Idiosyncratic toxicity associated with potentiated sulfonamides in the dog

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Idiosyncratic toxicity to potentiated sulfonamides occurs in both humans and dogs, with considerable clinical similarities. The syndrome in dogs can consist of fever, arthropathy, blood dyscrasias (neutropenia, thrombocytopenia, or hemolytic anemia), hepatopathy consisting of cholestasis or necrosis, skin eruptions, uveitis, or keratoconjunctivitis sicca. Other manifestations seen less commonly include protein-losing nephropathy, meningitis, pancreatitis, pneumonitis, or facial nerve palsy. The pathogenesis of these reactions is not completely understood, but may be due to a T-cell-mediated response to proteins haptenated by oxidative sulfonamide metabolites. Our laboratory is working on tests to characterize dogs with possible idiosyncratic sulfonamide reactions, to include ELISA for anti-drug antibodies, immunoblotting for antibodies directed against liver proteins, flow cytometry for drug-dependent anti-platelet antibodies, and *in vitro* cytotoxicity assays. The management of idiosyncratic sulfonamide toxicity involves client education to identify clinical signs early and allow rapid drug discontinuation, supportive care to include possibly ascorbate and glutathione precursors, and avoidance of subsequent re-exposure. It is important to realize that only antimicrobial sulfonamides, such as sulfamethoxazole, sulfadiazine, and sulfadimethoxine, share this clinical syndrome. There is no evidence for cross-reactivity with drugs that have different underlying structures but share a sulfonamide moiety, such as acetazolamide, furosemide, glipizide, or hydrochlorthiazide.

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INTRODUCTION

Sulfonamide antimicrobials such as sulfamethoxazole (SMX), sulfadiazine (SDZ), and sulfadimethoxine (SDM), potentiated by combination with either trimethoprim (TMP) or ormetoprim (OMP), have a broad spectrum of activity, to include urinary, prostatic, skin, and respiratory tract bacterial pathogens; opportunistic mycobacterial infections; and protozoal infections (Greene, 1998; Plumb, 2002). However, the use of potentiated sulfonamides as first-line antimicrobials is limited by concern over the relatively high incidence of adverse reactions to these agents. This review will focus primarily on idiosyncratic reactions, with a brief discussion of dose-dependent reactions included for comparison.

DOSE-DEPENDENT REACTIONS

Adverse reactions to sulfonamides that are dose-dependent include nonregenerative anemia, hematuria, and inhibition of thyroid hormone synthesis. In humans, high prolonged dosages of potentiated sulfonamides lead to megaloblastic anemia, which is caused by inhibition of the synthesis of reduced folate by both the sulfonamide (which acts as a false folate analog) and by TMP (which inhibits the enzyme dihydrofolate reductase). Although aplastic anemia has been documented in dogs receiving prolonged therapeutic and supratherapeutic doses of potentiated sulfonamides experimentally, the anemia is generally normocytic, not megaloblastic, and appears to be related to folate deficiency (Craig & White, 1976; Lording & Bellany, 1978). In reported clinical cases of aplastic anemia in dogs given TMP/SDZ at dosages of 30–60 mg/kg/day, the anemia was normocytic, normochromic, and nonregenerative (Weiss & Adams, 1987; Fox *et al.*, 1993). Serum folate concentrations were not evaluated in these clinical cases, and it is unclear whether the anemia reported in these cases was due to derangements in folate metabolism or whether some of the clinical cases may have involved immune-mediated reactions directed at marrow stem cells.

Sulfonamides, particularly SDZ, may form insoluble crystals in acid urine, leading to tubular obstruction and hematuria in

humans (Abeshouse & Tankin, 1946; Biorn, 1952). This risk is greatest at high doses, or with pre-existing acidosis or dehydration. Sulfonamides have even been associated with uroliths composed of drug and drug metabolites (Albala *et al.*, 1994). These complications have not been reported in dogs, possibly because the *N*-acetylated metabolite appears to be the major offender, and dogs lack the necessary enzyme to make this metabolite (Trepanier *et al.*, 1997).

Sulfonamides have been shown to interfere with thyroid hormone synthesis in several species, including dogs (MacKenzie & MacKenzie, 1943; Roepke *et al.*, 1957; Milne & Greer, 1962; Nishikawa, 1983; Fullerton *et al.*, 1987). Sulfonamides act as a substrate for, and reversibly inhibit, thyroid peroxidase (Doerge & Decker, 1994) and thus interfere with iodination and coupling of tyrosine residues (Kobayashi *et al.*, 1973). Therapeutic doses of sulfonamides appear to have only modest effects on thyroid function in dogs. One study using TMP/SMX at 60 mg/kg/day for 6 weeks found suppression of serum T4 levels below the reference range in 57% of dogs with pyoderma (Hall *et al.*, 1993), while another study using TMP/SDZ dosed at 30 mg/kg/day for 4 weeks found no effect on serum total T4 or free T4 concentrations, or response to exogenous TSH (Panciera & Post, 1992). However, there is at least one report of clinical hypothyroidism in a dog given prolonged therapeutic doses of sulfonamides (two-courses of TMP-SDZ at 48 mg/kg/day, totaling 10 weeks of treatment) (Gookin *et al.*, 1999). In addition, acute hypothyroidism has been reported in human patients as part of a hypersensitivity reaction seen after treatment with one of several sulfonamides (Gupta *et al.*, 1992). In these more unusual cases, the sulfonamide was converted by thyroid peroxidase into a reactive metabolite, which caused local thyroid damage. The hypothyroid state in these cases was reversible with time and removal of the offending sulfonamide. It is not known whether the thyroid is a target for similar toxicity in dogs.

IDIOSYNCRATIC REACTIONS

In contrast to dose-dependent reactions, idiosyncratic reactions to sulfonamides may be seen after just 5 or more days of therapy at standard dosages, and can have severe multisystemic manifestations. These reactions have also been termed 'hypersensitivity' because of their clinical resemblance to delayed immunologic reactions in humans (onset after 5 or more days of treatment; fever, rash, eosinophilia; and more rapid relapse after re-exposure) (Shapiro & Shear, 1996). For the purposes of this review, the terms sulfonamide hypersensitivity and idiosyncratic toxicity will be used interchangeably.

In dogs, sulfonamide hypersensitivity reactions develop an average of 12 days after initiation of sulfonamide treatment (with a range of 5–36 days in a recent survey of 40 dogs; Trepanier *et al.*, 2003a). It is important to note that clinical signs are sometimes noted after a 7–10-day course of sulfonamide has just been completed. Signs in dogs can include fever, polyarthropathy, skin eruptions, hepatotoxicity, thrombocytopenia, neutropenia, hemolytic anemia, uveitis, lymphadenopathy, facial swelling, and proteinuria, with pancreatitis, meningitis, focal retinitis, facial palsy, and polymyositis seen less commonly (Giger *et al.*, 1985; Cribb, 1989; Kunkle *et al.*, 1995; Cribb *et al.*, 1996; Trepanier, 1999; Trepanier *et al.*, 2003a) (Table 1). Although fever was the most common sign in our survey of 40 dogs with sulfonamide hypersensitivity, this is observed in only about 50% of dogs with this presentation (Noli *et al.*, 1995; Trepanier *et al.*, 2003a); therefore, the lack of a fever should not be used to rule out the possibility of sulfonamide hypersensitivity.

Idiosyncratic toxicity reactions to sulfonamides are, by definition, relatively uncommon events. In one retrospective study of dogs and cats referred to the University of Utrecht, an overall incidence of 0.25% (2.5 cases per 1000 prescriptions) was estimated (Noli *et al.*, 1995). However, compared with other antimicrobials and other drugs in general, potentiated sulfonamides appear to be a relatively common cause of hypersensitivity reactions in dogs. One small survey of adverse drug reactions in veterinary patients in the UK attributed almost 10% of all reported reactions to sulfonamides, and among antimicrobials, 82% of reactions were attributed to sulfonamides (Gray, 1991). Interestingly, in this same survey, no adverse reactions in cats were attributed to sulfonamides. In another survey of owners administering antimicrobials to their pets, TMP–SDZ was associated with an 18% adverse reaction rate in dogs, although many of these were nonspecific (gastrointestinal upset, loss of appetite, personality change) and were not followed up with biochemical testing (Kunkle *et al.*, 1995). In the same survey, TMP–SDZ was associated with a 25% adverse reaction rate in cats, with all of these signs being nonspecific (gastrointestinal upset, depression, salivation). Sulfonamide hypersensitivity appears to be much less common in cats than in dogs, although mucocutaneous ulceration and ulcerative dermatitis have been reported in cats (Noli *et al.*, 1995).

All of the potentiated sulfonamides commercially available in the US have been implicated in sulfonamide hypersensitivity, to include TMP/SDZ (Tribrissen, Schering Plough Animal Health, Union, NJ, USA), OMP/SDM (Primor, Pfizer Animal Health, Groton, CT, USA), and human generic formulations (TMP/SMX). It is not entirely clear whether the sulfonamides themselves are exclusively responsible for these reactions, as TMP alone can cause skin eruption or hepatopathy in humans, although much less commonly than sulfonamides alone (Lim, 1992; Lindgren, 1994). In one rechallenge study in a dog with hypersensitivity associated with potentiated sulfonamides, clinical signs were associated with the sulfonamide but not the TMP component (Giger *et al.*, 1985). Interestingly, SDM has been used for decades in dogs as a cocciodiostatic agent without OMP (Albon®, Roche Pharmaceuticals, Nutley, NJ, USA), without reports of drug hypersensitivity in the literature.

Polyarthropathy

One of the most commonly documented idiosyncratic reactions to potentiated sulfonamides in the dog is a syndrome of nonseptic polyarthritis, usually with fever, which involves the distal joints (elbow, stifle, carpal, and tarsal) (Werner & Bright, 1983; Giger

et al., 1985; Lees *et al.*, 1986; Grondalen, 1987; Harvey, 1987; Gray, 1990; Medleau *et al.*, 1990; Trepanier *et al.*, 2003a). In reported cases, the time from drug initiation to onset of lameness ranged from 7 to 21 days, although sulfonamide re-exposure can lead to recurrence of clinical signs in hours to days (Werner & Bright, 1983; Grondalen, 1987). Synovial fluid analysis reveals a predominance of neutrophils without toxic change or organisms seen (Giger *et al.*, 1985; Lees *et al.*, 1986). Improvement is seen within 1–3 days after discontinuation of the sulfonamide, with or without glucocorticoid administration (Werner & Bright, 1983; Giger *et al.*, 1985).

Large breed dogs appear to be predisposed to sulfonamide arthropathy, with Dobermans over-represented. Of 41 cases of sulfonamide-related polyarthritis reported in dogs, 10 cases were in Doberman pinschers (Giger *et al.*, 1985; Whur, 1987; Cribb & Spielberg, 1990; Gray, 1990; Trepanier *et al.*, 2003a), and the majority were in large breeds, including Labrador retriever, Golden retriever, Great Dane, Dalmation, Giant Schnauzer, Briard, Weimaraner, Irish setter, Flat coated retriever, Gordon setter, Springer spaniel, German short haired pointer, and Airedale (Lees *et al.*, 1986; Grondalen, 1987; Harvey, 1987; Gray, 1990; Noli *et al.*, 1995; Trepanier *et al.*, 2003a). Only two cases have been reported in smaller dogs (one Cocker spaniel and one Pekingese: Gray, 1990; Little & Carmichael, 1990). In Dobermans, additional signs of lymphadenopathy, retinitis, protein-losing nephropathy, leukopenia, and modest thrombocytopenia have also been observed (Giger *et al.*, 1985).

The prevalence of large breeds reported with sulfonamide-associated polyarthropathy could represent breeds for which sulfonamides are more commonly prescribed, or a predisposition for immune-mediated polyarthritis in large and hunting breed dogs (Taylor, 2003). Alternatively, it may represent increased weight bearing in these large dogs, with an increased likelihood of either development of lesions in weight bearing joints, or the manifestation of clinical signs associated with such lesions, as has been suggested for fluoroquinolone-associated cartilage damage (Brown, 1996).

The arthropathy component of sulfonamide hypersensitivity syndrome appears to be unique to dogs, and is not reported in humans. The overall incidence of sulfonamide polyarthropathy in dogs is difficult to estimate, as most of these dogs are treated with simple drug discontinuation, and these cases are likely to be under-reported. One survey reported a 4% incidence of lameness in pet dogs given TMP/SDZ, as noted by owners (Kunkle *et al.*, 1995), but polyarthropathy was not confirmed by physical exam or synovial fluid analysis.

Blood dyscrasias

Thrombocytopenia, neutropenia, and Coomb's-positive hemolytic anemia have all been observed in dogs given potentiated sulfonamides (Trepanier, 1999; Trepanier *et al.*, 2003a). In cases referred to veterinary medical teaching hospitals with a final diagnosis of sulfonamide hypersensitivity, thrombocytopenia was a poor prognostic sign, with only 63% recovery compared with 90% recovery in dogs without thrombocytopenia

(Trepanier *et al.*, 2003a). The mechanism of thrombocytopenia is presumed to be immune-mediated destruction, although vasculitis, with secondary platelet activation and consumption, may also contribute in some dogs (Giger *et al.*, 1985). Anti-megakayocyte antibodies are positive in some cases (unpublished observations). One case report of sulfonamide-associated thrombocytopenia in a dog described increased mean platelet volume and platelet fragmentation indices, megakaryocytic hyperplasia on bone marrow cytology, and *in vitro* lysis caused by the convalescent serum (taken during the recovery phase) of the affected dog toward normal dog platelets (Sullivan *et al.*, 1992), suggesting a humoral mechanism. In humans with sulfonamide-associated thrombocytopenia, drug-dependent antibodies against the platelet glycoprotein IIb/IIIa complex have been demonstrated (Curtis *et al.*, 1994). We developed a flow cytometric assay for the presence of drug-dependent anti-platelet antibodies in dogs, and we are now able to screen serum from dogs with sulfonamide-associated thrombocytopenia.

Neutropenia may accompany sulfonamide hypersensitivity in dogs, often as an early and transient finding, and can range from mild neutropenia to agranulocytosis. In one case of severe sulfonamide-associated neutropenia observed by the author, bone marrow cytology suggested acute marrow depopulation with left shift of the granulocytic/monocytic lines, but peripheral neutrophil counts recovered rapidly (within 24 h). In our recent review of 40 dogs with sulfonamide hypersensitivity, 27% of dogs had documented neutropenia, which may be an underestimate of the prevalence of this finding, as not all dogs were evaluated with complete blood counts when clinical signs were first noted.

Hemolytic anemia may accompany sulfonamide hypersensitivity, although this appears to be less common than thrombocytopenia or neutropenia. Eight dogs had evidence of hemolytic anemia (spherocytosis with regenerative anemia, without coagulopathy or blood loss, and/or positive direct Coomb's test) in our series of 40 sulfonamide-hypersensitive dogs (Trepanier *et al.*, 2003a). Six dogs recovered, one was lost to follow-up, and one died of pulmonary thromboembolism. TMP-SMX has also been associated with hemolytic anemia in a horse (Thomas & Livesay, 1998).

Hepatopathy

One of the most dramatic manifestations of idiosyncratic toxicity due to potentiated sulfonamides in dogs is acute hepatopathy. Like thrombocytopenia, this clinical presentation is associated with decreased survival (46% recovery), compared with sulfonamide-hypersensitive dogs without hepatopathy (89% recovery: Trepanier *et al.*, 2003a). The clinical pattern may be acute parenchymal damage with moderate to severe increases in serum alanine aminotransferase (ALT) activity, acute cholestasis with jaundice, or a combination of the two (Toth & Derwelis, 1980; Anderson *et al.*, 1984; Rowland *et al.*, 1992). The most common reported finding on histopathology is marked hepatic necrosis (Thornburg, 1988; Thomson, 1990; Bureau of Veterinary Drugs, 1995; Twedt *et al.*, 1997), although marked

cholestasis or marked lymphoplasmacytic inflammation have also been observed (Rowland *et al.*, 1992; Trepanier *et al.*, 2003a).

Although sulfonamide hepatopathy appears to be relatively uncommon overall, sulfonamides are associated with a significant percentage of drug-induced hepatopathies in dogs. In 1988–90, TMP/SMX was implicated in over 20% of the adverse hepatic drug reactions in dogs reported to the Center for Veterinary Medicine at the FDA (Bunch, 1993). Because of the severity of the reactions, controlled re-challenge has not been performed, although inadvertent re-challenge, with a second episode of hepatopathy, was reported in one Schnauzer (Thornburg, 1988).

Skin eruptions

In one survey of dogs given potentiated sulfonamides, a 0.5% incidence of skin rash and a 1.5% incidence of facial swelling was reported by owners (Kunkle *et al.*, 1995). Severe skin eruptions, to include toxic epidermal necrolysis, erythema multiforme, and pemphigus folliacious, have also been associated with potentiated sulfonamides in dogs, and less commonly, in cats (Scott *et al.*, 1986; Noli *et al.*, 1995; White *et al.*, 2002). In one dog with sulfonamide-associated erythema multiforme, manifested as severe mucocutaneous ulceration and epidermal necrosis, indirect immunofluorescence testing indicated auto-antibody directed against the intercellular matrix of the stratified squamous epithelium (a pemphigus-like pattern: Scott *et al.*, 1986). Urticaria has also been noted in dogs on secondary challenge with potentiated sulfonamides (Werner & Bright, 1983). Other presentations include ulcerative dermatitis, mucosal ulcerations, follicular necrosis, hyperkeratosis, and exudative dermatitis with subcorneal pustules and basement membrane IgG deposition (Scott *et al.*, 1976; Medleau *et al.*, 1990; Noli *et al.*, 1995; Scott *et al.*, 1995). Antinuclear antibody titers have been negative when performed (Scott *et al.*, 1986; Noli *et al.*, 1995). Fever, peripheral eosinophilia, and other systemic signs are variably present. Inadvertent re-challenge has lead to relapse of signs within days (Medleau *et al.*, 1990). Although most dogs recover with drug withdrawal, severe lesions can lead to systemic complications or scarring (Medleau *et al.*, 1990).

Ocular manifestations

Keratoconjunctivitis sicca (KCS) is a fairly common side effect of potentiated sulfonamides in dogs, with an estimated incidence of 15% in treated dogs (Berger *et al.*, 1995). The pathogenesis of sulfonamide-induced KCS in dogs may be distinct from other sulfonamide-associated idiosyncratic toxicity, in that time to onset of signs is often months to years, rather than days to weeks (Morgan & Bachrach, 1982; Diehl & Roberts, 1991). In addition, structurally related arylamine compounds such as phenazopyridine have been shown to be directly cytotoxic to lacrimal gland tissue (Slatter, 1973). However, doses of TMP/SDZ as high as 300 mg/kg/day for 20 days, or 90 mg/kg/day for 8 weeks, have

not reportedly lead to the development of KCS in dogs (Craig & White, 1976; Lording & Bellany, 1978). Therefore, it is unclear whether sulfonamide-associated KCS is primarily idiosyncratic or dose-dependent. In our recent series of 40 dogs with sulfonamide toxicity, KCS was seen as a sole sign in four dogs, but accompanied systemic hypersensitivity (fever, thrombocytopenia, or hepatopathy) in an additional five dogs (Trepanier *et al.*, 2003a). Therefore, it is important to evaluate Schirmer tear tests in dogs suspected of adverse reactions to sulfonamides, as KCS is reversible in some cases if the drug is discontinued promptly.

Uveitis has also been observed in dogs with sulfonamide hypersensitivity, and in some cases may be a sequela to KCS or intraocular bleeding from thombocytopenia. In other dogs without these underlying conditions, uveitis may represent a distinct manifestation of drug hypersensitivity. In humans, uveitis has rarely been reported as the sole manifestation of sulfonamide hypersensitivity (Tilden *et al.*, 1991). Finally, focal retinitis, characterized by retinal edema and reduced tapetal hyper-reflectivity, has been reported in one Doberman with sulfonamide-associated arthropathy, and was reproducible with drug re-challenge (Giger *et al.*, 1985). The lesions resolved with drug discontinuation.

Miscellaneous clinical manifestations

Proteinuria has been associated with sulfonamides (Giger *et al.*, 1985; Trepanier *et al.*, 2003a), and has been attributed to possible drug-induced glomerulonephritis, although renal biopsies have not been performed. Neurologic signs are not common manifestations of sulfonamide hypersensitivity, but fever with neck pain (suggestive of meningitis) and facial nerve palsy have been observed (Giger *et al.*, 1985; Trepanier *et al.*, 2003a). We have also observed pancreatitis and eosinophilic pneumonitis in a few cases; each of these presentations has been reported rarely in humans (Wang *et al.*, 1984; Werth *et al.*, 1995; Brett & Shaw, 1999).

PATHOGENESIS

The prevailing hypothesis regarding the pathogenesis of sulfonamide hypersensitivity is that the parent sulfonamide is bioactivated to reactive metabolites, which haptenize tissue proteins, leading to antigen presentation and T-cell proliferation. There is some evidence for humoral involvement in sulfonamide hypersensitivity, in that human patients with hypersensitivity have been shown to have antibodies that recognize native hepatic microsomal proteins (Cribb *et al.*, 1997), platelet proteins (Kiefel *et al.*, 1987; Curtis *et al.*, 1994), or SMX itself (Daftarian *et al.*, 1995). However, anti-drug antibodies are neither sensitive nor specific for these reactions, as they are absent in many hypersensitive patients (Gruchalla *et al.*, 1998), and may be present in patients who are tolerant to sulfonamides (Daftarian *et al.*, 1995). One dog with sulfonamide-associated thrombocytopenia was reported to have a positive direct ELISA for platelet-bound antibodies (Lewis *et al.*, 1995). We have been

unable to find drug-specific IgG or IgM by ELISA in a group of 20 dogs with sulfonamide hypersensitivity (Trepanier *et al.*, 2002).

There is much more evidence to support a delayed cell-mediated pathogenesis for sulfonamide hypersensitivity, particularly for skin eruptions. Studies of human patients with sulfonamide hypersensitivity document drug- and metabolite-specific T-cell clones (Schnyder *et al.*, 2000; Farrell *et al.*, 2003). In patients with skin rash, these T cells are cytotoxic toward autologous lymphocytes and keratinocytes (Schnyder *et al.*, 1998). In addition, rodents and rabbits dosed with SMX metabolites generate metabolite-specific T cell clones with memory (Farrell *et al.*, 2003). Fixed drug eruption from TMP-SMX has been linked to a specific human leukocyte antigen class I haplotype in humans (Ozkaya-Bayazit & Akar, 2001). Similar studies have not been performed in dogs.

Several metabolic factors may contribute to the individual risk of sulfonamide hypersensitivity in dogs. First, all dogs (in fact, all canids) lack the genes that express *N*-acetylation enzymes, and so cannot detoxify sulfonamides via *N*-acetylation of the parent drug (Trepanier *et al.*, 1997). This is a significant defect, as *N*-acetylation is a major pathway of detoxification of sulfonamides, procainamide, dapsone, hydralazine, and other structurally related drugs in humans (Weber, 1987). Decreased *N*-acetylation activity appears to be a risk factor for sulfonamide hypersensitivity in human patients (Rieder *et al.*, 1991; Wolkenstein *et al.*, 1995a). Therefore, absent *N*-acetylation may put dogs as a species at greater risk for sulfonamide toxicity, but this defect does not explain individual risk among dogs.

Most studies suggest that parent sulfonamides themselves are not the primary mediators of sulfonamide hypersensitivity, although this is somewhat controversial (Britschgi *et al.*, 2003). Sulfonamides that do not undergo *N*-acetylation are subject to oxidation by cytochrome P450 in the liver (via CYP2C9 in humans) (Cribb *et al.*, 1995). This oxidation also occurs in dogs, but the involved P450 has not been characterized (Cribb & Spielberg, 1990). The product of this enzymatic oxidation, the hydroxylamine (Fig. 1), is cytotoxic at high concentrations *in vitro* (Rieder *et al.*, 1989), and weakly immunogenic in rodents (Gill *et al.*, 1997). Although increased P450-mediated hydroxylamine generation would be a potential risk factor for sulfonamide toxicity, 'rapid' isoforms of CYP2C9 have not been found, and the gene encoding CYP2C9 is not different in sulfonamide-hypersensitive vs. sulfonamide-tolerant human patients (Pirmohamed *et al.*, 2000). We evaluated enzymatic oxidation of SMX in the livers of two dogs that died or were killed with severe multisystemic signs of sulfonamide hypersensitivity; however, hydroxylamine generation was not different from normal control livers (unpublished observations). Although the particular enzyme(s) involved in the hepatic generation of sulfonamide hydroxylamines have not been characterized in dogs, this would be a potential source of individual variability among dogs.

Besides cytochrome P450, myeloperoxidase, present in activated leukocytes and platelets, can also generate sulfonamide hydroxylamines (Cribb *et al.*, 1990; Hofstra & Uetrecht, 1993).
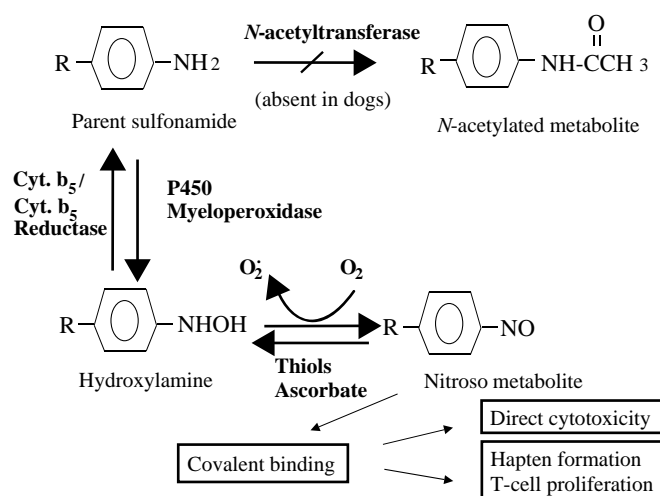

Fig. 1. Metabolic disposition of sulfamethoxazole and other sulfonamide antimicrobials in humans. Disposition is similar in dogs except that the *N*-acetylation detoxification pathway is absent.

Similar reactive metabolites for other drugs (e.g. clozapine) have been shown to accelerate neutrophil apoptosis (Williams *et al.*, 2000). Alternatively, oxidative metabolites could haptenize neutrophil or platelet membranes, with subsequent generation of antibodies or sensitized T-cell clones. Such mechanisms could contribute to the thrombocytopenia or transient neutropenia seen in sulfonamide hypersensitivity in dogs. Anti-myeloperoxidase antibodies have been demonstrated in cats with propylthiouracil-associated neutropenia, suggesting the generation of locally reactive metabolites (Waldhauser & Uetrecht, 1996), but this possible mechanism remains to be evaluated in dogs given sulfonamides.

Sulfonamide hydroxylamines spontaneously oxidize to a nitroso metabolite, and this is thought to be the proximate toxin and immunogen in hypersensitivity (Fig. 1). The nitroso metabolite is directly cytotoxic at relatively high concentrations; both apoptosis and necrosis are observed (Rieder *et al.*, 1995; Naisbitt *et al.*, 1999, 2002). At concentrations (low μM) expected after therapeutic dosing, nitroso-SMX rapidly binds to surface proteins of lymphoid and hepatic cells (Naisbitt *et al.*, 1999; Manchanda *et al.*, 2002), and, following internalization and processing, is presented in association with major histocompatibility complex (MHC) molecules (Naisbitt *et al.*, 2002), therefore acting as a hapten. The nitroso metabolite, more so than the hydroxylamine, is immunogenic in rats, and leads to anti-drug antibodies and drug-specific T cells in this animal model (although clinical signs of hypersensitivity are not seen) (Gill *et al.*, 1997; Naisbitt *et al.*, 2001).

Nitroso metabolites of sulfonamides are primarily detoxified by nonenzymatic reduction, either by cysteine, glutathione, or ascorbate (Cribb *et al.*, 1991; Naisbitt *et al.*, 2000; Trepanier *et al.*, 2003c), as well as by NADH or NADPH (unpublished observations). Glutathione and ascorbate also prevent the generation of nitroso-SMX from the hydroxylamine (Cribb *et al.*, 1990, 1991), and decrease cellular haptenization,

cytotoxicity, and T-cell proliferation induced by nitroso *in vitro* (Naisbitt *et al.*, 1999, 2002; Reilly *et al.*, 2000; Burkhart *et al.*, 2001; Manchanda *et al.*, 2002; Farrell *et al.*, 2003). In patients with HIV infection, who have a relatively high incidence of sulfonamide hypersensitivity (Gordin *et al.*, 1984; Carr & Cooper, 1995–96), deficiency in glutathione, cysteine, and ascorbate have all been reported (Buhl *et al.*, 1989; Allard *et al.*, 1998; Naisbitt *et al.*, 2000), and plasma ascorbate deficiency correlates with impaired nitroso reduction (Trepanier *et al.*, 2003c). Therefore, lowered antioxidant levels may be an acquired risk factor for these reactions in humans. Whether genetic or acquired deficiencies in thiols, ascorbate, or other antioxidants play a role in dogs with sulfonamide hypersensitivity remains to be determined.

Although nitroso metabolites are thought to be mediators of sulfonamide hypersensitivity, hydroxylamine metabolites may also be important, as they can redox cycle with nitroso metabolites, generating superoxide radicals (Cribb *et al.*, 1991) and further depleting ascorbate and other antioxidants. Therefore, defects in their detoxification may also contribute to the risk of sulfonamide toxicity. Hydroxylamines are detoxified by enzymatic reduction by cytochrome b_5 reductase and cytochrome b_5 (Trepanier *et al.*, 2003b). This pathway is present in both humans and dogs, with activity in dogs about three- to fivefold higher than that seen in humans (Trepanier & Miller, 2000). One might speculate that an increased ability to reduce hydroxylamines could represent an adaptation to absent *N*-acetylation in dogs. We are now looking for differences in cytochrome b_5 reductase and cytochrome b_5 expression and activity in dogs and humans with sulfonamide hypersensitivity. We hypothesize that these differences could be acquired (e.g. in HIV-infected patients), or genetic (e.g. in breeds of dogs predisposed to sulfonamide toxicity), and could lead to increased levels of hydroxylamine and nitroso metabolites, antioxidant depletion, immunologic activation, and subsequent hypersensitivity.

The hypothesis that impaired detoxification of hydroxylamine or nitroso metabolites could be a factor in sulfonamide hypersensitivity is supported by *in vitro* studies of leukocyte cytotoxicity in human patients (Spielberg, 1984, 1986; Rieder *et al.*, 1989; Wolkenstein *et al.*, 1995b). For example, when lymphocytes from human patients with sulfonamide hypersensitivity are incubated with hydroxylamine or nitroso metabolites, they are significantly more susceptible to cell death than are control lymphocytes (Spielberg, 1984, 1986; Shear *et al.*, 1986). Interestingly, this increased cytotoxicity can be documented months to years after the episode of hypersensitivity, and has also been observed in family members who have never been challenged with the drug (Shear *et al.*, 1986; Wolkenstein *et al.*, 1995b). These results suggest that a detoxification defect is expressed in these hypersensitive patients prior to drug exposure, and that this defect is involved in a step after the oxidation of the parent compound to the hydroxylamine. Interestingly, similar results were found in a small study of Doberman pinschers (Cribb & Spielberg, 1990), although these results need to be confirmed in a larger number of dogs.

A group of researchers in Switzerland has recognized that some human patients with sulfonamide hypersensitivity have T-cell clones that recognize the parent drug, rather than the oxidative metabolites, in a relatively unstable, noncovalent, association with MHC classes I or II molecules (Britschgi *et al.*, 2003). These findings suggest the possibility that SMX itself may be immunogenic in some human patients, although further work is needed to determine the relative importance of this mechanism vs. the metabolite-hapten concept.

CONFIRMATION, CLINICAL MANAGEMENT, AND PREVENTION

Our laboratory is working on several potential confirmatory tests for drug hypersensitivity in dogs, to include an *in vitro* lymphocyte cytotoxicity assay, ELISA for anti-drug antibodies, immunoblotting for anti-liver antibodies, and flow cytometry for anti-platelet antibodies. Until we have more information about the predictive value of these tests, we must rely on clinical parameters for diagnosis. A presumptive diagnosis of sulfonamide hypersensitivity can reasonably be made based upon a clinical presentation of polyarthropathy, blood dyscrasia, hepatopathy, skin eruption, or other unexpected systemic signs 5 or more days after exposure to potentiated sulfonamides, with elimination of other systemic cause for the signs, such as sepsis, vasculitis, rickettsial infection, lymphoma, or other neoplasia. A careful chronologic history is important. Signs may occur a few days after a course of sulfonamides has been discontinued, but generally not more than a week after discontinuation, and usually not more than 30 days after drug initiation.

The clinical management of sulfonamide hypersensitivity begins with educating the owner to look for any signs of decrease in appetite, vomiting or diarrhea, lameness, lethargy, ocular discomfort, or change in urine color, during or shortly after a course of potentiated sulfonamides. This is really the most important step, because a failure to recognize toxicity early can lead to needless continuation of the drug and, sometimes, a fatal outcome (Kreisberg, 1995). Although drug discontinuation is critical, it alone may not be sufficient in some cases. Dogs with suspicious clinical signs should be evaluated with complete physical exam including fundic evaluation, CBC, biochemical panel, urinalysis, and Schirmer tear test. If finances are restricted, then a PCV/TP, blood smear, ALT, serum alkaline phosphatase, bilirubin, serum albumin, and urine dipstick are indicated, along with Schirmer tear test. Because of the relative rarity of these reactions in dogs and the usual short course of therapy, routine serial monitoring of complete blood counts or liver enzymes in patients without adverse clinical signs is probably not warranted. However, patients being treated with prolonged courses of sulfonamides should be monitored for the development of nonregenerative anemia, (reversible) clinical hypothyroidism, and KCS.

For dogs with arthropathy, nonsteroidal anti-inflammatory drugs can alleviate pain and inflammation for the first few days until the reaction subsides (these are contraindicated, however, if

significant thrombocytopenia is present). Thrombocytopenia and hemolytic anemia can be managed with platelet concentrate or packed red cell transfusions, respectively, and, although efficacy has not been proved in this setting, several weeks of a tapering course of immunosuppressive dosages of glucocorticoids. Hepatopathy often requires aggressive support, to include low sodium fluids, fresh frozen plasma, gastroprotectants such as H_2-blockers, and control of coagulopathy and encephalopathy. In addition, *N*-acetylcysteine (as a precursor of glutathione) may be helpful. The author has managed one dog with sulfonamide-associated hepatic necrosis for which a course of i.v. *N*-acetylcysteine, as recommended for acetaminophen toxicity (Plumb, 2002), was associated with resolution of histologic signs of necrosis on repeat liver biopsy. In addition, because of our finding that ascorbate can reduce the nitroso metabolites of sulfonamides, i.v. ascorbate may also theoretically be beneficial, again using the same protocol (30 mg/kg, i.v. q. 6 h) recommended for acetaminophen hepatotoxicity (Hjelle & Grauer, 1986).

Skin eruptions require support for secondary pyoderma, and if severe, may warrant fluid and oncotic support, debridement of lesions, control for sepsis, and, depending on clinical presentation, anti-inflammatory doses of glucocorticoids (Medleau *et al.*, 1990). Keratoconjunctitilis sicca may be reversible in some cases (Morgan & Bachrach, 1982; Diehl & Roberts, 1991; Trepanier *et al.*, 2003a,b,c), particularly with topical cyclosporine therapy.

NOT ALL SULFONAMIDES ARE IMPLICATED

Other drugs besides sulfonamide antimicrobials, such as acetazolamide, furosemide, glipizide, hydrochlorthiazide, celecoxib, and tolbutamide, have a sulfonamide moiety in their chemical struct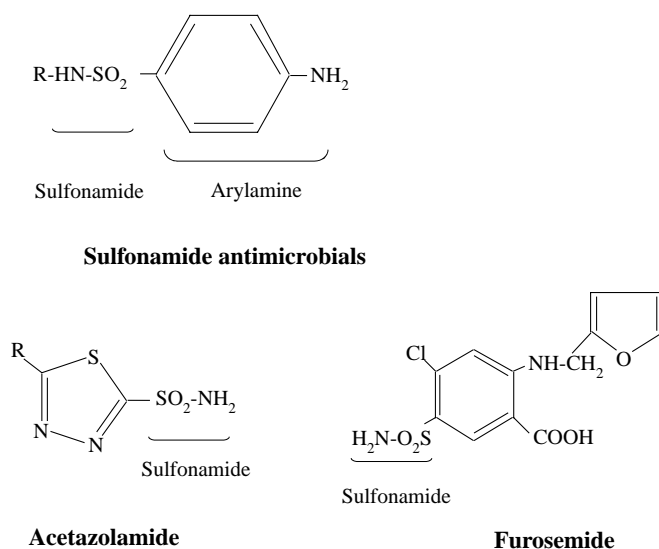ure (Strom *et al.*, 2003) (Fig. 2). However, these drugs do not appear to cross react with sulfonamide antimicrobials, or produce hypersensitivity syndromes with a similar pathogenesis (Uetrecht, 2002; Strom *et al.*, 2003). This is most likely because it is the primary arylamine structure that leads to hydroxylamine and nitroso generation (and presumably leads to hypersensitivity), not the sulfonamide portion of the drug, and these other sulfonamides do not contain primary arylamines.

Although idiosyncratic toxicity associated with potentiated sulfonamides is relatively uncommon, concern over the risk of adverse reactions limits the clinical use of these antimicrobials. Much of the work done to date suggests a delayed T-cell mediated pathogenesis for these reactions, although direct cytotoxicity and humoral mechanisms could contribute in some cases. Further work will determine what genetic or acquired factors lead to the development of these reactions in dogs, and may suggest improved preventative and management strategies.

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Sulfonamide antimicrobials

Acetazolamide   **Furosemide**

Fig. 2. Structural differences between sulfonamide antimicrobials (for which the arylamine portion of the molecule is related to toxicity) and other sulfonamide drugs (which are not arylamines, and do not share the same spectrum or mechanisms of toxicity).

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