide accelerates wound healing (Luo et al., 2004). One strategy suggested to decrease superoxide levels is to enhance the conversion of superoxide to hydrogen peroxide by increasing the activity of superoxide dismutase (SOD) (Brownlee, 2001).

Hydrogen peroxide influences wound healing through multiple mechanisms. These include altered angiogenesis, neutrophil infiltration, fibroblast migration and myofibroblast activation (Fujiwara et al., 2013). Some of the effects of hydrogen peroxide on wound healing are concentration dependent. For example, lower concentrations (0.1-10 µM) promote angiogenesis whereas higher concentrations (>125 µM) inhibit angiogenesis and produce vascular endothelial cell injury (Huang and Zheng, 2006; Yasuda et al., 1998). Factors that affect hydrogen peroxide levels and thereby influence angiogenesis are listed in Table 1.

The stimulation of angiogenesis by hydrogen peroxide requires proangiogenic factors such as vascular endothelial growth factor (VEGF) and transforming growth factor-β-1 (TGF-β-1) (Kim and Byzofa, 2014). VEGF induces angiogenesis which restores tissue microcirculation and oxygen release, thereby improving the metabolic derangements of the wound environment (Detmar et al., 1977). TGF-β-1 influences numerous
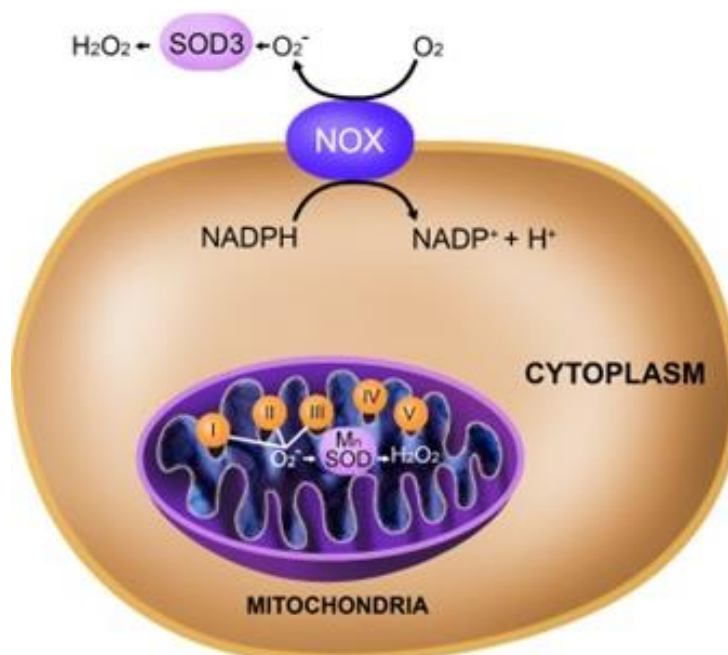
Figure 2. Generation of reactive oxygen species.
Source: Illustration by Huțanu Alexandru Adrian.

Table 1. Factors influencing angiogenesis.

Factor	Effect on H_2O_2	Effect on angiognesis
$H_2O_2 > 125$ µM	↑↑	↓
↓SOD	↓	↓
↓NOX	↓	↓
NOX inhibitors	↓	↓
N-acetylcysteine	↓	↓
↑↑SOD	↑↑	↓
$H_2O_2 = 0.1 – 10$ µM	↑	↑
↑SOD	↑	↑
↓myeloperoxidase conversion of H_2O_2 to HOCl	↑	↑

↑ = increase; ↑↑ = large increase; ↓ = decrease.
Source: Huang Zheng (2006), Yasuda et al. (1998) and Brownlee (2001).

cellular functions including proliferation, differentiation, motility, and apoptosis. This cytokine also regulates angiogenesis, enhances the repair of injured tissue, and promotes wound healing (Chin, 2004).

Based on the previous discussion regarding the pathophysiology of DFUs, an ideal agent to treat DFUs would be one that reduces serum glucose levels, decreasing superoxide while increasing hydrogen peroxide to levels that reduce oxidative stress, stimulates angiogenesis, reduces neuropathy, decreases inflammation, treats infection, and restores normal immune functioning. One agent that shows promise in this regard is chlorine dioxide.

CHLORINE DIOXIDE

History of chlorine dioxide

Chlorine dioxide (ClO_2) is a synthetic molecule first produced in 1802 by the Irish chemist Richard Chenevix who mixed sulfuric acid with potassium chlorate (Sidgwick, 1950). Nearly a decade later, Sir Humphrey Davy combined potassium chlorate with hydrochloric acid

and produced a gas he called "euchlorine" that contained a mixture of ClO_2 and chlorine (Davy, 1832).

During the second half of the 19th century, ClO_2 was utilized as a water treatment in Europe (Benarde et al., 1965). After studies in the 1940's and 1950's demonstrated ClO_2's broad spectrum biocidal efficiency is at least equal to, if not superior, to that of chlorine, the use of this agent expanded further. By the 1970's, more than 500 water treatment plants throughout the world were utilizing ClO_2 to purify water (Clarke and Berman, 1983).

Today, in addition to treating water, ClO_2 is employed as a pigment removing agent for wood pulp (Gall, 1978), flour (Fukayama et al., 1986) and textiles (Jonnalagadda and Nadupalli, 2014), a disinfectant in the food and beverage industry (Drechsler et al., 1990; Gómez-López et al., 2009), a sterilant in the medical field (Lowe et al., 2013), a treatment for halitosis (Frascella et al., 2000), and a room deodorizer (Ogata and Shibata, 2009). ClO_2 has also been employed to clean ancient documents (Gettens, 1952), inactivate biological warfare agents (Gordon and Rosenblatt, 2005), prolong the longevity of honeybees (Lackett et al., 1972), and as an over-the-counter treatment for viral, bacterial, and fungal infections (Frontier Pharmaceutical, Inc.). ClO_2 has also been suggested as a decontaminant for the SARS-CoV-2 virus that causes COVID-19 (Ogata and Miura, 2020) as well as a prevention and treatment for COVID-19 (Ogata and Miura 2021; Aparicio-Alonso et al., 2021). Based upon its safety and broad-spectrum efficacy, ClO_2 has been termed "the ideal biocide" (Simpson et al., 1993).

One of the more recent applications of ClO_2 is as a treatment for DFUs. This paper reviews the rationale for the use of ClO_2 as a treatment for DFUs.

Chemistry of chlorine dioxide

ClO_2 is a symmetric, triatomic molecule consisting of one chlorine and two oxygen atoms. At room temperature, ClO_2 is a yellowish gas that is highly soluble in water (PubChem, 2018).

ClO_2 is quickly broken down in high temperatures and is subject to photodecomposition, especially by ultraviolet light. In the air this molecule breaks down into chlorine gas and oxygen, whereas in water it is relatively stable. However, above a pH of 9 to 10 aqueous ClO_2 disproportionates to produce chlorite (ClO_2^-) and chlorate (ClO_3^-) ions (U.S. Department of Health and Human Services, 2004).

ClO_2 has an odd number of electrons with 19 valence electrons. A single electron occupies its highest molecular orbital, and this unpaired electron is shared among all three atoms. Most of the single electron density is located on one or the other oxygen atom. This unique arrangement provides ClO_2 a single unpaired electron and two reaction centers (oxygen and chlorine) upon which to react. The single unpaired electron renders the molecule a free radical, which helps explain its reactive nature (Flesch et al., 2006). ClO_2 is also a highly reactive oxidant, which means it can accept electrons via oxidation-reduction reactions.

ClO_2 undergoes a one electron transfer reaction forming chlorite (ClO_2^-) and thus reacts through oxidation rather than addition or substitution. ClO_2 reacts with a relatively narrow range of organic reactants including thiols, phenols, nitrogen heterocycles and aliphatic tertiary amines. ClO_2 readily reacts with the free amino acids cysteine, tyrosine and tryptophan while other amino acids react at a much slower rate. These features enable ClO_2 to undergo unique and somewhat selective oxidation reactions of organic molecules.

After accepting one electron, ClO_2 is converted to ClO_2^- (chlorite). This occurs following the oral ingestion of ClO_2. Chlorite exists in many present-day medications including tetrachlorodecaoxygen complex (TCDO), WF10, OXO-K993, Ryoxon®, and Oxoferin®. TCDO is a chlorite-oxygen reaction product that consists of chlorite (ClO_2^-) (4.25%), chloride (Cl^-) (1.9%), chlorate (ClO_3^-) (1.5%), sulfate (SO_4^{2-}) (0.7%), and sodium anions (Na^+) (Ennen et al., 1993). The chlorite ion, which has been demonstrated to be the active principle in these medicines, is metabolized in the body into non-toxic products (that is, chloride, oxygen and water) (Schempp et al., 2001).

TCDO was first synthesized by F. W. Kühne to treat venous ulcers without additional anti-infectious therapy (Hinz et al., 1984a; Tissot et al., 1990). Clinical studies found TCDO stimulates the formation of granulation tissue and promotes wound healing (Hinz et al., 1984b). Additional studies revealed TCDO enhances macrophage phagocytosis (Woerly et al., 1986), exhibits anti-inflammatory properties (Tissot et al., 1990), regulates cellular and humoral immunity (Gillissen et al., 1986), and triggers the release of O_2 in hemoglobin-containing solutions (Mueller-Klieser and Vaupel, 1987). The medicine WF10 (Immunokine) is an aqueous solution of TCDO used for intravenous administration. Chlorite is the active principle in this solution which contains 63 mmol/L of chlorite. The reaction of WF10 with hemoproteins has been suggested to be the central step in the activation of the drug (Schempp et al., 2001). Oxoferin was approved in 1983 in Germany for topical use to enhance healing of chronic wounds, such as diabetic foot ulcers (Hinz et al., 1986; Schempp et al., 2001). OXO-K993 was approved in Thailand as an intravenous infusion therapy for postradiation syndrome and for supportive care in patients undergoing treatment for cancer (Schempp et al., 2001). Nearly a quarter century ago, WF10 was found to reduce the hospitalization rate and mortality rate in individuals with AIDS (Raffanti et al., 1998).

Another medicine relevant to this topic is sodium chlorite ($NaClO_2$), also known as NP001. This molecule serves as both a substrate for the production of ClO_2 and

functions as a medicine currently being explored to treat neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) (Miller et al., 2014, 2015).

Although a number of methods are used to synthesize ClO_2, one of the most common involves mixing sodium chlorite ($NaClO_2$) with an acid such as phosphoric acid (H_3PO_4) or hydrochloric acid (HCl) (American Chemistry Council, 2023). $NaClO_2$ is a molecule that is chemically related to both table salt (NaCl) and bleach (NaClO).

Kinetics of chlorine dioxide and chlorite

Pharmacokinetic studies of chlorine dioxide and chlorite in humans are scarce. Most kinetic studies have been carried out in animal models, primarily in rats. A series of studies conducted in the 1970's and 1980's examined the kinetics of ClO_2 in rats and chickens using the radioactive tracer $^{36}ClO_2$. Investigators found that following oral administration of $^{36}ClO_2$ in rats, radioactivity was rapidly absorbed from the gastrointestinal tract. ClO_2 was then converted to chloride (Cl^-), chlorite (ClO_2^-), and chlorate (ClO_3^-). At 12- and 24-hours following administration of ClO_2, the ratio of ClO_2^- to Cl^- was 1:5. However, at 72 hours the ratio in urine and plasma changed to 1:4. ClO_3^- was detected only in the 12-24 urine. Cl^- reached a peak in plasma at ½ hour after the oral administration of $^{36}ClO_2$, whereas ClO_2^- reached a peak plasma level at 2 h. At 72 h post-administration, the distribution of ClO_2^- was highest in whole blood, packed RBCs, plasma, stomach, testes, skin, lung, kidney, duodenum, carcass, spleen, ileum, brain, bone marrow and liver. Regarding elimination, it was found that following oral administration of $^{36}ClO_2$, 75% of the recovered dose was found in the urine and 25% was found in the feces. When studying ClO_2^-, 87 and 13% of the administered dose were found in urine and feces, respectively. No Cl compounds were recovered from expired air. The elimination half-life was 43.9+2.3 h for ClO_2 and 35.2+3.0 h for ClO_2^- (Abdel-Rahman, 1979a, 1979b; Couri et al., 1982).

Safety/toxicity

The safety of chlorine dioxide is dependent upon the route of administration and the dose. When administered topically at low doses, chlorine dioxide is non-toxic. A study using equine fibroblasts found chlorine dioxide is less toxic than chlorhexidine, which is a commonly used skin wound antiseptic solution (Redding and Booth, 1991). A study exploring the use of chlorine dioxide 50 ppm as a wound irrigant in humans found this biocide was safe and was not associated with adverse events (Valente et al., 2014).

A series of studies in the 1980's explored the safety of oral chlorine dioxide and chlorite. Low doses were found to be safe, but high doses caused adverse effects. Based on these studies, the US Environmental Protection Agency (EPA) determined a no-observed-adverse-effect level (NOAEL) of 3 mg/kg/day and a lowest-observed-adverse-effect level (LOAEL) of 5.7 mg/kg/day for ClO_2 (EPA 2000). A few years later, the Agency for Toxic Substances and Disease Registry (ATSDR) lowered the NOAEL level to 2.9 mg/kg/day (US Department of Health and Human Services, 2004).

Human studies found no adverse effects in individuals who drank low concentrations (0.04-0.34 mg/kg/day) of chlorine dioxide or chlorite in experimental studies (Lubbers et al., 1981, 1982, 1984a, 1984b) or ingested water disinfected with chlorine dioxide (Tuthill et al., 1982).

A Phase I study examining the safety and tolerability of acute intravenous administration of sodium chlorite found escalating doses up to 3.2 mg/kg/day were generally safe and well tolerated (Miller et al., 2014). In a subsequent multicenter, randomized, double-blind Phase II study involving 136 individuals with amyotrophic lateral sclerosis (ALS), sodium chlorite (NP001) 2 mg/kg/day was administered intravenously as a single daily dose for 6 months. This study resulted in Class I evidence that sodium chlorite was generally safe and well tolerated. Pain at the injunction site and transient dizziness were the only significant adverse events (Miller et al., 2015).

A small double-blind, placebo-controlled trial compared treatment for end-stage acquired immunodeficiency syndrome (AIDS) with chlorite (WF10) in 10 individuals and placebo in 9 individuals. Chlorite was administered intravenously at 0.5 mL/kg for 5 days every 3 weeks for a total of 4 cycles over 3 months to 10 individuals. No serious adverse events occurred (Raffanti et al., 1998).

MECHANISMS OF ACTION OF CHLORINE DIOXIDE AND CHLORITE IN THE TREATMENT OF DFUS

Chlorine dioxide/chlorite exhibits multiple effects that may enhance healing of DFUs. These include improved glucose control (Maraprygsavan et al., 2016; Yingsakmongkol et al., 2021), decreased production of superoxide ions while increasing the production of hydrogen peroxide (Tissot et al., 1990), increased tissue oxygen tension (Tissot et al., 1990), reduced inflammation (Giese et al., 2004; Schönberg et al., 2016; Tissot et al., 1990; Yingsakmongkol, 2013), antimicrobial effects (Alvarez and O'Brien, 1982; Benarde et al., 1965; Sanekata et al., 2010; Zu et al., 2019), and improved wound healing (Al-Bayaty and Abdulla, 2012; Hinz et al., 1986; Kenyon et al., 1986; Wilkins, 2014; Yingsakmongkol et al., 2011).

Improve glucose control

Chlorite has been demonstrated to reduce hemoglobin A1c levels in individuals with DM who suffer with severe DFUs. A retrospective study of 12 individuals who had

Table 2. Reduction in Hgb A1c levels following treatment with chlorite (WF10)*.

Weeks following treatment	HgbA1c (%)	Unpaired student's T-test
Baseline	9.1±1.6	
4	6.7±1.4	$p < 0.001$
8	6.2±1.1	$p < 0.01$
12	6.8±1.0	$p < 0.01$
16	7.3±1.5	$p < 0.05$

*Maraprygsavan et al. (2016).

been diabetic for an average of 16 years examined the effects of chlorite (WF10) for the treatment of DFUs. These individuals had severe ulcers with gangrenous toes and osteomyelitis. Eight had been referred for below the knee amputation. The study found 11 of these 12 individuals showed either complete healing of the wounds or significant improvement following treatment with WF10. In 8 of the 12 individuals, complete wound healing was observed. None of the individuals required below the knee amputation. Furthermore, 11 of the 12 individuals demonstrated a statistically significant reduction in Hemoglobin A1c (HbA1c) levels at 4 to 16 weeks post-treatment (Table 2). A decrease in hematocrit was observed in some individuals, but this resolved with blood transfusion. Four of the 12 individuals received a blood transfusion (Maraprygsavan et al., 2016).

A prospective, non-controlled study using chlorite (WF10) as adjunct treatment for DFUs found the treatment decreased HbA1c values by >2%, reduced fasting blood glucose (FBG) levels, and accelerated healing of DFUs as demonstrated by a Wound Severity Score reduction from 8.0 to 1.4 after 12 weeks (Yingsakmongkol et al., 2021).

Reduce superoxide levels and increase hydrogen peroxide

The effects of chlorite on the production of ROS are species-specific. For example, sodium chlorite (TCDO) injected into rats significantly reduced superoxide generation while at the same time raised hydrogen peroxide levels (Tissot et. al., 1990). This may have occurred as a result of increased SOD activity (which increases the conversion of superoxide to hydrogen peroxide) and impairment of myeloperoxidase activity (which converts hydrogen peroxide to hypochlorous acid) (Ali and Mahmood, 2017; Schempp et al., 2001).

A recent study from Egypt explored the effects of topical ClO_2 gel on the healing of full-thickness wounds in albino rats (Mawas et al., 2022). This study consisted of 4 groups: Control non-diabetic, control diabetic untreated, sesame oil and ClO_2 gel. The results showed that SOD activity was higher in the group treated with ClO_2 than in the diabetic untreated group, and wound closure was greater in the ClO_2 group than in all the other groups.

Increase tissue oxygen tension

Hypoxia has been reported to increase the risk of infection in diabetic tissue due to impaired host defense mechanisms and reduced expression of hypoxia inducible factor-1α (HIF-1α) and hypoxia inducible factor-1 (HIF-1) target genes resulting in impaired cellular responses to hypoxia (Pozzilli and Leslie, 1994; Davis et al., 2018). Thus, increasing oxygen tension would be expected to reduce the risk of infection.

Attempts to increase tissue oxygenation using hyperbaric oxygen have produced mixed results. Despite marked elevations in blood oxygen levels, many individuals fail to achieve an increase in tissue oxygen pressure, and some even demonstrate a paradoxical reduction in tissue oxygen concentration. These effects have been hypothesized to occur as a result of vasoconstriction resulting from increased production of ROS and decreased levels of nitric oxide. Whereas superoxide and hydrogen peroxide induce vascular constriction; nitric oxide causes vasodilation (Efrati et al., 2009).

The effects of sodium chlorite on ROS production show dose-dependent effects. Rats injected with 1.5 µmoles of sodium chlorite (TCDO) demonstrated a marked reduction in superoxide generation after 4 h (Tissot et al., 1990) whereas human erythrocytes incubated in a solution of 0.1 to 3.0 mM of sodium chlorite demonstrated a 3 to 21 fold increase in the generation of ROS (Ali and Mahmood, 2017). Thus, lower doses of chlorite would be expected to reduce generation of ROS thereby decreasing vasoconstriction and improving hypoxia. Higher doses, on the other hand, would be expected to increase ROS levels and increase vasoconstriction resulting in increased hypoxia.

Reduce inflammation

ClO_2 and chlorite alter the immune response via modifications in the functioning of multiple immune cells (McGrath et al., 1998). Chlorite exerts dose-dependent

inhibitory effects on T-lymphocyte proliferation *in vitro* (Giese et al., 2004) and inhibits cytotoxic T-cell-mediated target cell killing (Wabnitz and Samstag, 2016). Chlorite also exhibits anti-inflammatory properties such as inhibiting the proinflammatory type of M1 macrophages (Schönberg et al., 2016), inhibiting polymorphonuclear (PMN) migration (Tissot et al., 1990), increasing the phagocytic activity of macrophages (Yingsakmongkol, 2013), and down-regulating pro-inflammatory genes in monocytes (Giese et al., 2004). Chlorite (WF10) is activated in reactions with hemoproteins, particularly myeloperoxidase, resulting in the production of taurine chloramine (TauCl), a strong inducer of apoptosis that also exerts anti-inflammatory effects (Giese et al., 2004; Schempp et al., 2001; Yingsakmongkol, 2013). Chlorite also stimulates natural killer (NK) cell cytotoxicity (Kühne et al., 2011).

A study exploring the treatment of AIDS with chlorite (WF10) found individuals exhibited significant downregulation of T-cell immunologic activation markers such as CD38, CD28 and DR as well as a decrease in DR+ circulating MO and B lymphocytes (McGrath et al., 1998).

Sodium chlorite (NP001) has been safely administered intravenously to patients with amyotrophic lateral sclerosis (ALS). In Phase I and phase II studies, this medicine was demonstrated to reduce blood monocyte immune activation markers CD16 and HLA-DR and slow the progression of ALS in a subset of patients with elevated neuroinflammation (Miller et al., 2014, 2015). The suggested mechanism of action for this medicine in the treatment of neurodegenerative diseases is the conversion of pro-inflammatory macrophages (M1) to non-inflammatory macrophages (M2) (Neuvivo, 2022).

Antimicrobial effects

ClO_2 has been demonstrated to exert potent antimicrobial effects (Alvarez and O'Brien, 1982; Benarde et al., 1965; Sanekata et al., 2010; Zhu et al., 2019). Chlorite (TCDO) exhibits antiviral and virucidal activity against a number of viruses including Herpes Simplex Virus-1 (HSV-1), Herpes Simplex Virus-2 (HSV-2), Influenza A, Polio-1 (Dargan and Subek-Sharpe, 1992), and human immunodeficiency virus (HIV) (Ennen et al., 1993). Chlorite (TCDO) possesses dose-dependent and species-specific antibacterial activity against aerobic and anaerobic bacteria (Ullmann and Kühne, 1985).

Gillissen et al. (1986) found chlorite (TCDO) increased the survival rate in mice experimentally infected with *Candida albicans* or *Peptostreptococcus intermedius* while enhancing both humoral and cellular immune responses. This was achieved in part by increasing the number of IgM and IgG forming spleen cells.

A double-blind, randomized placebo-controlled trial examining intravenous chlorite (WF10) as a treatment for DFUs in humans found individuals treated with chlorite

exhibited a decrease in wound severity compared with the placebo group after 9 weeks of treatment. Statistically significant findings included decreased infection (Yingsakmongkol et al., 2011).

ClO_2 exerts antibacterial, antiviral and antifungal effects. These antimicrobial effects are attributed to ClO_2's actions as an oxidant. Oxidants are agents that accept or receive electrons from reducing agents (that is, electron donors). ClO_2 oxidizes DNA, RNA and proteins leading to interruption of protein synthesis, disruption of cell membranes and cell death (Peredo-Lovillo et al., 2023).

Improve wound healing

Chlorine dioxide and chlorite improve wound healing and enhance granulation tissue formation in various types of wounds (Hinz et al., 1986; Kenyon et al., 1986). Wilkins (2014) described three cases in which ClO_2 (Ciderm) was used as part of a post-operative wound care management program. They found ClO_2 effectively treated and prevented infection, while preserving viable tissue.

A study conducted in Malaysia explored the wound healing activities of ClO_2 gel in streptozotocin-induced diabetic rats (Al-Bayaty and Abdulla, 2012). The authors experimentally induced excision wounds in adult male Sprague Dawley rats, then applied chlorine dioxide gel topically twice daily. A second group of rats was treated with a hyaluronic acid gel, while a third group was treated with Intrasite gel. A control group was treated with sterile distilled water. Wound tissue was collected on day 10 to measure the activities of antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD). After 10 days of healing, the size of the wound was significantly smaller in the group treated with ClO_2 than in the control group. Also, granulation tissue from the treatment group contained fewer inflammatory cells, more collagen, and more proliferating capillaries than granulation tissue from the control group. Additionally, the activity levels of GPx and SOD were significantly increased in the ClO_2 group relative to the control group. A double-blind, randomized placebo-controlled trial exploring intravenous chlorite (WF10) as a treatment for DFUs found individuals in the treatment group exhibited a decrease in wound severity compared with the placebo group after 9 weeks of treatment. Statistically significant findings included decreased infection, inflammation, and necrotic tissue along with an increase in granulation tissue (Yingsakmongkol et al., 2011).

POTENTIAL CHALLENGES ASSOCIATED WITH THE USE OF CLO₂ AS A TREATMENT FOR DFUS

Despite the potential safety and efficacy of ClO_2 as a treatment for DFUs, obstacles exist that may limit the use

Table 3. Proposed mechanisms of action for ClO_2/ClO_2^- in the treatment of DFUs.

Beneficial effect	Mechanism of action
Improved glucose control	Unknown
Improve vasculopathy	Increase angiogenesis and tissue oxygen tension
Slow the progression of neuropathy	Improve blood supply to neurons
Reduce inflammation	Inhibit PMN migration
	Increase the phagocytic activity of macrophages
	Down-regulate pro-inflammatory genes in monocytes
	Increase production of taurine chloramine
	Increase conversion of pro-inflammatory macrophages (M1) to non-inflammatory macrophages (M2)
Reduce infection	Interruption of protein synthesis
	Destabilization of cell membranes
	DNA/RNA/protein oxidation
	Cell death
Improve wound healing	Enhance formation of granulation tissue
	Regeneration of healthy tissue

Source: Maraprygsavan et al., (2016); Yingsakmongkol et al., 2021; Ali and Mahmood (2017); Tissot et al., (1990); Giese et al., (2004); Schönberg et al., (2016); Alvarez and O'Brien (1982); Bernarde et al., (1965); Sanekata et al., (2010); Zu et al., (2019); Al-Bayaty and Abdulla (2012); Hinz et al., 1986; Kenyon et al., (1986); Wilkins (2014)

of ClO_2 as a treatment for DFUs. One potential obstacle is the cost of this medicine. The problem is not that ClO_2 is expensive to manufacture or distribute. In fact, the opposite is true. ClO_2 is inexpensive and cannot be patented. Thus, it is unlikely that any pharmaceutical company will pursue research with this medicine because of the difficulty involved in recovering the large investment required to bring this therapeutic to market.

A second potential obstacle is the US FDA's critical and fear-inducing comments about "sodium chlorite products." During the COVID-19 pandemic, the FDA cautioned that sodium chlorite products are "dangerous" and "can make you sick." The FDA website warns "sodium chlorite and chlorine dioxide are the active ingredients in disinfectants and have additional industrial uses. They are not meant to be swallowed by people." The FDA equates drinking sodium chlorite products with drinking bleach (U.S. Food and Drug Administration, 2019).

Although it is true that ingesting high doses of sodium chlorite or chlorine dioxide can cause adverse effects, it is also true of every other medicine available today. As the physician and chemist Paracelsus stated over 500 years ago, "Solely the dose determines that a thing is not a poison" (Borzelleca, 2000, p. 3). Furthermore, the FDA's warning ignores the fact that millions of people throughout the world drink low doses of chlorine dioxide in their municipal water every day and chlorite has already been approved as a medicine in Germany and Thailand. Thus, research into potential therapeutic applications of chlorine dioxide will have to confront negative perceptions of this medicine and resistance to its use.

DISCUSSION

The objective of this narrative review was to evaluate the safety and efficacy of chlorine dioxide as a treatment for DFUs and to describe potential mechanisms of action by which these agents may treat DFUs. After reviewing the literature, it was found that when used in low doses, chlorine dioxide and chlorite show promise as safe and effective treatments for DFUs (Table 3).

We hypothesize chlorine dioxide and chlorite can promote healing of DFUs through multiple mechanisms including:

(1) improve glucose control and reduce hyperglycemia thereby improving vasculopathy, neuropathy, and immune system functioning (Maraprygsavan et al., 2016; Yingsakmongkol et al., 2021)
(2) improve vasculopathy by increasing angiogenesis and tissue oxygen tension by increasing the conversion of superoxide to hydrogen peroxide via enhancement of superoxide dismutase along with increasing levels of cytokines VEGF and TGF-β-1
(3) slow the progression of neuropathy by improving blood supply to neurons

(4) reduce inflammation via inhibition of PMN migration (Schönberg et al., 2016), increase the phagocytic activity of macrophages (Yingsakmongkol, 2013), down-regulating pro-inflammatory genes in monocytes (Giese et al., 2004), increase production of taurine chloramine (Giese et al., 2004; Schempp et al., 2001; Yingsakmongkol, 2013), increase conversion of pro-inflammatory macrophages (M1) to non-inflammatory macrophages (M2) (Neuvivo, 2022)

(5) reduce infection via antimicrobial effects (Alvarez and O'Brien, 1982; Benarde et al., 1965; Dargan & Subek-Sharpe, 1992; Ennen et al., 1993; Gillissen et al., 1986; Sanekata et al., 2010; Yingsakmongkol et al., 2011; Zhou et al., 2019)

(6) improve wound healing via enhanced formation of granulation tissue along with regeneration of healthy tissue (Al-Bayaty and Abdulla, 2012; Hinz et al., 1986; Kenyon et al., 1986; Yingsakmongkol et al., 2011)

CONCLUSION

DFUs are associated with significant morbidity and mortality and constitute a severe global health problem. Chlorine dioxide and chlorite show promise as safe and effective treatments for DFUs. Randomized, placebo-controlled studies are needed to explore these medicines' safety, efficacy, and optimal dosage ranges. Studies examining different routes of administration (e.g. oral vs topical vs intravenous vs multiple simultaneous routes of administration) are also needed. The results of this review strongly encourage such research and we hope investigators around the world will continue to explore the potential benefits of chlorine dioxide and chlorite.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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