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Peroxidase-Dependent Oxidation of Sulfonamides by Monocytes and Neutrophils From Humans and Dogs

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SUMMARY

The hydroxylamine and nitroso metabolites formed by N^1-oxidation of sulfonamides are thought to be involved in the pathogenesis of idiosyncratic reactions to this class of drugs. Idiosyncratic reactions to sulfonamides are characterized by multisystemic toxicity, including hepatitis, nephritis, dermatitis, and blood dyscrasias (aplastic anemia, agranulocytosis). We have previously shown that cytochrome P-450 in the liver metabolizes sulfamethoxazole to its hydroxylamine metabolite. In this paper we report the N^1-oxidation of sulfamethoxazole by activated monocytes and neutrophils (human and canine) to form sulfamethoxazole hydroxylamine and nitrosulfamethoxazole. The presumed nitroso intermediate was not detected. Purified myeloperoxidase and prostaglandin H synthase were also capable of mediating the oxidation of sulfamethoxazole. The present studies suggest that myeloperoxidase is responsible for the observed oxidation by phagocytic cells. Oxidation by neutrophils may play a role in agranulocytosis, and oxidation by monocytes may facilitate antigen presentation. Extrahepatic bioactivation of sulfonamides by peroxidases in phagocytic cells and other tissues may be important in determining the range of adverse reactions to sulfonamides that occur.

Adverse reactions to sulfonamides occur in approximately 5% of treated patients, with serious idiosyncratic reactions occurring at an incidence of less than 1% (1). Idiosyncratic reactions in humans include hepatitis, nephritis, blood dyscrasias, drug-induced lupus, and serum sickness; the sulfonamide hypersensitivity syndrome is characterized by fever, skin rash, lymphadenopathy, and multisystemic toxicity occurring 7 to 14 days after start of therapy (1, 2). Hematological disorders may occur as part of a generalized hypersensitivity reaction or as an isolated clinical event (1). Acute hemolytic anemia in humans is usually related to glucose-6-phosphate dehydrogenase deficiency but may occur in apparently normal individuals. Agranulocytosis and aplastic anemia occur at an incidence of 1/1000 or less. There is no clear dose dependency and the exact pathogenesis is unclear (1). In dogs, the only other species in which idiosyncratic reactions to sulfonamides are well documented (3), polyarthritis and fever are the major manifestations of adverse reactions to sulfonamides.

The formation of reactive intermediates is thought to play a crucial role in the pathogenesis of idiosyncratic reactions (4). N^1-Oxidation of sulfonamides to the hydroxylamine and nitroso metabolites is thought to be an important route of bioactivation (5). A decreased ability to detoxify these intermediates may play a role in determining susceptibility to idiosyncratic reactions (2, 6). Traditionally, hepatic metabolism has been considered the major source of reactive intermediates responsible for xenobiotic toxicity (see Ref. 7). However, there is considerable evidence that extrahepatic metabolism is important in the tissue-specific toxicity of many compounds. MPO (8) and PGS (9) have been implicated in the bioactivation of several compounds, including aromatic amines. Such metabolism has been implicated in the myelotoxicity of benzene (10), the nephrotoxicity of acetaminophen (11), and the induction of bladder cancer in dogs by aromatic amines (9). The aromatic amines dapsone and procainamide are metabolized to their corresponding hydroxylamines by MPO (12, 13). It seems likely, then, that similar bioactivation of sulfonamides may take place. It has previously been suggested that the hydroxylamine of SDZ may be formed by a MPO-dependent reaction (12); however, substantiating data have not been presented. The proposed N^1-oxidation of sulfonamides is presented in Fig. 1.

The aim of this study was to determine whether monocytes and neutrophils from humans and dogs, two species that are

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ABBREVIATIONS: MPO, myeloperoxidase; CMF, calcium- and magnesium-free phosphate-buffered saline; HBSS, Hanks' buffered salt solution; PMA, phorbol myristate acetate; SMX, sulfamethoxazole; SDZ, sulfadiazine; SOD, superoxide dismutase; DMSO, dimethylsulfoxide; PGS, prostaglandin H synthase; HPLC, high performance liquid chromatography; SMX-HA, hydroxylamine metabolite of sulfamethoxazole; SMX-nitro, nitro metabolite of sulfamethoxazole.

known to experience idiosyncratic reactions, are capable of oxidizing sulfonamides to known reactive cytotoxic intermediates. When phagocytic cells are activated during phagocytosis or by phorbol esters, they undergo an oxidative burst, producing significant quantities of active oxygen species, including superoxide anion and hydrogen peroxide (14). MPO is released into intracellular phagosomes or into the extracellular space (15, 16). In addition, arachidonic acid is released from the cell membrane, activating the PGS pathway (17). Thus, in these cell types, metabolism of sulfonamides could be mediated by active oxygen species, by MPO, or by PGS.

Materials and Methods

Isolation of leukocytes. For each experiment, 60 ml of heparinized blood were collected by venipuncture from normal human subjects, with informed consent, or from two healthy mature female dogs (cared for under the Guidelines of the Canadian Council for Animal Care). The preparation of monocytes (18) and neutrophils (19) followed standard procedures. For preparation of monocytes, the blood was diluted 1:1 with 0.9% NaCl. Thirty-five milliliters of diluted blood were layered over 15 ml of Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) and centrifuged at 500 × *g* for 30 min. The mononuclear leukocyte layer was harvested and washed twice with CMF (pH 7.4). The cell pellets were resuspended in 3.5 ml of CMF and mixed with 6.7 ml of Sepracell medium (Sepratech Corp., Oklahoma City, OK). After centrifugation at 1500 × *g* for 20 min, the monocytes were collected from just under the meniscus and washed three times before use. Human monocytes were washed in HBSS (GIBCO Laboratories, Grand Island, NY) and resuspended in HBSS at the desired cell concentration. Canine monocytes were washed with CMF (to prevent clumping) and resuspended in HBSS before use. The isolated cells were confirmed to be monocytes by morphology, α-naphthyl acetate esterase staining (20), the ability to adhere, and the ability to undergo a respiratory burst when stimulated with PMA (21). A commercially available monoclonal antibody against the surface antigen CD14 (IOM2; AMAC Inc., Westbrooke, ME) was also used to confirm the identity of isolated monocytes. By fluorescent microscopy, the preparations contained approximately 70–75% monocytes. The major contaminating cell type was lymphocytes.

Neutrophils were isolated from the red cell pellet obtained after centrifugation over Histopaque-1077. The red cell pellet was mixed with 2% dextran in 0.15 mM NaCl and red cells were allowed to sediment for 40 min at room temperature. The neutrophil-rich supernatant was collected and centrifuged at 300 × *g* to pellet the neutrophils. Contaminating red cells were lysed by the addition of 1 ml of double-distilled water, followed by the immediate addition of 45 ml of CMF. The neutrophils were washed twice in CMF and resuspended in HBSS. The identity of the neutrophils was confirmed by morphological examination. Preparations were greater than 95% pure, with eosinophils being the major contaminating cell type. Viability of monocytes and neutrophils was assessed by the ability to exclude propidium iodide and was at least 95%.

Leukocyte incubations. Standard incubations contained 2.0×10^6 monocytes or 1×10^6 neutrophils, 25 ng of PMA (Sigma Chemical Co.), and 500 μM SMX (Sigma Chemical Co.) (added in 5 μl of DMSO) in 500 μl of HBSS. Some incubations contained 1 mM ascorbic acid. The suspension was incubated for 45 min at 37° in a shaking water bath. A protein-free filtrate was obtained by centrifugation at 2000 × *g* through a micropartition system (Centrifree; Amicon, Danver, MA) at 0° for 10 min.

The following compounds were added to some incubations, at concentrations indicated in the figure legends: catalase, SOD, indomethacin, ibuprofen, methimazole, and sodium azide. Compounds were dissolved in 5 μl of either HBSS or DMSO, depending on solubility. When DMSO was used as a vehicle, a similar quantity was added to the control incubation.

Enzyme incubations. SMX was incubated with purified MPO (Alfa Therapeutics Corp., Los Angeles, CA) and PGS (Oxford Biomedical Research, Inc., Oxford, MI). For MPO, standard incubations contained 500 μM SMX, 1 mM ascorbic acid, and 1.56 units of MPO, in 500 μl of phosphate buffer (pH 7, 0.05 M). Incubations were carried out at room temperature for 15 min, after initiation of the reaction by the addition of 200 μM H_2O_2. Conditions were varied as indicated in the figure legends. For PGS, 500 μM SMX was incubated with 150 units of PGS, 1 μM hematin, 1 mM ascorbic acid, and either H_2O_2 (200 μM) or arachidonic acid (200 μM added in 5 μl of ethanol). Incubations were conducted for 10 min at 37° in 0.1 M sodium phosphate buffer, pH 7.4. All incubation mixtures were treated before analysis as described above for leukocytes.

Analytical procedures. The hydroxylamine and nitro metabolites of SMX for use as standards were synthesized and identified as previously described (22). Ten to forty microliters of the ultrafiltrate obtained above were injected into the high performance liquid chromatograph without further preparation. HPLC was performed with a Waters 501 pump and a Lambda-Max model 481 detector (Waters Associates, Milford, MA). A C_{18} Ultrasphere ODS 5-μm column (150 × 4.6 mm) (Beckman Instruments, Mississauga, Canada) was used in all analyses. For detection of SMX-HA, a mobile phase of water/acetonitrile/acetic acid/triethylamine (80:20:1:0.05, v/v), at a flow rate of 1 ml/min, was used. Under these conditions, SMX-HA is well separated from the parent compound (Fig. 2). The retention times for SMX-HA

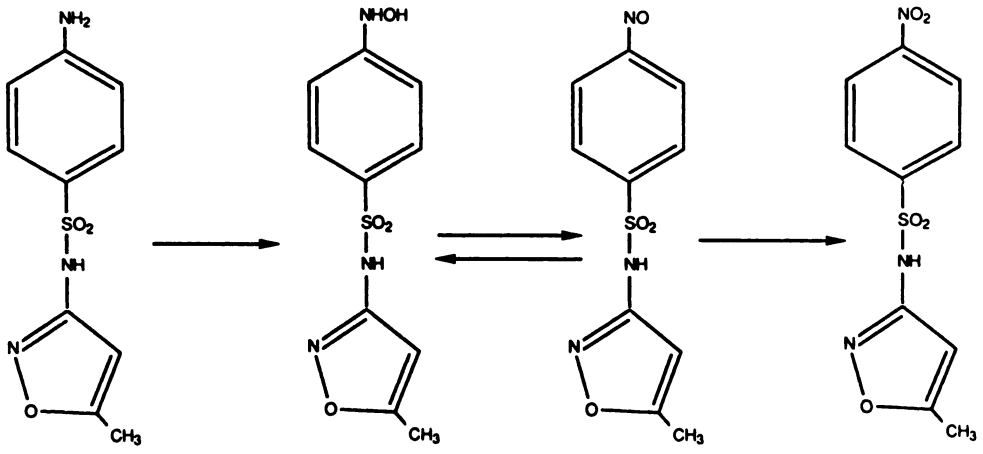

Fig. 1. Oxidative metabolites of SMX.

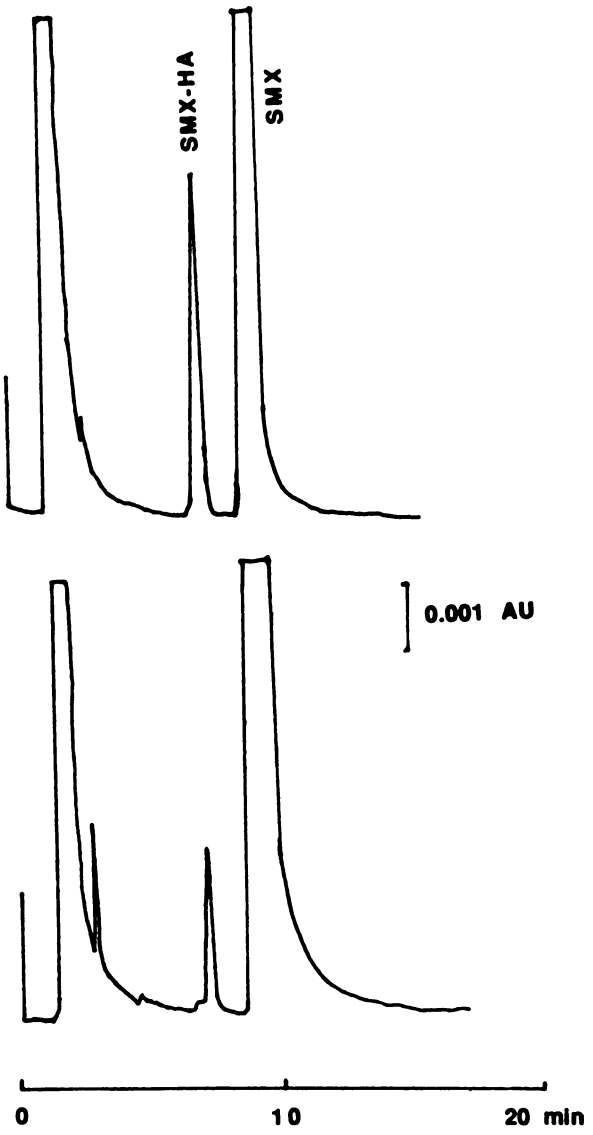

Fig. 2. Typical HPLC tracings for SMX and SMX-HA. *Upper*, chromatogram of aqueous standards of 1 μM SMX-HA and 500 μM SMX. *Lower*, typical chromatogram of SMX-HA formed from 500 μM SMX under experimental conditions (with MPO). *AU*, absorbance units.

and SMX were 7.2 and 9.4 min, respectively. The limit of detection was less than 20 pmol formed when 10 μl were injected. For SMX-nitro, the solvent ratio was 60:40:1:0.05. The retention times were 3 and 5.8 min for SMX and SMX-nitro, respectively. Metabolites were detected at a wavelength of 260 nm for the hydroxylamine and 280 nm for the nitro metabolites. Standard curves for SMX-HA and SMX-nitro were performed on each day of analysis. They were linear over the range of concentrations used (r = 0.99–1.0) and were highly reproducible from day to day.

Results

Metabolism of SMX by activated monocytes. When PMA-activated monocytes (human and canine) were incubated with SMX, two products, which coeluted with authentic SMX-HA and SMX-nitro, were formed. The retention times of the products formed were the same as those of their respective standards under markedly different HPLC conditions (i.e., the products coeluted with authentic standards under both HPLC conditions used, despite marked differences in retention times). Inclusion of ascorbic acid (1 mM) in the incubation medium prevented formation of the product coeluting with SMX-nitro and increased the formation of the product coeluting with SMX-HA. This is consistent with the behavior of the nitro and hydroxylamine metabolites of other aromatic amines formed under similar conditions (12, 13). When the pH of the ultrafiltrate was raised above pH 10 by addition of NaOH, the product coeluting with SMX-HA was eliminated and, if sufficient product was present, a new peak coeluting with SMX-nitro was formed. Conversion to the corresponding nitro derivative at basic pH is a characteristic reaction of hydroxylamines. UV profiles of the product and authentic SMX-HA eluted from the HPLC column showed a single peak, with a maximum at 258–260 nm. Chemical ionization mass spectral analysis of SMX-HA showed a base peak at m/z 254 and an M + 1 peak at m/z 270. An additional peak was present at m/z 268. Loss of 2 and 16 mass units is consistent with the presence of a hydroxylamine. Therefore, the product coeluting with SMX-HA is identified as SMX-HA and the product coeluting with SMX-nitro is identified as SMX-nitro.

In preliminary experiments, SMX-nitro was not consistently formed, as has been described in the metabolism of other aromatic amines by monocytes (12, 13). The inclusion of 1 mM ascorbic acid increased the yield of SMX-HA by 80% (Fig. 3). Although SMX-nitro was formed, the presumed intermediate of this further oxidation, nitroso-SMX, was not detected. Similar results were obtained when SDZ was used as a substrate (data not shown) and, therefore, SMX was studied as the model sulfonamide. Experiments with monocytes were carried out in the presence of ascorbic acid to maximize yield of SMX-HA and to stabilize the product during analysis. Under these conditions, SMX-nitro is not formed and only SMX-HA was quantified in the following experiments.

In general, no oxidation of SMX occurred in the absence of PMA. However, the occasional cell preparation formed a small amount of SMX-HA in the absence of PMA, probably the result of activation during isolation. This is reflected as a small degree of background formation of SMX-HA when the results

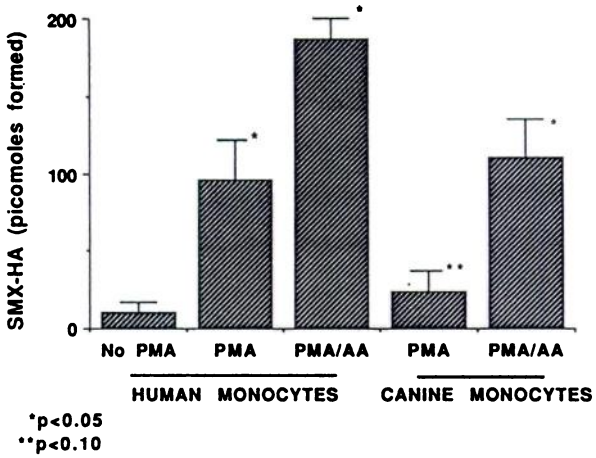

Fig. 3. Metabolism of SMX by human and canine monocytes. Monocytes (2×10^6) were incubated with 500 μM SMX and either 25 ng of PMA or 25 ng of PMA and 1 mM ascorbic acid (*AA*). Incubations were carried out for 45 min at 37°. Results are the mean ± standard error of five experiments with human monocytes and three experiments with canine monocytes.

are pooled (see Fig. 3). The addition of catalase, which decreases available hydrogen peroxide, and methimazole, an inhibitor of peroxidases, inhibited formation of the hydroxylamine (Fig. 4). SOD had no effect on the formation of the hydroxylamine when ascorbic acid was present. These findings suggest that oxidation of SMX is dependent on the presence of hydrogen peroxide and is catalyzed by a peroxidase. Indomethacin (1 mM) significantly inhibited the oxidation of SMX (Fig. 5), but ibuprofen had no effect. Aspirin interfered with detection of the metabolites and could not be used. Neither ibuprofen nor indomethacin significantly inhibited the oxidative burst of monocytes under the conditions employed (data not shown). These results strongly suggest that formation of SMX-HA in monocytes is mediated by MPO.

Metabolism of SMX by activated neutrophils. When activated by PMA in the absence of ascorbic acid, human neutrophils oxidized SMX to SMX-nitro (Table 1). Significant quantities of SMX-HA were not detected, as has been previously reported for procainamide (13). This suggests that the oxidative potential of neutrophils is greater than that of monocytes. The addition of ascorbic acid produced a dose-dependent decrease in formation of SMX-nitro and an increase in the formation of SMX-HA. However, the recovery of SMX-HA did not equal formation of SMX-nitro. Therefore, SMX-nitro was quantitated, because it gave a better indication of the overall

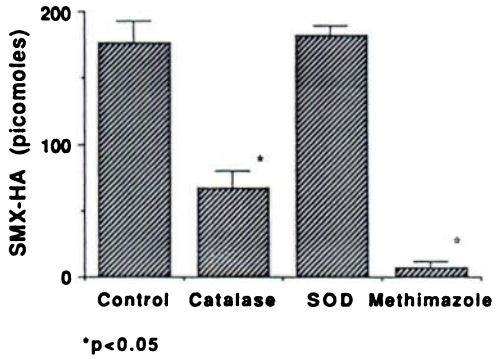

Fig. 4. Effect of catalase, SOD, and methimazole on the metabolism of SMX by activated human monocytes. Control incubations contained 25 ng of PMA and 1 mM ascorbic acid. Catalase (5000 units/ml), SOD (300 units/ml), and methimazole (500 μM) were added before PMA. Results are the mean ± standard error of three experiments performed in duplicate. Similar results were obtained with canine monocytes (not shown).

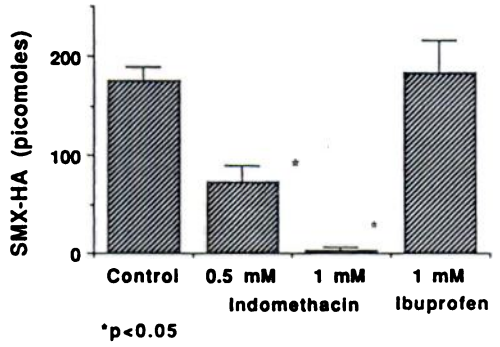

Fig. 5. Effect of PGS inhibitors on metabolism of SMX by activated human monocytes. Control incubations contained 25 ng of PMA and 1 mM ascorbic acid. Indomethacin and ibuprofen were added before PMA. Results are the mean ± standard error of three experiments performed in duplicate. Similar results were obtained with canine monocytes (not shown).

TABLE 1

Metabolism of SMX by activated human neutrophils

Neutrophils (1 × 10^6) were incubated with 25 ng of PMA for 45 min, as described in Materials and Methods. Results represent the mean ± standard error of five experiments performed in duplicate. Inhibitors were used at the concentrations indicated. Results are the mean of two experiments performed in duplicate and are expressed as the percentage of SMX-nitro formed relative to control incubations containing PMA.

Reaction conditions	SMX-nitro
	pmol formed
No PMA	Not detectable
PMA	823 ± 46
Inhibitors	
	% of control
Catalase	
500 units	42
5000 units	17
Methimazole (500 μM)	Not detectable
Sodium azide (1 mM)	9
Ibuprofen (1 mM)	74
Indomethacin (1 mM)	24

metabolism of SMX (Table 1). Activated canine neutrophils also oxidized SMX to SMX-nitro, but their activity was less than that of human neutrophils (159 pmol formed versus 823 pmol formed). As in monocytes, catalase and methimazole inhibited the oxidation of SMX (Table 1). Because an unidentified metabolite of methimazole had a retention time similar to that of SMX-nitro, sodium azide was also assessed as an inhibitor of MPO in neutrophils and produced significant inhibition. Indomethacin markedly inhibited the formation of SMX-nitro, but ibuprofen was not effective (Table 1).

Metabolism of SMX by purified MPO. When purified MPO was incubated with SMX in the presence of hydrogen peroxide and 1 mM ascorbic acid, SMX was oxidized to SMX-HA (Fig. 6). The combination of hydrogen peroxide and MPO was required for maximal oxidation. These incubations were carried out in chloride-free phosphate buffer. Thus, chloride is not necessary for the oxidation of SMX. In fact, addition of 100 mM NaCl inhibited the formation of SMX-HA (Table 2), suggesting that it may compete with oxidation of SMX. When ethanol or buffer alone was used as the solvent for SMX in the presence of high chloride concentrations, several additional peaks were detected by HPLC. These were presumably chlorinated metabolites of SMX. However, because these metabolites were not detected in whole-cell incubations when ethanol was used as a solvent, they were not investigated further.

As in whole-cell incubations, catalase and methimazole inhibited the formation of SMX-HA (Table 2). Indomethacin significantly inhibited the MPO-mediated oxidation of SMX, but ibuprofen had no effect.

Metabolism of SMX by purified PGS. Purified PGS was also capable of oxidizing SMX to hydroxylamine and nitro metabolites. In the absence of hematin (which restores heme lost in the purification of the enzyme), no oxidation was detectable. When phenol was added to the incubation as recommended by the manufacturer (Oxford Biomedical Research, Inc.), no detectable oxidation of SMX took place. However, unidentified peaks were detected, suggesting that oxidation of phenol had occurred. Oxidation of phenol by peroxidases has been previously reported (10). Therefore, it is probable that oxidation of phenol competitively inhibited the oxidation of SMX. The final incubation mixture contained PGS (150 units/ml), 1 μM hematin, 1 mM ascorbic acid, and hydrogen peroxide

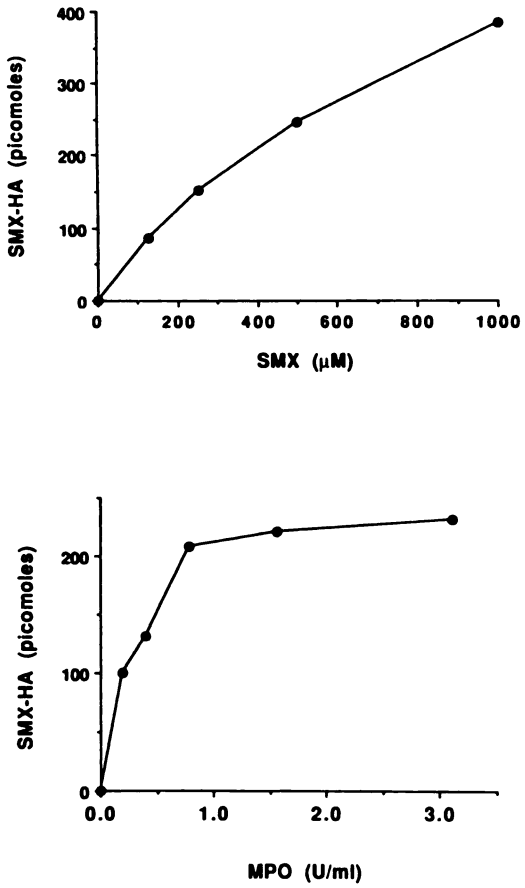

Fig. 6. Metabolism of SMX by purified MPO. Reaction conditions were as described in Materials and Methods. *Points* represent the mean of duplicate determinations. *Top*, the formation of SMX-HA was dependent on the concentration of SMX when MPO was kept constant at 1.56 Units. *Bottom*, increasing quantities of MPO resulted in increased formation of SMX-HA when the concentration of SMX was kept constant at 500 μM.

TABLE 2

Effect of inhibitors on formation of SMX-HA by purified MPO

Control incubations contained 500 μM SMX, 200 μM H_2O_2, 1 mM ascorbic acid, and 1.56 units of MPO in 500 μl of phosphate buffer (pH 7, 0.05 M). Incubation was for 15 min at room temperature. Under these conditions, 230 pmol of SMX-HA were formed. Results are the mean ± standard error of three experiments performed in duplicate.

Reaction conditions	SMX-HA formed
	% of control
Control	100
Indomethacin (1 mM)	48 ± 2
Ibuprofen (1 mM)	105 ± 3
Methimazole (500 μM)	Not detectable
Catalase (5000 units)	Not detectable
NaCl (100 mM)	24 ± 1

or arachidonic acid (Table 3). Both arachidonic acid and hydrogen peroxide were capable of stimulating the oxidation of SMX to the hydroxylamine and nitro metabolites. In the absence of ascorbic acid, SMX was oxidized to SMX-nitro. The ability of hydrogen peroxide to serve as a substrate indicates that the peroxidase portion of PGS is responsible for the oxidation of SMX. This was confirmed by the ability of methimazole to serve as an inhibitor of SMX oxidation (Table 3).

TABLE 3

Metabolism of SMX to SMX-HA by purified PGS

Purified PGS was incubated with 500 μM SMX, as described in Materials and Methods. Substrates (200 μM) were added as indicated. Inhibitors (indomethacin, 1 mM; methimazole, 500 μM) were added before initiation of the reaction. Results are the mean of duplicate determinations.

Reaction conditions	SMX-HA
	pmol formed
PGS (75 units), H_2O_2	268
PGS (150 units), H_2O_2	473
PGS (150 units), arachidonic acid	205
PGS, arachidonic acid, indomethacin	Not detectable
PGS, arachidonic acid, methimazole	Not detectable
PGS, H_2O_2, methimazole	Not detectable

Discussion

Several drugs have been reported to be metabolized by activated phagocytic cells to products that may have therapeutic or toxic properties (8, 10, 12, 13, 23, 24). To this list can now be added sulfonamides. SMX and SDZ were oxidized to hydroxylamine- and nitrosulfonamides by activated monocytes and neutrophils isolated from dogs and humans, two species in which idiosyncratic reactions to sulfonamides occur.

Because activation of the phagocytic cells was required for metabolism to occur, it is unlikely that cytochrome P-450 contributes significantly to the oxidation of SMX. It has been suggested that cytochrome P-450-mediated metabolism of drugs can occur in macrophages (25), but it does not appear to be a significant route for the majority of drugs (13). Catalase, azide, and methimazole inhibited the oxidation of SMX by monocytes and neutrophils, indicating that the oxidation was dependent on the presence of hydrogen peroxide and a peroxidase. The combination of MPO and hydrogen peroxide was also capable of oxidizing SMX.

The effects of PGS inhibitiors on the metabolism of SMX suggest that PGS does not play a significant role in oxidation by phagocytic cells. The relatively high concentrations of indomethacin and ibuprofen were chosen because they correspond to concentrations used by other investigators in assessing the oxidation of arylamines by phagocytic cells (12, 13). Indomethacin (1 mM) inhibited the oxidation of SMX. However, as has been previously reported for phenol (10), indomethacin also inhibited the MPO-mediated oxidation of SMX. Therefore, the inhibitory effect of indomethacin is not specific for PGS at this concentration. Because indomethacin has not been shown to affect the MPO-mediated metabolism of other aromatic amines (12, 13), inhibition may occur through competition for the role of electron donor rather than through a direct effect on the enzyme. Ibuprofen did not significantly inhibit oxidation of SMX; therefore, it appears that the cyclooxygenase portion of PGS does not contribute significantly to its oxidation. This is not surprising, though, because the release of arachidonic acid is small compared with the major respiratory burst (17). The peroxidase portion of PGS may still contribute to metabolism, because co-oxidation of SMX can occur when hydrogen peroxide, rather than the normal prostaglandin hydroperoxide, serves as a co-substrate. In conclusion, the oxidation of SMX in activated peripheral blood monocytes and neutrophils is mediated by the activity of MPO.

The ability of catalase to inhibit the oxidation of SMX indicates that a significant portion of oxidation occurs extracellularly. However, only partial inhibition occurred in mono-

cytes, despite the very high concentration of catalase used; this suggests that intracellular metabolism may also be occurring. PMA is a nonphysiological activator of phagocytic cells and can stimulate the extracellular release of MPO, particularly in neutrophils. Although such extracellular release may occur when phagocytic cells engulf bacteria at the sites of infection, such extracellular release is unlikely to be a systemic occurrence *in vivo*. Therefore, intracellular metabolism is likely to be of greatest biological significance.

The formation of hydroxylamine requires a two-electron oxidation. MPO and PGS are known to catalyze one-electron and two-electron oxidations of arylamines (8, 9, 12). It is possible, therefore, that one-electron oxidation intermediates are formed and these may be involved separately in covalent binding and/or toxicity (8, 24) or may lead to the formation of the products detected (see below). Attempts to identify hydroxylated metabolites of carcinogenic arylamines formed through peroxidative metabolism have generally not been successful (26), but *N*-hydroxylation of other aromatic amines occurs readily (12, 13). It appears then that the chemical structures of the substituents of the aromatic ring play an important role in determining the exact products of oxidation of the aromatic amine. Such a discussion, however, is beyond the scope of this paper.

The inclusion of ascorbic acid was necessary for the detection of SMX-HA in neutrophils and purified enzyme preparations. We hypothesize that the increased yield of SMX-HA is the result of prevention of further oxidation to the nitroso and nitro metabolites. Ascorbic acid has been reported to increase the activity of MPO towards some substrates (27). However, in neutrophils, ascorbic acid decreased the overall metabolism of SMX. Thus, increased activity of MPO is an unlikely explanation for the increased yield of SMX-HA. The direct formation of a nitroso metabolite without an intermediate hydroxylamine is possible, as has been proposed for 2-aminofluorene (26). However, the detection of the hydroxylamine in monocyte incubations without the addition of ascorbic acid suggests that the hydroxylamine is formed initially but that it is rapidly oxidized by hydrogen peroxide (enzymatically or spontaneously). Further, the formation of a nitroso metabolite without a hydroxylamine intermediate would require either a four-electron oxidation, which cannot be mediated by peroxidases, or a series of one-electron intermediates (see Ref. 26). One-electron intermediates would likely be reduced by ascorbic acid, preventing the formation of the nitroso metabolite and, thus, SMX-HA. The concentrations of ascorbic acid used are physiologically relevant, inasmuch as the intracellular concentration of ascorbic acid in neutrophils and monocytes is between 0.7 and 4 mM (8, 27). Hence, the hydroxylamine metabolite is formed under conditions encountered *in vivo* and its formation is, therefore, physiologically relevant.

The hydroxylamine and nitroso metabolites of sulfonamides are known to be toxic to mononuclear leukocytes (22, 28, 29) and other cell types, including renal and hepatocyte cell lines.[1] In a previously reported study of individuals susceptible to idiosyncratic reactions, SMX-HA was toxic to isolated mononuclear leukocytes at concentrations of 1–3 μM (2, 6). These low concentrations of SMX-HA are achievable under our experimental conditions, despite the low rate of the reaction. However, the role of peroxidative metabolism in the toxicity of sulfonamides, and indeed of most other drugs, is largely speculative.

The hydroxylamine of dapsone is toxic to bone marrow cells in culture (30) and is thought to be responsible for the hemolytic anemia associated with dapsone therapy (31). It is likely then that the hydroxylamine metabolites of sulfonamides are involved in the hemolytic anemias associated with sulfonamide therapy in some individuals. Metabolism within the bone marrow could result in the local formation of the cytotoxic hydroxylamine metabolites and lead directly to agranulocytosis (10). Alternatively, local production of reactive metabolites could result in covalent binding to the surface of leukocytes and lead to an immune response directed against neoantigens thus formed.

It has been suggested that bioactivation of drugs by monocytes is an important step in the occurrence of generalized hypersensitivity syndromes (32). It is hypothesized that attachment of a drug or its metabolites to the surface of B lymphocytes and/or antigen-presenting cells could lead to the stimulation of autoantibody production or formation of antibodies against drug metabolites (33). Antinuclear antibodies and a lupus-like syndrome have been described in patients receiving sulfonamides, but they are not hallmarks of the sulfonamide hypersensitivity syndrome. Except in the case of anaphylactic reactions to sulfonamides (34), antibodies to sulfonamide metabolites have not yet been described. Therefore, the relative *in vivo* importance of direct cytotoxicity or indirect antibody-mediated toxicity remains unknown.

In patients with acquired immunodeficiency syndrome, the incidence of neutropenia and other adverse reactions to sulfonamides is significantly higher than in the general population (35). In one report, over 50% of treated patients developed leukopenia and 25% developed thrombocytopenia (35). The presence of an underlying infection (e.g., *Pneumocystis carinii*) could result in the activation of granulocytes, with the subsequent production of cytotoxic metabolites. Glutathione is thought to be important in protecting cells against the toxic effects of hydroxylamines (22). Preliminary observations suggest that patients with acquired immunodeficiency syndrome may have a deficiency in systemic glutathione (36). This, when coupled with increased local formation of toxic metabolites, could lead to the observed high incidence of neutropenias and other adverse reactions.

Tissues other than monocytes and neutrophils also contain peroxidases and there is supporting evidence that local production of reactive metabolites by peroxidases may contribute to tissue-specific toxicity. We have previously reported the unique occurrence of hypothyroidism in a group of patients experiencing idiosyncratic reactions to sulfonamides (37). Subsequently, we have shown that thyroid peroxidase is capable of mediating the oxidation of SMX to its hydroxylamine,[1] which is toxic *in vitro* to thyroid cells (37). *In vivo*, patients develop antithyroid peroxidase antibodies, supporting the concept that SMX covalently binds to the peroxidase to form a neoantigen. Thus, both direct and antibody-mediated toxicity may contribute to the occurrence of clinical signs, with the local peroxidase-dependent oxidation of SMX being a necessary component of the reaction.

Developing monocytes contain large amounts of peroxidase. However, as they mature and migrate to tissues to become

[1] Cribb A. E., Spielberg, S. D., Unpublished observations.

macrophages, gene expression for MPO is lost (38). Therefore, if oxidation of sulfonamides occurs in tissue macrophages, it is likely to occur through the activity of PGS. Similarly, as has been suggested for the oxidation of benzene metabolites (39), PGS may be important in the bone marrow. The ability of purified PGS to mediate the oxidation of SMX clearly suggests that PGS present in other tissues could play a role in the bioactivation of sulfonamides. Further work is required to determine whether PGS is important in macrophage-mediated oxidation of sulfonamides.

Sulfonamides can cause keratoconjunctivitis sicca ("dry eye") in dogs (40); approximately 25% of human patients with toxic epidermal necrolysis develop "dry eye" and approximately 10% show decreased saliva production (41). Sulfonamides are a well recognized cause of toxic epidermal necrolysis and the less severe but related Stevens-Johnson syndrome (42, 43). Lacrimal and salivary glands are known to contain significant amounts of lactoperoxidase (43), which has been shown to bioactivate aromatic amine carcinogens (44) in a similar fashion to MPO and PGS. Formation of the hydroxylamine metabolite in the lacrimal and salivary glands could contribute to toxicity in these glands. Such toxicity usually shows a delayed onset, similar to that seen in sulfonamide-induced hypothyroidism.

In summary, we have shown that N^4-oxidation of sulfonamides to reactive metabolites can be mediated by MPO in activated monocytes and neutrophils and by purified PGS. The peroxidase-mediated metabolism of sulfonamides may be involved in tissue-specific idiosyncratic toxicities and may contribute to the range of clinical signs. However, it is clear that further work is needed to define the role of tissue-specific metabolism in the pathogenesis of idiosyncratic reactions and the relative importance of hepatic versus extrahepatic metabolism. Because it would appear that all individuals are capable of producing reactive metabolites, the ability or lack of ability to detoxify these reactive metabolites may be the most important metabolic factor in determining the susceptibility of an otherwise normal individual to idiosyncratic toxicity, whereas tissue-specific metabolism may be important in defining the range of clinical toxicities experienced.

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Erratum

Volume 37, No. 3 (1990), in the article "Changes in Expression of mRNA Coding for Glutathione *S*-Transferase Subunits 1-2 and 7 in Cultured Rat Hepatocytes" by Yves Vandenberghe, Fabrice Morel, Sally Pemble, John B. Taylor, Vera Rogiers, Damrong Ratanasavanh, Antoine Vercruysse, Brian Ketterer, and André Guillouzo, pp 372–376: the affiliations should be listed as follows:

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