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## Hemolysis and Anemia Induced by Dapsone Hydroxylamine

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### 1. Introduction

Dapsone (4,4'-diaminodiphenylsulfone, DDS) has been used for over half a century in the treatment of leprosy, for anti-inflammatory conditions and, in the chlorproguanil-dapsone and artesunate-dapsone-proguanil combinations, for treating malaria. It is also a second-line treatment for AIDS-related *Pneumocystis pneumonia* (Sangiolo et al., 2005), and is increasingly applied to a variety of immuno-related conditions (Bahadir et al., 2004; Ujiie et al., 2006), despite its well-documented toxicity, which is closely related to its routes of biotransformation.

Dapsone is mono and diacetylated and the monoacetylated derivative and the parent drug can be oxidised by cytochrome P (CYP) family to hydroxylamines, both of which are methaemoglobin formers. However, both dapsone and mono-N-acetyl dapsone are 97% to 100% bound to plasma proteins. Both hydroxylamines are auto-oxidisable to nitroso arenes, which can covalently bind proteins. In erythrocytes, hydroxylamines react with hemoglobin to form methemoglobin and nitrosoarenes and produce reactive oxygen species (ROS). In turn, ROS reacts with glutathione (GSH) and with hemoglobin thiols to generate thiyl radicals (RS· where R is residue from glutathione or hemoglobin cysteine residue). The thiyl free radicals are responsible for glutathione-protein mixed disulfide and skeletal protein-hemoglobin disulfide formation, which causes alterations in cell morphology (McMillan et al., 2005; Bradshaw et al., 1997) (Fig. 1).

Mono- and diacetylated metabolites of dapsone (MADDS and DADDS) are not associated with toxicity (Coleman et al., 1991), although N-hydroxylation of the parent drug and MADDS lead to the formation of the toxic hydroxylamines DDS-NHOH and MADDS-NHOH (Israili et al., 1973; Coleman et al., 1989) (Fig. 1). These species, formed either by CYP2C9 (Winter et al., 2000), one isoform of the cytochrome P450 (CYP) family, or other oxidative enzyme systems, are linked with several immune-mediated hypersensitivity reactions (Vyas et al., 2006). The hydroxylamines are also responsible for the clinical methaemoglobinaemia associated with dapsone therapy (DT) (Israili et al., 1973; Schiff et al., 2006).

DDS-NHOH cannot be directly detected in human plasma as it is rapidly taken up by erythrocytes prior to its redox cycling with haemoglobin, forming methaemoglobin (Coleman & Jacobus, 1993). In any case, the metabolic elimination of dapsone is N-

hydroxylation, which accounts for between 30% and 40% of an oral dapsone dose, and the efficiency of N-hydroxylation is related to dapsone clearance (May et al., 1990; May et al., 1992; Bluhm et al., 1999). Dapsone therapy includes a daily administration of 50-100 mg for leprosy and 100-300 mg for dermatitis herpetiformis (Leonard and Fry, 1991), leading to serum concentrations of 0.5-5 mg/L (equivalent to 2-20  $\mu\text{M}$ ); therapeutical doses up to 400 mg have been reported in literature (Elonen et al., 1979; Zuidema et al., 1986), as well as some cases of intoxication with DDS, such as after an overdose with 10 g of DDS, leading to serum concentrations of 120 mg/L (about 0.5 mM, comparable to those used in our in vitro experiments). Another case of intoxication produced methaemoglobinemia at serum concentrations of 18.8 mg/L (76  $\mu\text{M}$ ) (Woodhouse et al., 1983). The acetylation ratio (MADDS:DDS) shows a genetically determined bimodal distribution, allowing the definition of 'slow' and 'rapid' acetylators (Zuidema et al., 1986).

## 2. DDS-NHOH toxicity

Adverse effects of dapsone therapy are the cause of an idiosyncratic reaction, called dapsone hypersensitivity syndrome (DHS) (Orion et al., 2005; Sener et al., 2006), and, more frequently, dose-related methaemoglobinaemia and haemolytic anemia (Cream, 1970).

DHS includes a number of adverse effects including fever, rash, and internal organ involvement, all related to the bioactivation of DDS into DDS-NHOH (Prussick R & Shear NH, 1996). Bioactivated drug represent the first step in the formation of toxic intermediates, which bind covalently to or modify various molecules through the process defined haptenation, where a small molecule can elicit an immune response by attaching to a large carrier, such as a protein. Once the body has generated antibodies to a hapten-carrier adduct, it will usually initiate an immune response.

It has been recently demonstrated that skin (Roychowdhury et al., 2007) and human keratinocytes are able to convert DDS to hydroxylamine by the action of myeloperoxidase (MPO). Once formed, these highly reactive metabolites can bind to cellular proteins and act as haptens, promoting autoimmunity in susceptible individuals (Vyas et al., 2006).

DDS mediated haemolytic anemia is closely related to erythrocyte membrane alterations leading to premature cell removal, which can occur both extravascularly, by spleen-mediated subtraction of damaged erythrocytes, or intravascularly, by DDS induced cell fragility. All haematological side effects reported for DDS therapy are due to the N-hydroxy metabolites of the drug, dapsone hydroxylamine (DDS-NHOH).

## 3. Erythrocytes and DDS-NHOH toxicity

### 3.1 In vitro alterations of normal erythrocyte membranes

DDS-NHOH undergoes a coupled oxidation-reduction reaction with haemoglobin and molecular oxygen yielding methaemoglobin and ROS formation (ferryl haem and hydroxyl radicals) (Fig. 1), respectively (Bradshaw et al., 1997).

To date, no direct evidence of the mechanism whereby DDS-NHOH shortens the erythrocyte lifespan has ever been reported. Only the fact that DDS-NHOH affects the integrity of the erythrocyte lipid bilayer has been excluded, since neither lipid peroxidation nor phosphatidylserine (PS) externalisation have ever been detected (McMillan et al., 1998; McMillan et al., 2005).

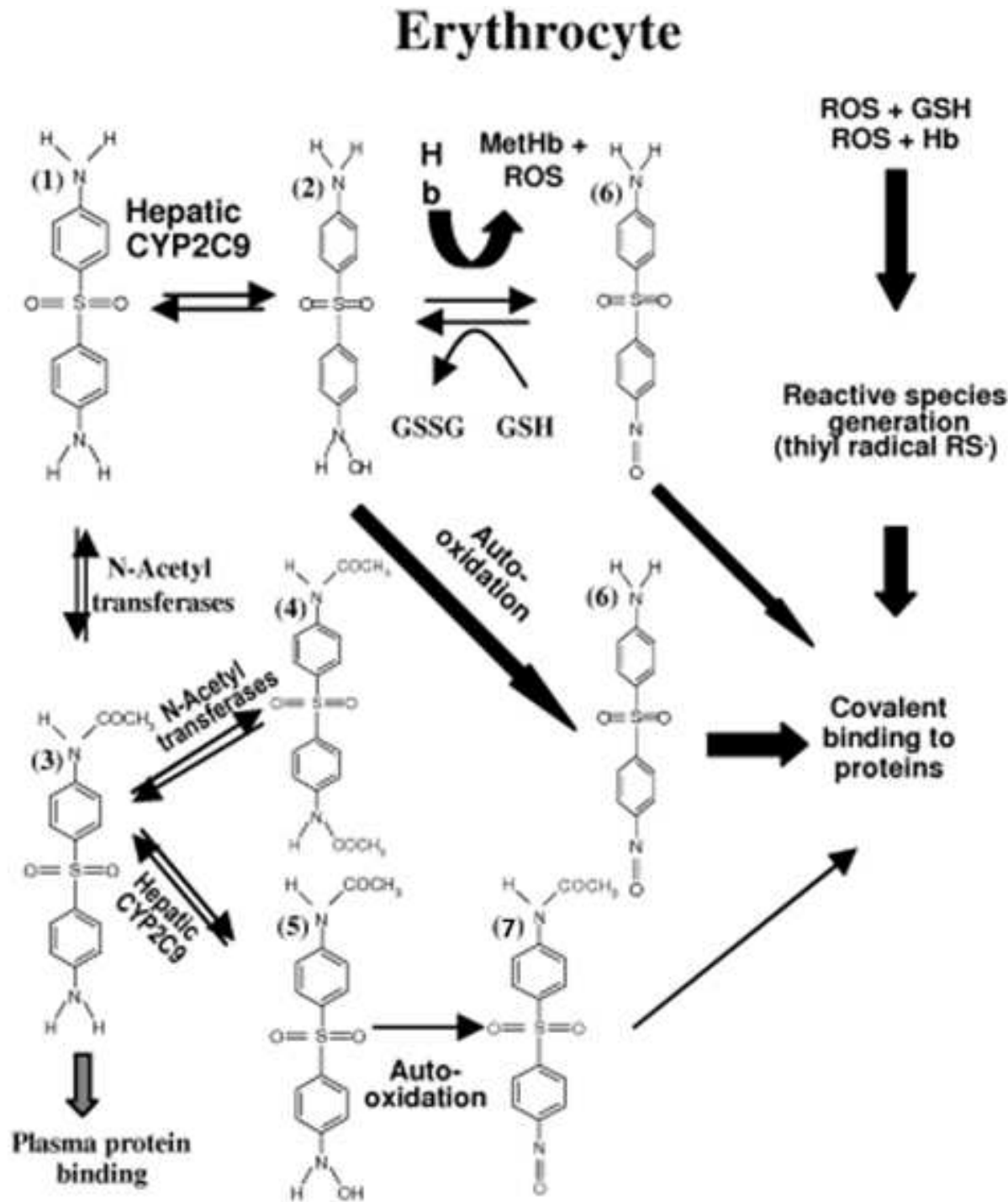


Fig. 1. Scheme showing main features of metabolic fate of dapsone in man. (1) Dapsone; (2) dapsone hydroxylamine; (3) monoacetyl dapsone (MADDS), (4) diacetyl dapsone (DADDS); (5) monoacetyl dapsone hydroxylamine; (6) dapsone nitrosoarene derivatives (7) . monoacetyl dapsone nitrosoarene derivative.

In a recent report (Bordin et al., 2010a) we proposed tyrosine phosphorylation (Tyr-P) level of erythrocyte membrane as diagnostic method to evaluate erythrocyte membrane status. In human erythrocytes, Tyr-P of membrane proteins is the result of the antithetic actions of protein tyrosine kinases (TPKs) and protein tyrosine phosphatases (PTPs) and involves mainly

band 3 protein. This is the most abundant membrane protein of red blood cells and is divided into three regions: an external domain, enriched in glycosyl chains that probably allow band 3 protein to be recognised as a specific antigens (Bratosin et al., 1998); a transmembrane domain, representing the anionic exchanger of cells; and a cytosol portion (Wang, 1994), containing all phosphorylatable residues. Although serine/threonine (Ser/Thr)-phosphorylation of the band 3 cytosol domain has been demonstrated to regulate the anion flux rate (Baggio et al., 1993a; Baggio et al., 1993b), Tyr-P is involved in multiple functions, including regulation of glycolysis (Low et al., 1993), alteration of erythrocyte morphology (Bordin et al., 1995) and volume (Musch et al., 1999), and senescence (Bordin et al., 2009; Pantaleo et al., 2009).

When triggered by oxidative (diamide) or hyperosmotic stress, the band 3 Tyr-P level can predict both pathological and particular physiological conditions. In glucose-6-phosphate dehydrogenase (G6PD) deficiency, the higher band 3 Tyr-P level, compared with normal control cells, correlates well with chronic impairment of cell anti-oxidative defences (Bordin et al., 2005b); conversely, the lower band 3 Tyr-P level observed in pregnancy is synonymous of characteristically increased anti-oxidative defences (Bordin et al., 2006).

Methemoglobinemia occurs to some extent in all patients receiving DDS and becomes less pronounced as treatment is continued because of an adaptative increase in the activity of NADH-dependent reductase in erythrocytes (Orion et al., 2005). Methemoglobin (MetHb) production is due to oxidation of hemoglobin by nitroso species which react with NADPH (Kiese et al., 1966) or glutathione (GSH) (Coleman et al., 1994) to regenerate hydroxylamines. Reilly and co-workers (Reilly et al., 1999) showed that GSH, rather than NADPH, is the key reducing specie responsible for regenerating hydroxylamine metabolites and that any GSH consumed must be rapidly regenerated.

We observed that DDS-NHOH, when added to intact erythrocytes in *in vitro* experiments, triggered the formation of both MetHb and Tyr-P level of band 3 (Bordin et al., 2010b). This last process was time and dose-dependent by DDS-NHOH but only for the early 30 minutes of incubation and to 0.3 mM concentration. Increasing incubation time (50 min) and effector dose (0.6 mM), band 3 Tyr-P decreased to negligible level.

We compared these effects with those induced by diamide (Bordin et al., 2005a), which increased protein phosphorylation level by inhibiting tyrosine phosphatase activities by directly oxidising cysteine located in the catalytic domain of the enzyme (Hecht & Zick, 1992), and by inducing immediate band 3 clustering (Bordin et al., 2006; Fiore et al., 2008).

Our findings showed that both Tyr-kinase and phosphatase activities were promptly inhibited by DDS-NHOH in both dose- and time-dependent manners, and total inactivation was reached in both after 60 min incubation with 0.15 and 0.3 mM. At 0.6 mM, DDS-NHOH treatment was almost completely inhibitory after only 15 minutes of incubation. This suggests that the triggering of band 3 Tyr-P is not due to an imbalance between enzymatic activities but, more probably, by a favoured substrate-kinase interaction, at least up to 0.3 mM within 30 min. Longer incubation times or higher compound concentrations resulted in the total disappearance of band 3 Tyr-P, as well as total enzyme inhibition. This time-dependent increasing effect of DDS-NHOH indicated that there is progression in the action mechanism of the compound.

In addition, it has been previously demonstrated that band 3 structural alterations can be useful to further reveal the status of membranes (Bordin et al., 2006). DDS-NHOH treatment induced band 3 aggregation in high molecular weight aggregates (HMWA) mainly located in the Triton-soluble part of the membrane. This effector differentiated greatly from diamide: its time-dependent effect increased in a sort of amplifying system, leading to



further increases in band 3 HMWA, but, more interestingly, also to their total relocation within the membrane, accompanied by reorganization of both PTKs (Brunati et al., 2000) and PTPs (Bordin et al., 2002), independently from band 3 Tyr-P level. This new membrane set up was easily recognized and marked by autologous IgG, representative of damaged cells (Bordin et al., 2010b).

This raises the hypothesis that the gradual band 3 Tyr-P tailing off within the first 45 min may represent the time threshold between the formation of two differently located band 3 aggregates - Triton-soluble, and, successively, cytoskeleton bound. Accordingly, the Tyr-phosphorylative process may be considered a cellular defence against the incoming oxidative modifications induced by DDS-NHOH. In this process, introduction of negative charges, represented by phosphate groups, to band 3 protein would slow down its aggregation, at least up to the total arrest of the phosphorylative process. Subsequently, modifications would continue more profoundly, inducing not only more marked clustering of band 3 but also totally redistributing HMWA from soluble to insoluble (cytoskeleton) membrane fractions. This is further suggested by total rearrangement of band 3 HMWA at 0.6 mM DDS-NHOH: in these conditions, band 3 Tyr-P is very slight, and band 3 HMWA were located in the cytoskeleton even after 30 min incubation (Bordin et al., 2010b).

This may fit the hypothesis that reactive radicals also generate a second species of radicals, probably a thiyl radical (McMillan et al., 2005), more reactive and efficacious in generating so many and drastic alterations in membrane structure and composition.

Taken together, the direct evidence of the mechanism whereby DDS-NHOH shortens the erythrocyte lifespan is consistent with progressive oxidative alteration starting from cytosol, where it induces methaemoglobin formation (Israili et al., 1973; Schiff et al., 2006), glutathione oxidation, and initial impairment of Tyr-protein kinase and phosphatase activities. Later, the effect of DDS-NHOH advances, with progressive reorganisation of membrane/proteins, as evidenced by enzyme recruitment and the formation of band 3 aggregates (HMWA) (Bordin et al., 2010b). Lastly, general membrane reorganisation is achieved, with protein relocation from the Triton-soluble compartment to the cytoskeleton and with autologous antibody recognition (Bordin et al., 2010b). The fact that DDS-NHOH affects the integrity of the erythrocyte lipid bilayer has been excluded, since neither lipid peroxidation nor phosphatidylserine externalisation have ever been detected (McMillan et al., 1998; McMillan et al., 2005).

### **3.2 Erythrocyte membrane alterations in Glucose-6-Phosphate Dehydrogenase (G6PD) deficient patients in dapsone therapy**

In order to verify whether the above mechanism of DDS-NHOH-induced membrane reorganisation was the mechanism effectively leading to erythrocyte denaturation/removal *in vivo*, we analysed membranes from two patients in dapsone treatment (DT) for dermatitis herpetiformis (Bordin et al., 2010b). The two patients were diagnosed as suffering from dermatitis herpetiformis (DH) according to skin biopsies and cell surface deposition of IgA, and were given oral dapsone. At admission, both had normal blood and urine samples. Their treatment started with 100 mg/day DT, as usual dose (Leonard & Fry 1991).

Patient 1 remained successfully in treatment for the length of the study; blood was withdrawn before and during dapsone administration (after 14 days' treatment).

Patient 2, was hospitalised for a haemolytic episode following 3 days of 100 mg/day DT (P2<sub>100</sub>). His laboratory tests revealed that he had Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, class II, according to the WHO directive (Betke et al., 1967). G6PD residual activity in red cells was < 10%, measured spectrophotometrically at 340 nm on a

Sigma diagnostic kit (Sigma-Aldrich, Italy). Dapsone was discontinued for a month, after which laboratory test results had returned to normal range. Dapsone treatment (DT) was later re-administered, starting with two days with 30 mg/day, and then 50 mg/day, with partial relief but not total remission of symptoms.

Blood samples from both patients were taken before and during treatments. Samples from patient 1 were called P1 and P1<sub>100</sub> to indicate samples before administration and during 100 mg/day DT; erythrocytes from patient 2 were called P2, P2<sub>30</sub>, and P2<sub>50</sub> to indicate samples withdrawn before and after 2 days at 30 mg/day, or after 3 days at 50 mg/day DT, respectively. Erythrocytes were analysed for their band 3 HMWA and IgG bound contents. DT in patient 1 (P1) induced a slight increase in band 3 HMWA, which was correlated with an increase in bound IgG (Fig. 2, panel A). Erythrocyte membranes from patient 2 showed a higher level of basal band 3 HMWA (P2), which increased (+18%) during the 30 mg/day DT, but reached a dramatic level at 50 mg/day (+215%). The effect was correlated with a 30% increase in bound IgG in P2<sub>30</sub> and with more than 120% in P2<sub>50</sub>.

P1<sub>100</sub> was chosen as arbitrary unit to indicate erythrocyte membrane alterations (band 3 HMWA and IgG binding) induced by DT (A) or band 3 Tyr-P induced by diamide (B) in normal patients.

In addition, when analysed for Tyr-P level extent, membranes from erythrocytes of both patients showed that the basal level of band 3 Tyr-P was negligible. Successive analysis of glutathione content evidenced that DT induced a decrease in total GSH content in both patients (Bordin et al., 2010b). However, P1<sub>100</sub> maintained about 85% of total glutathione in reduced form (GSH), but P2 showed progressive depletion of glutathione, with an alarming rise in oxidised glutathione (GS-SG) which, at P2<sub>50</sub>, reached almost 60% of total glutathione. To induce weak oxidative stress, addition of 0.3 mM diamide to isolated erythrocytes from both patients was performed. P1<sub>100</sub> showed a reduction in total glutathione content and a rise of GS-SG. P2 and P2<sub>30</sub> highlighted a net reduction in the amount of total glutathione which, at P2<sub>50</sub>, was only 50%, compared with the glutathione content of P2. Diamide induced net increase in the GS-SG form, which reached almost 100% glutathione at P2<sub>50</sub>.

When analysed also for their Tyr-P content after 0.3 mM diamide treatment (inconsistent with Tyr-P triggering in normal subjects), patients presented clear differences (Bordin et al., 2006) (Fig. 2, panel B). The first patient showed a slight trace of band 3 Tyr-P only after DT (P1<sub>100</sub>). Instead, P2 evidenced net band 3 Tyr-P (as expected, due to his G6PD deficiency), which dramatically escalated on increasing DT (Fig. 2 panel B). Syk and SHP-2 content in membranes from P2 also rose after DT, in both the absence and presence of diamide incubation (Bordin et al., 2010b).

This is in line with what evidenced in vitro from normal erythrocytes: in normal subjects, therapy leads to weakening of anti-oxidant defences (as indicated by decreased GSH content) and triggers membrane reorganisation, as indicated by increased band 3 HMWA formation (Fig. 2, panel A) and higher sensitivity towards diamide-induced oxidative stress. When dapsone was administered to G6PD patient (P2), drops in both haemoglobin content and haematocrit were observed at P2<sub>50</sub>, suggesting the onset of the haemolytic process. This cannot be explained by the simple fall in GSH content since, even at 50 mg/day dapsone (P2<sub>50</sub>), almost one-third of total glutathione is in reduced form, but incapable of preventing DT-induced erythrocyte modification. In other words, glutathione is not sufficient to counteract membrane oxidation induced by dapsone, because its metabolite, DDS-NHOH, acts on different substrates in a time-dependent progressive ROS formation. That hydroxylamine is the responsible of the alterations is confirmed by the fact that DT induces the same membrane

alterations than those previously shown in *in vitro* experiments with DDS-NHOH, such as band 3 HMWA formation and IgG binding increase. Instead, band 3 Tyr-P was not detected, even in P2<sub>50</sub> erythrocytes, although Tyr-protein kinases and/or phosphatases were not inhibited in these conditions, as indicated by the following diamide-induced band 3 Tyr-P of patients' erythrocytes (especially in P2). This was probably because the concentration of this effector is insufficient to have immediate effects on the enzymes, like those evidenced in *in vitro* experiments, which would be representative of high toxicity. Band 3 Tyr-P level, therefore, is to be dependent on the net alteration of erythrocyte membrane following DT.

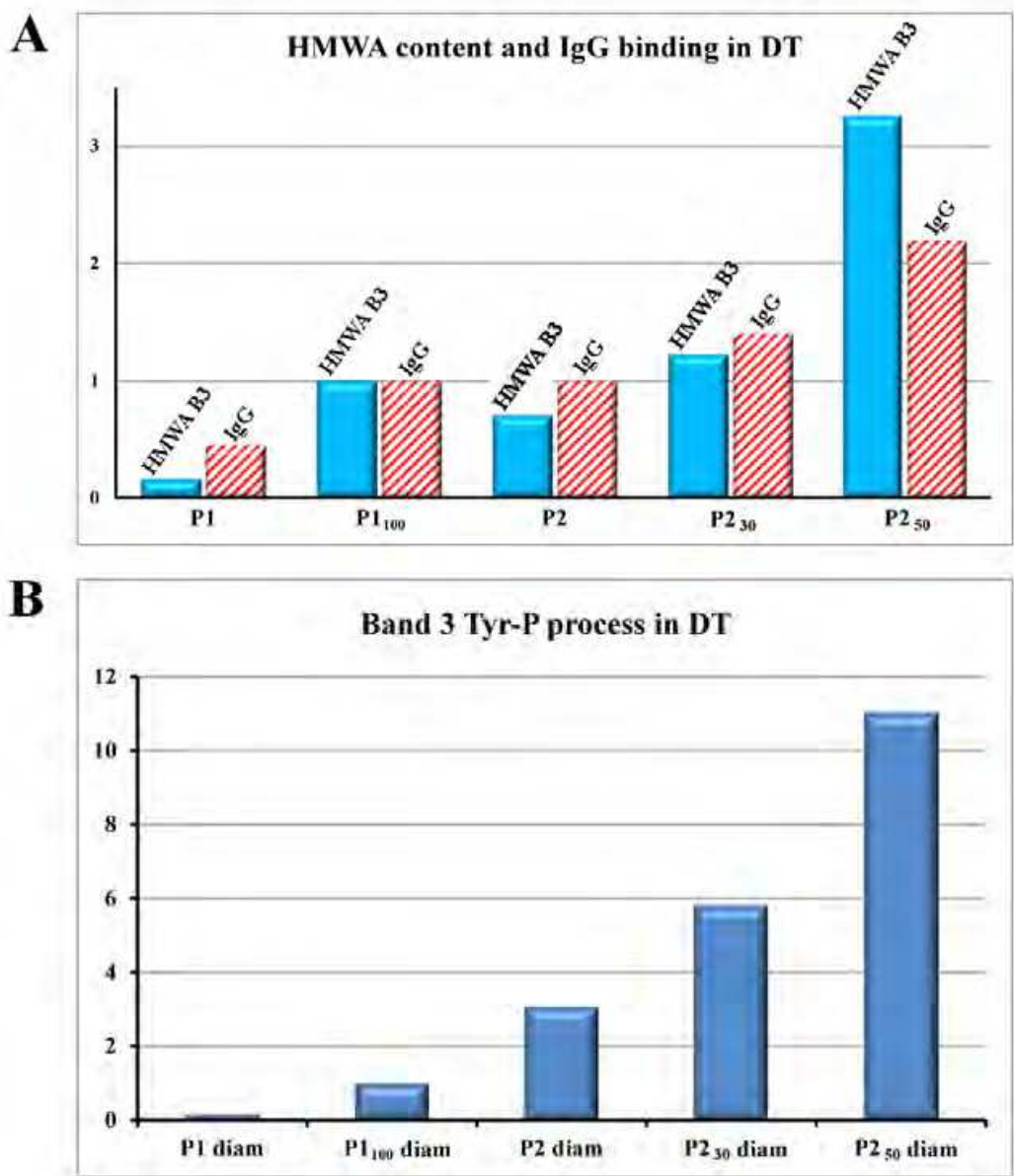


Fig. 2. Effect of dapsone treatment (DT) on erythrocyte membrane rearrangement. Erythrocytes from patients 1 and 2 before (P1 and P2) and after DT (P1<sub>100</sub> and P2<sub>30</sub> and P2<sub>50</sub>) were directly analysed for high molecular weight aggregate (HMWA) of band 3 and IgG binding (panel A), or incubated with 0.3 mM diamide to trigger band 3 Tyr-P level (panel B).



**3.3 DDS-NHOH-induced alterations in erythrocyte from endometriotic patients: Potential toxicity in inflammatory disease**

In the above paragraph, it has been reported that band 3 Tyr-P levels were negligible in erythrocytes from patients during DT, and diamide addition was useful to investigate membrane status, mainly cell capacity of counteracting additional oxidative stress.

To evidence the direct effect of pre-existing inflammatory status on DDS-NHOH treatment, we compared band 3 Tyr-P levels induced by increasing concentrations of DDS-NHOH on erythrocytes from endometriotic patients with that obtained in normal erythrocytes (Figures 3 and 4).

Figure 3 shows band 3 Tyr-P obtained with 0.15, 0.3 and 0.6 mM DDS-NHOH in erythrocytes from endometriotic patients (panel A, lanes b-d), which result much higher than that obtained in the control (lane a) with 0.3 mM (concentration able to induce maximum Tyr-P level in normal erythrocytes (Bordin et al., 2010b)).

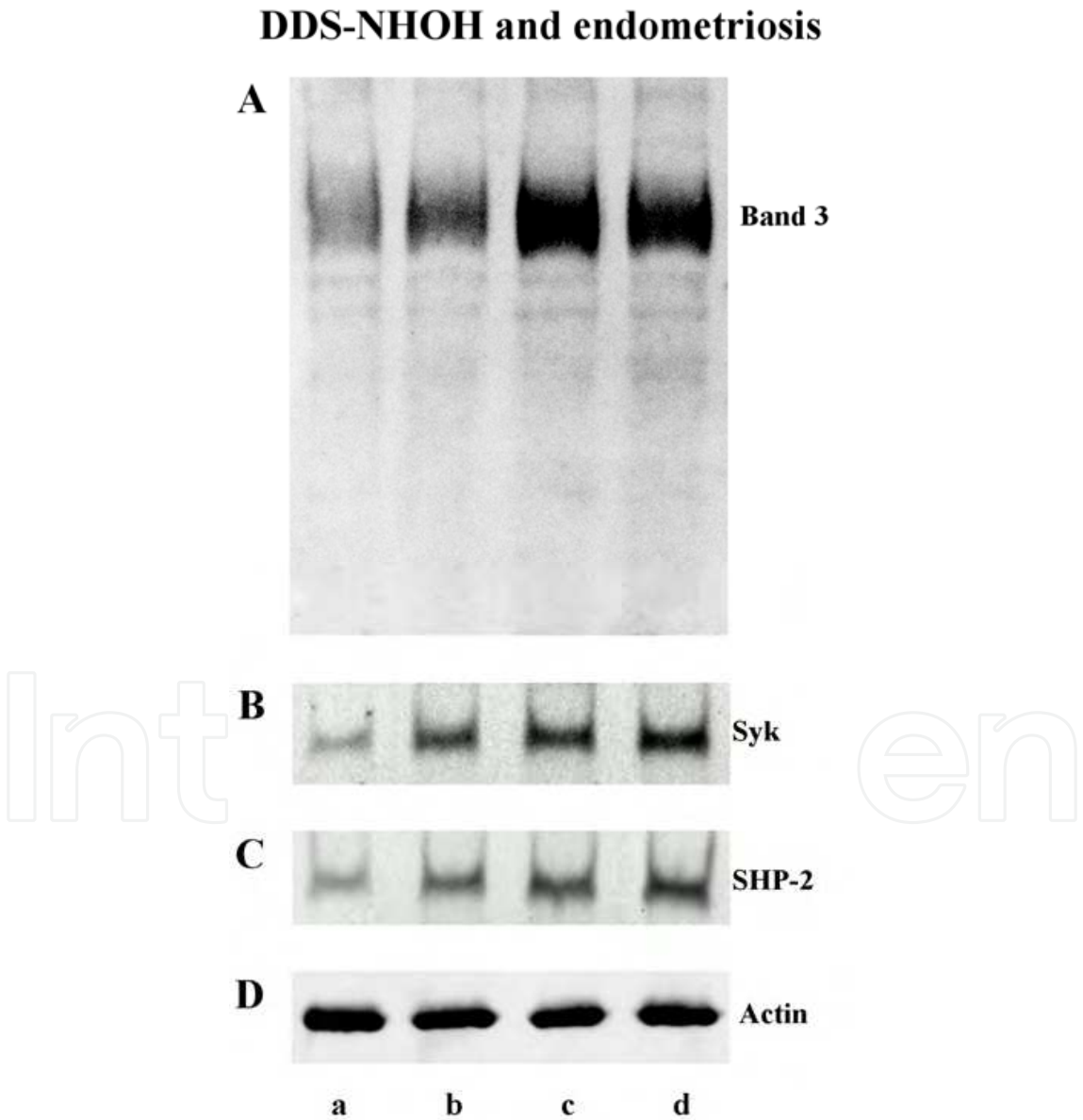


Fig. 3. DDS-NHOH effect on band 3 Tyr-P level (panel A), Syk (panel B) and SHP-2 (panel C) recruitments.

This higher sensitivity of endometriotic erythrocytes towards hydroxylamine was further confirmed by the increased amounts of enzymes, Syk PTK (panel B) and SHP-2 PTP (panel C) bound to membranes following DDS-NHOH treatment. In addition, band 3 HMWA, synonymous of a predisposition of the cell to be recognized by IgG and removed from circulation (Bordin et al., 2010b, Arese et al., 2005; Ciccoli et al., 2004; Kay, 2005; Lutz et al., 1987), were markedly higher in endometriotic cells (Fig. 4) following DDS-NHOH treatment (lanes b-d, compared with lane a, control erythrocytes incubated with 0.3 mM DDS-NHOH).

In order to verify if the patterns of figures 3 and 4 obtained in vitro would mirror potential toxicity for endometriotic patients in DT, we compared them with those obtained by incubating erythrocytes from G6PDd patients in the same above conditions (Fig. 5). Diamide-induced band 3 Tyr-P level and Syk and SHP-2 recruitments were very similar between G6PDd and endometriotic patients, the former reaching the highest values for all parameters, especially when compared with healthy controls.

The high similarity present in in vitro DDS-NHOH treatment between G6PDd and endometriosis erythrocytes strengthens the idea that inflammation status-related alteration would predispose cell to be highly sensitive to the presence of arylamine derivatives, which would lead to potential toxicity to DT.

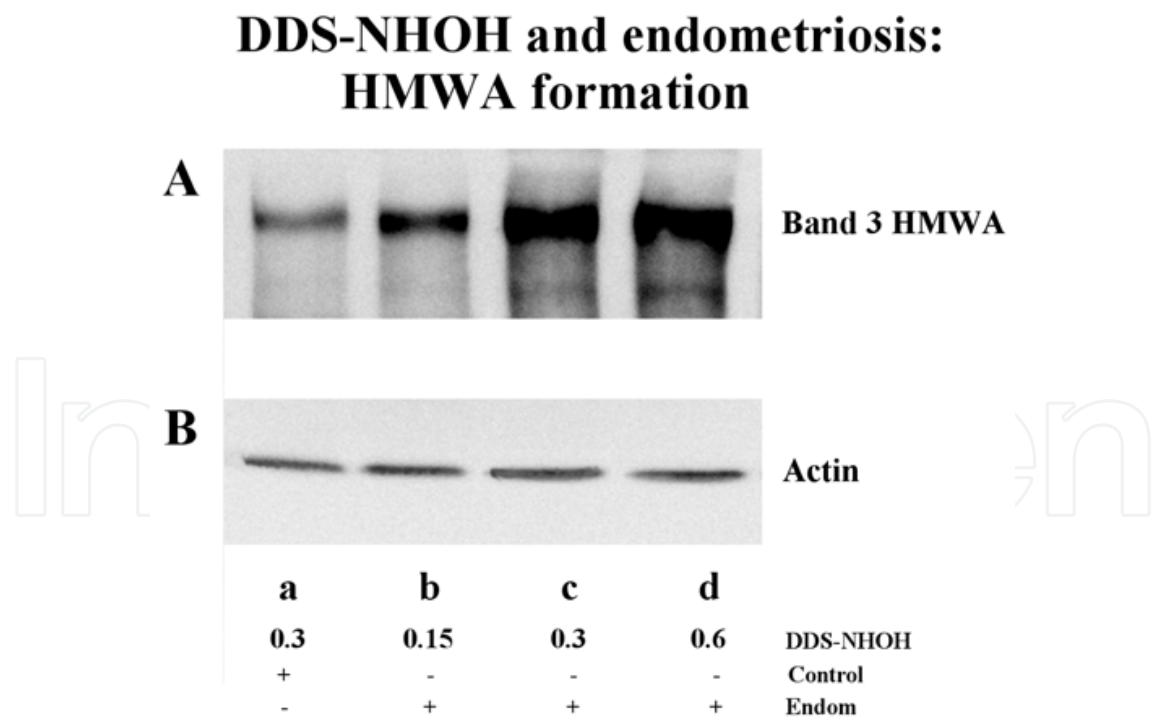


Fig. 4. Effect of increasing DDS-NHOH on band 3 HMWA formation in normal (lane a) and endometriotic patients (lanes b-d).

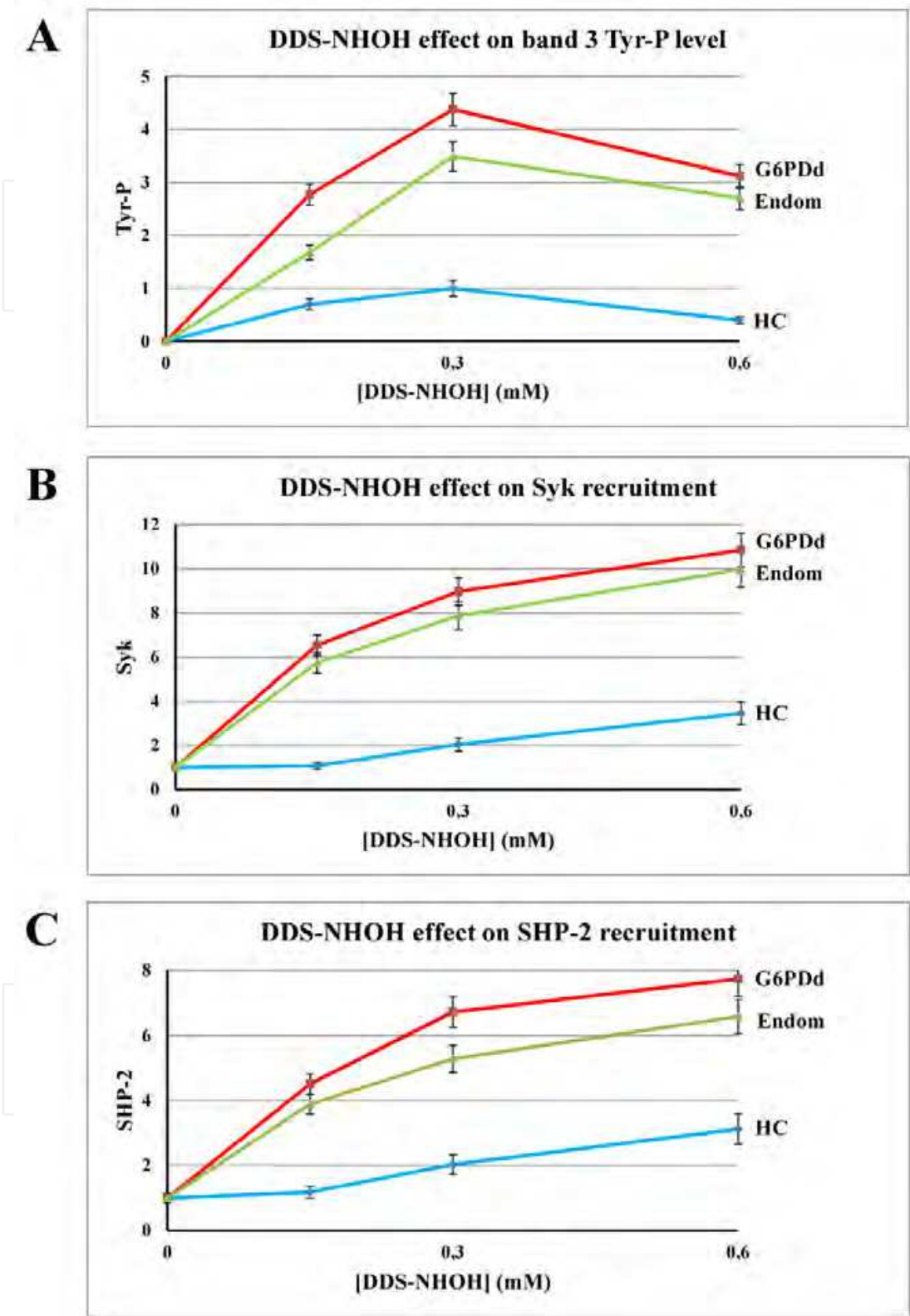


Fig. 5. DDS-NHOH effect on erythrocytes: membrane band 3 Tyr-P level (Panel A), Syk (Panel B) and SHP-2 (Panel C) recruitments, in in vitro experiments: comparison among Healthy Controls (HC), G6PDd and Endometriotic (Endom) patients.

#### 4. Conclusions

G6PD in the hexose (HMP) shunt regulates the production of NADPH, an obligatory substrate for several redox systems, in particular for glutathione, which protects the cell from oxidative stress. It has been previously shown that conditions of oxidative stress lowering NADPH content immediately raise the HMP shunt rate up to 30-fold. Red blood cells with G6PD deficiency cannot increase their shunt sufficiently during an oxidative load, and thus show a weakened cellular redox defence (Jacobasch & Rapoport 1996). In several antimalarial, antipyretics or analgesic drugs' treatments, G6PD deficient patients can not provide an adequate antioxidant defence and their erythrocytes present degenerative parameters, revealing the formation of anomalies in cell morphology and deformability (Jacobasch & Rapoport 1996). Oxidative stress induces haemoglobin (Hb) denaturation and membrane binding of hemichromes, Heinz body precursors, and provokes aggregation of band 3 and deposition of antibodies and complement C3c fragments. In fact, it has been described that membrane clustering of band 3 can allow immune recognition by naturally occurring antibodies, inducing antibody-dependent phagocytosis of senescent/alterate erythrocytes (Arese et al., 2005; Kay, 1984; Low et al. 1985; Schluter & Drenekhanh 1986; Lutz et al. 1988; Arese & De Flora 1990; Hebbel, 1990). Also, band 3 Tyr-P level induced by pathological conditions, could make structural alterations, which probably lead cell into apoptosis, by exposing new band 3 epitopes and favouring cell removal from circulation. Both can induce membrane alterations as well as binding of multivalent ligands, leading to hemolysis (Bottini et al., 1997).

All these facts, together with the G6PDd cell inability to response powerfully to oxidants, indicates that the physiological status of band 3 is essential for erythrocytes survival/apoptosis.

In G6PDd anti-oxidative defences are much lower than those present in endometriosis, which has been demonstrated to correlate with chronic oxidative assault induced by inflammation, rather than impairment in glutathione (GSH) restoring. In addition, pre-existing membrane alterations have been postulated even for endometriotic erythrocytes, as indicated by their higher sensitivity to diamide (Bordin et al., 2010a). In fact, diamide-triggered band 3 Tyr-P level was two or three times higher than those of controls, owed to an altered redox system, predisposing membrane proteins to be more markedly oxidized. This was confirmed by the observation that total cell glutathione does not differ from that of healthy controls (data not shown) but, once the erythrocytes are incubated with diamide, patients' GSH contents are far lower, probably due to membrane oxidative status alterations which retained glutathione under the form of protein glutathionylation (Bordin et al., 2010a).

Our study confirms previous reports, stressing that sensitiveness to the compound is clearly idiosyncratic and dependent on the patho/physiological patients' status (May et al., 1990; May et al., 1992; Wertheim et al., 2006).

From these considerations, the assessment of the pre-existent oxidative status of erythrocytes should be carefully evaluated prior to the choice of the appropriate therapy.

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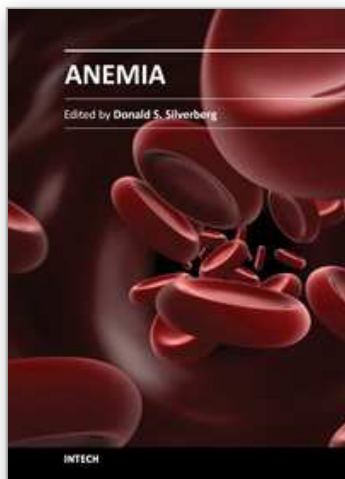
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## **Anemia**

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This book provides an up- to- date summary of many advances in our understanding of anemia, including its causes and pathogenesis, methods of diagnosis, and the morbidity and mortality associated with it. Special attention is paid to the anemia of chronic disease. Nutritional causes of anemia, especially in developing countries, are discussed. Also presented are anemias related to pregnancy, the fetus and the newborn infant. Two common infections that cause anemia in developing countries, malaria and trypanosomiasis are discussed. The genetic diseases sickle cell disease and thalassemia are reviewed as are Paroxysmal Nocturnal Hemoglobinuria, Fanconi anemia and some anemias caused by toxins. Thus this book provides a wide coverage of anemia which should be useful to those involved in many fields of anemia from basic researchers to epidemiologists to clinical practitioners.

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