

Clinical Applications and Methemoglobinemia Induced by Dapsone

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Dapsona é uma sulfona sintética que é utilizada como um antibiótico em seres humanos e animais para prevenir e tratar doenças, incluindo hanseníase, tuberculose, malária, e pneumonia por *Pneumocystis carinii* e encefalites por *Toxoplasma gondii* em pacientes com síndrome da imunodeficiência adquirida (AIDS), bem como em doenças anti-inflamatórias como dermatite herpetiforme. No entanto, este fármaco também está associado com vários efeitos adversos, incluindo a hemólise relacionada com a dose, metemoglobinemia, psicose, neuropatia periférica, agranulocitose, anemia aplástica, síndrome de hipersensibilidade, síndrome de sulfona, e outros. Destes efeitos, a metemoglobinemia é o mais comum efeito adverso da dapsona, que leva a anemia funcional e hipóxia celular com sintomas de cianose, dores de cabeça, fadiga, taquicardia, fraqueza e tonturas. Assim, esta revisão sumariza informações relevantes sobre a estrutura, mecanismo de ação, indicação clínica, e reações adversas de dapsona.

Dapsone is a synthetic sulfone that is used as an antibiotic in humans and animals to prevent and treat diseases including leprosy, tuberculosis, malaria, and *Pneumocystis carinii* pneumonia and *Toxoplasma gondii* encephalitis in acquired immune deficiency syndrome (AIDS) patients as well as in anti-inflammatory conditions, such as dermatitis herpetiformis. However, this drug is also associated with several adverse effects, including dose-related hemolysis, methemoglobinemia, psychosis, peripheral neuropathy, agranulocytosis, aplastic anemia, hypersensitivity syndrome, sulfone syndrome, and others. Of these effects, methemoglobinemia is the most common side effect of dapsone, which leads to functional anemia and cellular hypoxia with symptoms of cyanosis, headache, fatigue, tachycardia, weakness, and dizziness. Thus, this review summarizes relevant information on the structure, mechanism of action, clinical indication, and adverse reactions of dapsone.

Keywords: dapsone, methemoglobinemia, 4,4'-diamino-diphenylsulfone, dapsone hydroxylamine

1. Introduction

Dapsone was synthesized a century ago (1908) and has been used for treatment of several pathologies, including infectious processes such as leprosy, malaria, and *Pneumocystis carinii* pneumonia and *Toxoplasma gondii* encephalitis in acquired immune deficiency syndrome (AIDS) patients as well as in anti-inflammatory conditions, dermatitis herpetiformis and linear immunoglobulin A (IgA) disease.¹ However, this drug may lead to adverse effects such as dose-related hemolysis and methemoglobinemia.² Therefore, this review summarizes relevant information on the structure, mechanism of action, clinical indication, and adverse reactions of dapsone.

2. Molecular Structure and Mechanisms of Antimicrobial Action

Dapsone (4,4'-diamino-diphenylsulfone, DDS) is an aromatic amine that belongs to the family of sulfones, which also includes diadiphenyl sulfone, sulfonyl dianiline, and disulfone.³ Sulfone is a very weak Lewis base with no readily dissociable hydrogen ion (H⁺), which has the structure of the simplest of the sulfones: a sulfonyl group (a sulfur atom bound to two oxygen atoms by double bonds) linked to two carbon atoms, as illustrated in Figure 1. Dapsone is a white, odorless crystalline powder that darkens upon exposure to light but remains chemically unaltered. It is poorly water-soluble but easily soluble in alcohol.⁴ In addition, dapsone shows chemical similarity to the sulfonamides, where the presence of the sulfonyl group is

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essential for their pharmacological activity but also may be responsible for its toxicity.^{5,6}

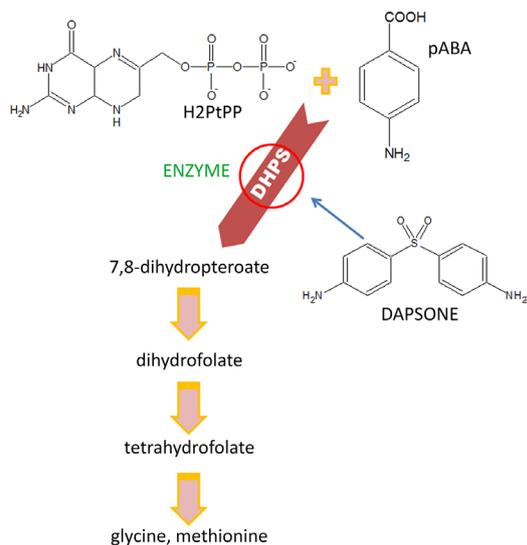


Figure 1. Antimicrobial mechanism of dapson. DDS: 4,4'-diaminodiphenylsulfone; pABA: *p*-aminobenzoic acid; H2PtPP: 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate; DHPS: 6-hydroxymethyl-7,8-dihydropterin synthase.

Eric Fromm and J. Whitmann were the first to describe the synthesis of sulfones, including dapson, from *p*-nitrosothiophenol in 1908;⁷ however, the synthesis of dapson from *p*-chloronitrobenzene was described in 1945.⁸ Therapeutic properties of this drug remained unknown until 1943, where the first *in vivo* studies showing the antimicrobial effect of dapson in streptococcal infections were reported by Buttle *et al.*⁹ That same year, Faget *et al.*¹⁰ reported that a dapson derivative, promin, can be useful for the treatment of leprosy. Therefore, in 1948, this drug was used as standard treatment for leprosy.¹¹ In 1976, during a workshop on leprosy chemotherapy, the World Health Organization (WHO) recommended the use of dapson, rifampicin, and clofazimine as a leprosy multidrug therapy,^{12,13} and, currently, dapson has been employed as a broad-spectrum antimicrobial agent.

The use of dapson as an antibiotic is due to its structural analogy of sulfa drugs, including sulfonamides and sulfones, to the substrate *p*-aminobenzoic acid (pABA). These drugs act as competitive antagonists of the enzyme 6-hydroxymethyl-7,8-dihydropterin synthase (DHPS) that is responsible for the conversion of pABA to folate, a purine precursor, thereby interfering in the production of DNA necessary for cell division.¹⁴⁻²¹ In this regard, DHPS catalyzes the formation of a C–N bond, joining pABA with 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate (H2PtPP) to form pyrophosphate and 6-hydroxymethyl-7,8-dihydropteroate. It is essential to

produce reduced folate cofactors by a *de novo* biosynthetic pathway present in prokaryotes and in some eukaryotes, such as protozoa, yeast, and plants, but it is absent in mammals.^{17,22-25} Ho *et al.*²⁶ reported that DDS was found to be approximately nine times more effective as a competitive inhibitor of DHPS than sulfonamides. These authors also suggested that this remarkable inhibitory activity of DDS can be attributed to its symmetry, which generates a single molecule with two identical planes quite similar to pABA, and this arrangement may facilitate the binding of DDS to the active site of DHPS. In addition, Bell and Roblin²⁷ have reported that a structural similarity between the sulfonyl group present in sulfones and sulfonamides and the carbon dioxide (CO₂) of pABA may be important to the antibacterial activity of these drugs. Thus, they suggested that high potency of DDS depended upon the negativity of the sulfone (SO₂) group and of steric factors that remain to be explained.

Structure-activity relationships and *in vivo* studies have shown that dapson is a symmetrical molecule and that the SO₂ group is essential for the pharmacological activity of this drug but is also responsible for its observed hemotoxicity.²⁸ Our group has shown through geometric properties of the structure of dapson that the symmetry of the SO₂ moiety is essential for the electron distribution between the two *para*-related aniline moieties. Molecular symmetry is the most significant aspect for the nucleophilicity of dapson, and the symmetric conformational isomer has lower energy than the asymmetric conformational isomer, showing the amine influence of conformation with participation of the SO₂ moiety by resonance effect on both aniline rings. Moreover, the SO₂ moiety may stabilize the radical formed during oxidation through conjugation (resonance effect), and the aniline ring is the nucleophilic moiety with possible biological properties through redox mechanisms, mainly electron transfer or oxidation for formation of the dapson hydroxylamine (DDS-NHOH) metabolite.^{29,30}

3. Clinical Experience and Recommended Use

Dapson is an effective drug that is used as an antibiotic in humans and animals to prevent and treat diseases.³¹ Moreover, DDS is a synthetic sulfone with bactericidal and bacteriostatic activity against *Mycobacterium leprae* and *M. ulcerans*, in high concentrations ($\geq 8 \text{ mg mL}^{-1}$), is also effective against *M. tuberculosis* including the multi-drug resistant (MDR) strains *M. intracellulare*, *M. kansasii*, *M. fortuitum*, and the *Mycobacterium avium* complex (MAC).³¹⁻³⁶ In combination with pyrimethamine, dapson has been used for malaria prophylaxis in chloroquine-resistant *Plasmodium falciparum* and *P. vivax* strains,

displaying activity against all multiplying stages in the plasmodium life cycle.^{37,38} Combinations, such as chlorproguanil-dapsone and artesunate-dapsone-proguanil, can be used in treatment and prophylaxis of malaria caused by *P. falciparum* and antimalarial action of schizonticides.³⁹⁻⁴¹ Furthermore, dapsone also may be used as a systemic adjuvant when it is combined with oral corticosteroids in the treatment of pemphigus vulgaris⁴² or in combination with pyrimethamine, in the treatment and prevention of opportunistic infections in AIDS patients, such as *Pneumocystis carinii* pneumonia and *Toxoplasma gondii* encephalitis, or for the treatment of Kaposi's sarcoma.⁴³⁻⁵¹

Besides use of dapsone as an antimicrobial agent, this drug may also be used as an anti-inflammatory agent. Since this activity is unrelated to its antibacterial action, it may, therefore, be used in non-infectious inflammatory diseases such as dermatitis herpetiformis, IgA dermatitis, rheumatoid arthritis, acne conglobata, chronic urticaria, vasculitis, leukocyte bullous systemic lupus erythematosus, and spider bite and urticarial vasculitis syndromes.^{4,20,49,52-56} Accordingly, dapsone alone or with concurrent systemic corticosteroids can also be used as efficacy therapy in other dermatologic conditions, such as mucous membrane pemphigoid, pemphigus vulgaris, bullous pemphigoid,⁵⁷ pyoderma gangrenosum (PG),⁵⁸⁻⁶⁰ subcorneal pustular dermatosis (SPD)⁶⁰⁻⁶² and Behcet's disease.^{63,64}

In these forms of dermatitis, the anti-inflammatory activity of dapsone is associated with polymorphonuclear leukocytes (PMNs) during the inflammatory process, such as cellular migration and apoptosis.^{56,65} In general, *in vitro* studies showed that dapsone stimulates neutrophil motility, and clinical studies reported that DDS mediated stimulation of PMN migration.^{60,66} In this regard, the therapeutic response of dapsone in PG resulted from inhibition of neutrophil chemotaxis and reduction of oxygen intermediates, thereby suppressing the inflammation,^{58,59} while for SPD and Behcet's disease, dapsone can inhibit the neutrophil cytotoxicity and neutrophil chemotaxis and alter the glycosaminoglycans, diluting inflammatory mediators.^{60,61,63,64} Moreover, the inhibition of neutrophil chemotaxis induced by dapsone can be explained due to the drug's interference with the activation or function of the G-protein that initiates the signal transduction cascade common to chemotactic stimuli.⁶⁷

In urticaria, dapsone may inhibit the neutrophils' functions due to its antimicrobial and anti-inflammatory effects by a mechanism that involves the down-regulation of leukotriene B4 and interference with the expression of the cluster of differentiation 11B (CD11B) molecule.^{68,69} Other studies indicate that dapsone may inhibit mitogen-

stimulated transformation of lymphocytes, the alternate pathway of complementary activation, by decreasing levels of complement 3 (C3) protein and C3 pro-activator and interfering with the myeloperoxidase-peroxidase-halide-mediated cytotoxic system within neutrophils.⁷⁰ In addition, *in vitro* studies showed that dapsone may prevent myeloperoxidase- and eosinophil peroxidase-mediated tissue injury at sites where the peroxidase enzymes are secreted and diluted into the neutral pH environment of the tissue interstitial space. Dapsone may inhibit tissue proteases because it is known to oxidize glutathione (GSH); this is important because reduced GSH is required for most proteolytic enzymes to function.^{71,72}

4. Pharmacokinetic Properties

Dapsone is administered orally, and about 80-85% is absorbed slowly from the gastrointestinal tract and uniformly distributed to all tissues. However, it tends to accumulate mainly in the skin, especially in the muscles, liver, and kidneys. Traces of this sulfone can be found up to three weeks after end of the treatment. This drug can cross both blood-brain and placental barriers and can be found in breast milk. Dapsone and its metabolic derivatives can be conjugated with glucuronic acid in the liver and excreted mainly by kidneys and approximately 10% as bile.^{4,56,65,73-76} In addition, about 70% of dapsone is bonded to plasma proteins with plasma concentrations ranging from 0.4-1.2 mg L⁻¹ 24 hours after ingestion of 100 mg of the drug, and the maximum plasma concentration is reached within 4-8 hours.^{75,77-80} Blood levels stabilize approximately 7-10 days after initiation of therapy.⁸¹ Dapsone shows a large half-life ranging from 24-36 hours and can be found in the organism after 35 days following the end of treatment.⁴

After absorption, DDS is transported through the portal circulation and is metabolized in the liver by two distinct routes: Nitrogen (*N*)-acetylation and *N*-hydroxylation (Figure 2). Dapsone acetylation is polymorphic and occurs via action of the liver cytosolic enzyme *N*-acetyltransferase (NAT), which is present in hepatic cells and in red blood cells.^{4,12} *N*-acetylation is the major route of biotransformation of drugs containing arylamine or anilines (Ar-NH₂).⁸² The aromatic amine is catalyzed by NAT enzymes present in two forms in humans (NAT1 and NAT2). These enzymes detoxify by converting the aromatic amines into amides, which are less toxic metabolites, producing the *N*-hydroxy metabolites, including monoacetyl dapsone (MADDS), formed by acetylation of the amine group (NH₂), and diacetyl-diaminosulfone (DADS), formed by di-acetylation. Both processes are reversible, yielding unmodified dapsone.⁸³

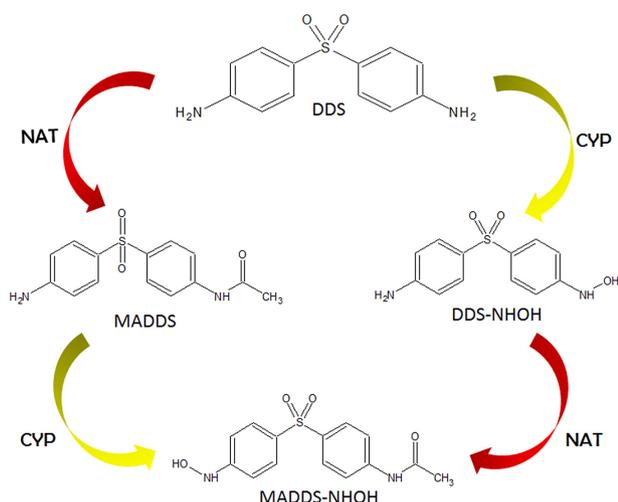


Figure 2. Possible metabolic pathways of dapsone involving NAT and CYP enzymes. NAT: *N*-acetyl transferase; CYP: Cytochrome P450; DDS: 4,4'-diamino-diphenylsulfone; DDS-NHOH: dapsone hydroxylamine; MADDs: monoacetyl dapsone; MADDs-NHOH: monoacetyl dapsone hydroxylamine.

The *N*-hydroxylation that occurs during the oxidative clearance of dapsone and its acetylated derivative is mainly mediated by hepatic cytochrome P450 (CYP)⁸⁴⁻⁸⁶ and glucuronidation, conjugation of glucuronic acid and uridine diphosphate (UDP) through the action of the enzyme UDP-glucuronosyltransferase (UGT).^{3,28} *N*-hydroxylated metabolites include dapsone hydroxylamine (DDS-NHOH) and monoacetyl dapsone hydroxylamine (MADDs-NHOH). These result from a process that occurs mainly by action of a cytochrome P450 isoform, such as CYP3A4, CYP2E1, CYP2C9, and CYP2C19.^{20,29,86,87} Recently, Schalcher *et al.*⁸⁶ showed by molecular modeling the interaction mechanism and possible binding of DDS with the active site of enzyme CYP2C19. However, other CYP isoforms are involved in the metabolism of DDS, such as CYP2E and CYP3A.^{84,88-90}

After oral administration, a constant MADDs/DDS is established, which tends to increase the rates of acetylation. In this regard, Coleman and Tingle⁹¹ reported that MADDs is not primarily responsible for the toxicity of DDS, unless the other amino group is *N*-hydroxylated. Dapsone and its metabolites are conjugated in the liver and excreted in the form of mono-*N*-glucuronide and mono-*N*-sulfamate and, in lower concentrations, excreted in saliva and breast milk.^{3,92} Its elimination rate after a single dose is 50% during the first 24 hours.^{79,80}

5. Adverse Effects

Adverse reactions related to the use of dapsone range from digestive problems such as nausea, vomiting, and stomatitis.⁹³ The occurrences of toxic hepatitis, cholestatic jaundice, cutaneous photosensitivity reactions, psychosis,

and a syndrome that became known as the “sulfone syndrome” (fever, malaise, jaundice, exfoliative dermatitis or morbilliform rash, hepatic dysfunction, lymphadenopathy, methemoglobinemia, hemolytic anemia and lymphocytosis with atypical lymphocytes) are unusual.^{54,93} The occurrence of liver disease associated with DDS is about 5% when administered alone, but the incidence of liver damage by DDS increased to 40% when the drugs were co-administered with other drugs such as trimethoprim for AIDS patients.⁹⁴ The most frequent adverse reactions caused by dapsone is methemoglobinemia production and hemolysis (Figure 3),^{3,95-97} showing inclusions resulting from the changes in hemoglobin called Heinz bodies.⁹⁶⁻¹⁰⁰ The recommended dose of dapsone is 50-100 mg *per day* (the dose should not exceed 300 mg *per day*), but the most serious side effects are observed when the dose is > 100 mg *per day*¹⁰¹ and agranulocytosis occurs.¹⁰² Dapsone taken orally in higher doses can generally cause nausea, vomiting, and epigastric pain.¹⁰³

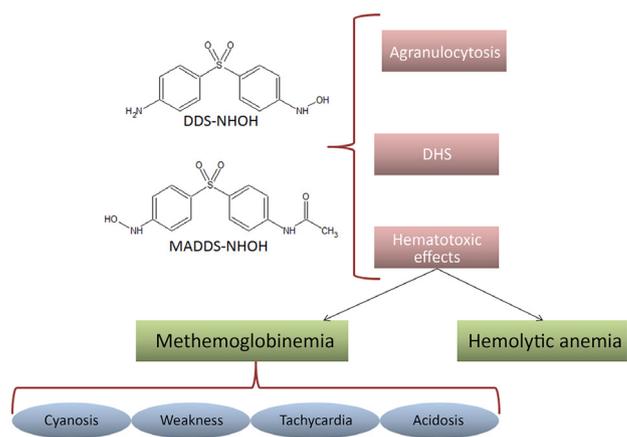


Figure 3. Hematological side effects associated with hydroxylated metabolites of dapsone. DDS: 4,4'-diamino-diphenylsulfone; DDS-NHOH: dapsone hydroxylamine; MADDs-NHOH: mono acetyl dapsone hydroxylamine.

Agranulocytosis is not a dose-dependent side effect of dapsone, with an unknown mechanism of development initiating after 4-12 weeks of treatment. It gradually progresses with symptoms of fever, swelling of the lymph nodes, inflammation and ulcers of the oral cavity, pharynx, and esophagus, increased susceptibility to sepsis, and death throughout the disease.^{100,104} Another adverse effect of DDS therapy is called dapsone hypersensitivity syndrome (DHS), caused by an idiosyncratic reaction^{105,106} related to metabolized products of DDS, as DDS-NHOH, which produces a great number of effects such as fever, rash, and internal organ involvement.^{107,108}

The most frequent and well-reported adverse effects of dapsone were dose-limiting hemotoxic effects, such as hemolytic anemia and methemoglobinemia, which

are associated with long-term administration of the drug at standard doses (100 mg *per day*), resulting in methemoglobinemia in about 15% of patients.^{109,110} The hemotoxic effects of DDS increase in a dose-dependent manner, especially in infants and elderly patients.^{44,46,111-113} After DDS therapy is initiated at the > 300-mg *per day* dose, the patient should present symptoms of hemolytic anemia as a dose-dependent side effect that usually occurs in 3-4 weeks; the mechanism is unknown but is associated with a reduction in the lifespan of red blood cells¹⁰¹ The most common side effect of DDS treatment is methemoglobinemia, which occurs when the concentration of methemoglobin (MetHb) in erythrocytes is more than 1%, leading to functional anemia and cellular hypoxia with symptoms of cyanosis at levels around 15% and headache, fatigue, tachycardia, weakness, and dizziness experienced in 30-40% of patients. Concentrations of approximately 60% may cause hypoxia leading to acidosis, paralysis, arrhythmias, coma, and convulsions, ultimately leading to death when concentrations reach 70-80% levels, due to the incapability of abnormal hemoglobin to carry oxygen or CO₂.^{114,115} *N*-hydroxy metabolites, DDS-NHOH and MADDS-NHOH, have been investigated as the predominant hemolytic metabolites responsible for hemotoxic reactions, mainly methemoglobinemia.^{20,89,90,116,117} In addition, dapsone is contraindicated in patients with low glucose-6-phosphate dehydrogenase (G6PD) levels, whereas gradually increasing the dose of dapsone (25-150 mg *per day* for three days) in patients with normal G6PD levels may lead to a degree of hemolytic anemia and associated symptoms.¹¹⁸

6. Methemoglobinemia

Methemoglobinemia is a clinical syndrome caused by increased blood concentration of MetHb.¹¹⁹ It occurs naturally without any tissue damage due to regulation by the enzyme nicotinamide adenine dinucleotide phosphate (NADPH)-dependent methemoglobin reductase; however, it can occur in congenital chronic form owing to changes in the synthesis or metabolism of hemoglobin, as acquired from disease in which there is an increase in the rate of oxidation of hemoglobin to methemoglobin¹²⁰⁻¹²⁵ or induced by agents with oxidizing action, such as DDS, sulfonamides, local anesthetics, methylene blue at high doses,^{20,30} primaquine, chloroquine, bupivacaine, nitroglycerin, lidocaine, and nitroprusside, among others.¹²⁶⁻¹²⁸ The changes to the MetHb molecule include the original ferrous (Fe²⁺) atom being oxidized to a ferric (Fe³⁺) atom, leading to an allosteric change in the heme portion of the oxidized hemoglobin molecule, decreasing its oxygen-binding capacity.^{120,121,123,124}

According to Kinoshita *et al.*,¹²⁹ the reduction of MetHb occurs by two main mechanisms: the NADPH-dependent pathway system, represented by NADPH-dependent methemoglobin reductase, and NADH-cytochrome b5 reductase (also known as NADH-dependent methemoglobin reductase). The NADPH-methemoglobin reductase is capable of reducing MetHb formed under normal conditions, since under conditions of high oxidation MetHb reduction is responsible for NADH-cytochrome b5 reductase.¹³⁰ The supply of NADH required for the reduction of MetHb is from the anaerobic glycolysis of the Embden-Meyerhof pathway from the glucose oxidation reaction that generates ATP and NADH, while for the other pathway the substrate is NADPH via the pentose-phosphate pathway from the activation of G6PD.

The most important pathway to reduce erythrocytes is that of NADH-cytochrome b5 reductase, influenced by the availability of NADH and cytochrome b5, a heme-protein present in the cytoplasm of cells with a primary role in the reduction of MetHb.¹³¹ In the case of MetHb formation, 40% up-regulation induced by oxidizing agents and some toxic compounds via NADH-cytochrome b5 reductase is compromised, thus patients with clinical manifestations, such as cyanosis and apnea, may be treated with methylene blue intravenously at 1-2 mg kg⁻¹ body weight during a 5-minute period.^{22,125,132} This is due to the methylene blue being a cofactor for the enzyme NADPH-methemoglobin reductase, thus the treatment results in the oxidation of the methylene blue by accepting an electron from NADPH in the presence of NADPH-methemoglobin reductase to leucomethylene blue, which acts as an artificial electron acceptor to MetHb, resulting in MetHb's conversion back to hemoglobin.^{123,125,133,134} However, this pathway is dependent on NADPH, and in patients with G6PD deficiency, methylene blue is ineffective and may still induce hemolysis¹³⁵ as well as symptoms including dyspnea, chest pain, and persistent cyanosis.¹³⁶

The methemoglobinemia occurs from the inability of MetHb to bind oxygen, causing a state of functional anemia, and increasing the binding affinity of the non-reduced ferrous heme for oxygen. This increase and spread of this process causes significant shortfalls in the supply of oxygen to the tissues, causing other important clinical manifestations, such as dyspnea, nausea, and tachycardia, when levels of MetHb reach up to 30%.¹³³ Lethargy, stupor, and unconsciousness result from levels of approximately 50-70% of MetHb that can cause cardiac arrhythmias, circulatory failure, and central nervous system depression. Levels greater than 70% usually lead to death.⁵⁴ Furthermore, it is possible to observe that in the process of MetHb formation, oxidative attack within

the erythrocyte may occur, causing denaturation of the hemoglobin and precipitation of polypeptides that form insoluble aggregates, so-called Heinz bodies.¹³⁷

Heinz bodies are characterized by the formation of hemichromes. These are the first products of denaturation and progressive globin dissociation, and in subsequent processes they take up another position in the molecule due to the change in the structure by denaturation of the α and β chains, forming irreversible hemichromes that may also precipitate with hemin and globin (free heme group).¹³⁷ The potential to induce methemoglobinemia in erythrocytes is related to the oxidation-reduction cycle with oxyhemoglobin and oxygen molecules and reactive oxygen species (ROS)-producing MetHb,¹³⁸ resulting in subsequent processes involving other cellular biomolecules, including proteins of the cytoskeleton and membrane lipids (lipid peroxidation). The combination of these factors is responsible for the hemolysis observed in this pathology.¹³⁹

Methemoglobinemia and anemia are the most common adverse reactions during treatment with DDS and its hydroxylated metabolite DDS-NHOH,^{106,138,140-146} and occur even at therapeutic doses (dapson 100 mg *per* day).¹⁴⁷⁻¹⁴⁹ Hansen *et al.*¹⁵⁰ showed that the patients had symptomatic methemoglobinemia during treatment with MetHb levels of 35 and 37%. In children presenting cases of acute intoxication with MetHb rates between 23.5-49.7%, clinical manifestations, such as cyanosis, tachycardia, vomiting, dyspnea, and agitation, were observed.¹⁵¹

Electronic properties, such as highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), ionization potential, molecular electrostatic potential (MEP), and spin densities were correlated to the redox properties of DDS and its derivatives on MetHb and shown that MetHb properties are linked to the aniline moiety at the *para*-position, and the lowest ionization potential is related with the increase of methemoglobinemia.³⁰

The major clinical problem associated with the use of DDS is a decreased lifetime of human erythrocytes. This effect may be the cause of anemia, thus causing increased morbidity and mortality^{152,153} associated with the formation of hydroxylamine metabolites with toxic effects on the red blood cells, producing high levels of MetHb.¹⁵⁴ The hydroxylated metabolites of DDS (DDS-NHOH and MADDS-NHOH) have similar toxic effects on human erythrocytes,¹⁵⁵⁻¹⁵⁸ although Rasbridge and Scott¹⁵⁹ claim that MADDS-NHOH was significantly more potent compared to DDS-NHOH when incubated with normal erythrocytes and G6PD deficiency.

Research assessing the damage of DDS and its metabolite confirm the significant increase in MetHb formation with both increased ROS by a redox cycle of

DDS-NHOH production and by reduction in the activity of enzymes involved in antioxidant defense^{58,138,144,158} with dose-dependent effects.¹³⁸

The probable mechanism of this process is that hemoglobin suffers oxidation of these compounds, leading to the formation of MetHb and a compound derived from DDS called nitrosobenzene, reduced by the NADH-methemoglobin reductase enzyme and back to GSH by DDS spreading the oxidation of Hb until GSH levels are depleted.²⁰ The G6PD from the oxidation provides electrons for NADH-methemoglobin reductase converting the Fe³⁺-hemoglobin to Fe²⁺-hemoglobin, reducing MetHb.¹⁶⁰

The development of hemolytic anemia induced by DDS-NHOH was correlated with inhibition of G6PD *in vitro* in human erythrocytes. In the study, DDS-NHOH was able to decrease the half-life of G6PD-deficient erythrocytes by inducing twice the anemic process when compared with normal erythrocytes exposed to this metabolite.^{83,161} Therefore, G6PD-deficient cells show increased susceptibility to oxidative damage since they are unable to reduce NADP⁺ to NADPH. The G6PD catalyzes the oxidation reaction of G6P to 6-phosphogluconolactone in a reaction that specifically uses NADP⁺ as coenzyme, which, after reduction, is converted into NADPH.

The NADPH cofactor is highly reductive; therefore, it is extremely important that erythrocytes maintain the reduced form of the GSH molecule, since it is necessary to the supply of glutathione reductase activity, which converts oxidized GSH (GSSG) into reduced GSH.¹⁶² As erythrocytes lack mitochondria or organelles, the only source of NADPH is the catalytic action of G6PD through the pentose-phosphate pathway, which favors the antioxidant defense capacity of erythrocytes by the action of GSH, which is able to detoxify peroxide as well as keep the cysteine residues of hemoglobin and other proteins of red blood cells in the reduced state.^{163,164}

Other mechanisms may be related to formation of anemia by use of DDS as those discussed by Coleman and Jacobus,²⁰ in which oxidative denaturation in the erythrocyte membrane acts by accelerating the processes of cell hemolysis as well as the induction of progressive changes in the erythrocytes. The latter occurs first via cytosolic domains of membrane proteins of erythrocytes through tyrosine phosphorylation of band 3 that is responsible for anionic exchange after the formation of MetHb,¹⁶⁵⁻¹⁶⁷ including the regulation of glycolysis and changes in morphology and other parameters in erythrocytes.¹⁶⁸⁻¹⁷²

New therapeutic alternatives are studied in order to minimize the effects of oxidative stress caused by administration of dapson. According to Lima *et al.*,¹⁷³ elements with known antioxidant potential, such as vitamins

A, C, and E, zinc, magnesium, and selenium, have produced good results regarding the decrease of methemoglobinemia; studies with cimetidine,⁹¹ isosorbide,¹⁷⁴ N-acetylcysteine, and arginine have shown that the search for new antidotes is valid and promising.

7. Conclusions

In conclusion, dapsone alone or combination therapies are effective to prevent and treat diseases, including leprosy, malaria and numerous dermatologic diseases. However, dapsone on high dosage can lead hemolytic toxicities, including methemoglobinemia and hemolytic anemia. Studies of structure-activity relationships have shown that dapsone is a symmetrical molecule and that the sulfone group is essential for the pharmacological activity of this drug but is also responsible for the observed hemotoxicity. In this review, it was shown that the process of MetHb formation can be associated mainly with DDS-NHOH to exert oxidative stress on human erythrocytes and it can result in the detoxification of the hydroxylamine by reduction to the amine.

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