SHORT COMMUNICATION

Further investigations of the role of acetylation in sulphonamide hypersensitivity reactions

Cindy E. Nuss, Denis M. Grant, Stephen P. Spielberg and Alastair E. Cribb

Sulphonamide hypersensitivity reactions are believed to be mediated through reactive intermediates derived from oxidation of the *para*-amino group to form sulphonamide hydroxylamines. Sulphamethoxazole hydroxylamine (SMX-HA) can be acetylated by *N*-acetyltransferase (NAT) enzymes to form an acetoxy metabolite (acetoxySMX). In the current studies, acetoxySMX was found to be not toxic over the concentration range of 0 to 500 μM towards a human lymphoblastoid cell line (RPMI 1788) or a human hepatoma cell line (HepG2). Further, transient expression of NAT1 in COS-1 cells or stable transfection of NAT1 and NAT2 in HepG2 cells did not alter the toxicity of SMX-HA *in vitro*. The activity of NAT1 in isolated mononuclear leucocytes (a reflection of systemic NAT1 activity) determined with *para*-aminobenzoic acid as a substrate was not different between controls ($n = 11$) or patients with a known hypersensitivity reaction ($n = 5$) (4.1 ± 1.2 nmol min^{-1} mg^{-1} vs 5.7 ± 1.4 nmol min^{-1} mg^{-1}). Thus, acetoxySMX is unlikely to play a significant role in mediating SMX hypersensitivity reactions and a constitutive deficiency in NAT1 activity is not a common finding in patients susceptible to SMX hypersensitivity reactions.

Keywords: *sulphamethoxazole, hydroxylamine, hypersensitivity, N-acetyltransferase, cytotoxicity.*

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Introduction

Sulphonamide hypersensitivity reactions (HR) are thought be mediated by reactive metabolites derived from the corresponding hydroxylamide metabolite (see Cribb *et al.* 1996a for a recent review). Much of the work in support of this hypothesis has been conducted with sulphamethoxazole (SMX) as a model compound. Pharmacogenetic and environmental variation in the formation and disposition of sulphamethoxazole hydroxylamine (SMX-HA) plays a role in susceptibility to SMX HR but not all the critical pathways have been identified. Mononuclear leucocytes (MNL) isolated from patients with sulphonamide (including SMX) HR were more susceptible to the toxic effects of sulphonamide hydroxylamines in an *in vitro* microsomal toxicity assay (Shear *et al.* 1986, Rieder *et al.* 1989, Riley *et al.* 1991). The *in vitro* microsomal toxicity assay tests the susceptibility of isolated MNL from patients to the toxicity of reactive metabolites formed by isolated hepatic microsomes. An association between susceptibility to HR *in vivo* and *in vitro* susceptibility of MNL patients to the toxicity of reactive metabolites of the causative drug has been found for several classes of drugs including the sulphonamides, aromatic anticonvulsants (Shear and Spielberg 1988) and cefaclor (Kearns *et al.* 1994). In all cases, the increased susceptibility is thought to be related to a metabolic defect but this has yet to be proven for any compound. Thus, while increased *in vitro* susceptibility of MNL isolated from patients to the toxicity of reactive metabolites of the causative drug is a biomarker associated with HR, its putative biochemical basis remains unproven.

We have been attempting to identify metabolic pathways that influence the *in vitro* toxicity of SMX-HA in order to identify a defective metabolic pathway to explain the increased *in vitro* susceptibility of patient MNL to SMX-HA toxicity and to serve as a marker of susceptibility to SMX HR. The metabolic pathways of SMX under investigation in this study are shown in Figure 1. In aqueous buffer at physiological pH, SMX-HA rapidly oxidizes to nitroso-sulphamethoxazole (nitrosoSMX) which is cytotoxic and readily reacts with protein (Cribb *et al.* 1991a, b). However, we have recently shown that SMX-HA may also be acetylated by the *N*-acetyltransferase enzymes NAT1 and NAT2 to an acetoxy metabolite (acetoxySMX) (Nakamura *et al.* 1995). Acetoxy metabolites of carcinogenic arylamines are unstable, reactive and are the ultimate carcinogens in many instances. Thus, acetoxySMX may potentially be involved in mediating SMX HR. In the presence of cytosolic and microsomal fractions, acetoxySMX is rapidly metabolized back to SMX-HA (approximately 80%) or to SMX (approximately 20%), probably by more than one deacetylase enzyme (Nakamura *et al.* 1995). Preliminary investigations using synthetic acetoxySMX suggested it was slightly more toxic than SMX-HA (Nakamura *et al.* 1995), however, a comprehensive study has not been performed.

The parent compound SMX is acetylated primarily by *N*-acetyltransferase type 1 (NAT1) (Cribb *et al.* 1992), but a deficiency in *N*-acetyltransferase type 2 (NAT2) (the classical 'slow acetylator' phenotype) is a relative risk factor for SMX HR (Rieder *et al.* 1991, Wolkenstein *et al.* 1995). The role of NAT1 in susceptibility to SMX HR has not been investigated. A deficiency in NAT1 could theoretically lead to decreased clearance of SMX and subsequently increased formation of SMX-HA. On the other hand, if acetoxySMX is highly toxic then increased NAT1 activity could lead to further bioactivation of SMX-HA and increase toxicity.

We therefore conducted a series of experiments to determine if (1) acetoxySMX is more or less cytotoxic than SMX-HA, (2) whether expression of NAT1 or NAT2 alters the *in vitro* toxicity of SMX-HA, and (3) whether NAT1 activity is altered in MNL of patients susceptible to SMX HR. NAT1 is the only NAT enzyme expressed in isolated MNL and activity

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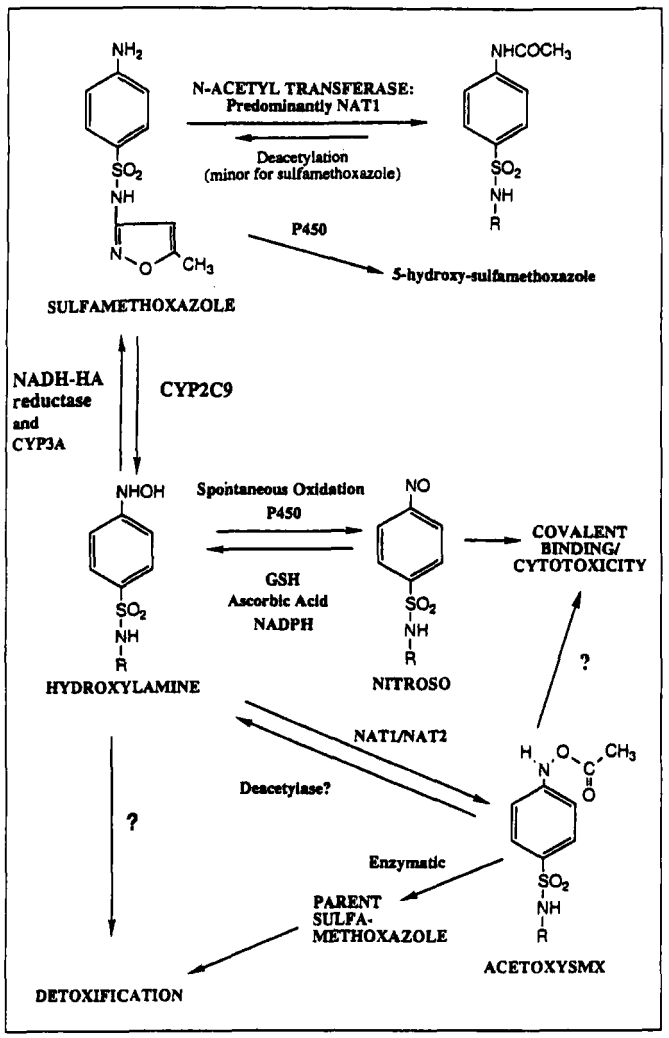

Figure 1. Partial scheme of metabolic pathways of SMX. Additional metabolic pathways have also been identified.

in MNL has been shown to correlate with the systemic activity of NAT1 (Cribb *et al.* 1991b).

MATERIALS AND METHODS

Chemicals

All routine chemicals were obtained from Sigma Chemical Co. (St Louis, MO) and HPLC solvents were obtained from Fisher Scientific (Malvern, PA). SMX-HA (> 98% pure) was obtained from Dalton Chemical Co. (Toronto, Ontario, Canada) and acetoxySMX (91% pure with approximately 4% SMX and 3% SMX-HA) was synthesized as previously described (Nakamura *et al.* 1995). Cell culture media were obtained from Mediatech, Inc. (Herndon, VA) and Gibco/BRL (Gaithersburg, MD). Foetal bovine serum (FBS) was from Hyclone (Logan, Utah). Restriction enzymes, T_4 ligase, and associated buffers were obtained from Promega (Madison, WI).

Cell lines

All cell lines used in these experiments were obtained from the American Type Culture Collection. RPMI 1788, a human lymphoblastoid cell line, was maintained as a suspension culture in RPMI 1640 medium with 10% foetal calf serum (FCS). COS-1, an African Green monkey kidney cell line, was maintained in DMEM with 10% FCS. HepG2, a human hepatoma cell line, was maintained in modified α-MEM medium with 10% FCS. All cell lines were maintained at 37 °C in 5% CO_2, except the COS-1 cells which were maintained in 10% CO_2.

Cytotoxicity assay

Cytotoxicity assays were performed in 96-well microtitre plates (RPMI 1788), 12.5 cm^2 flasks (COS-1 cells) and 12- or 24-well plates (HepG2). Cytotoxicity was determined by measuring the total content of lactate dehydrogenase (LDH) activity remaining in intact cells at the completion of the experiment and comparing to vehicle-treated controls (Chao *et al.* 1988, Ponsoda *et al.* 1991). LDH was measured using the CYTOTOX-96™ cytotoxicity assay (Promega, Madison, WI) according to the manufacturer's instructions. RPMI 1788 cells were plated in a 96-well microtitre plate at 100000 cells per well; HepG2 cells were plated at 100000 cells per well (24-well plate); and COS-1 cells were plated at 300000 cells per 12.5 cm^2 flask. Cells were allowed to incubate overnight, the media were removed, cells were washed and an appropriate volume of a HEPES-buffered medium containing the desired drug in 1% dimethylsulphoxide (DMSO) was added. After a 2-h incubation, the medium with drug was removed and replaced with the standard culture medium. The cells were allowed to incubate overnight (16 h), the medium removed, cells washed with buffer, and the total LDH content of the remaining cells was measured after lysis with 0.1% Triton X-100.

Plasmids for expression of human NAT1 and NAT2

The intronless coding regions for the NAT1 and NAT2 genes were cloned into the mammalian expression vector pcDNA3 (Invitrogen, San Diego, CA) for expression in mammalian cells. Bacterial expression phagemids containing the genomic coding regions for human NAT1 and NAT2 were purified from overnight cultures of *Escherichia coli* strains DMG 100 and DMG 200, respectively (Dupret and Grant 1992) using the Wizard Minipreps DNA Purification System (Promega). Both phagemids were restriction digested with ECoRI and Sal I and fragments separated on a 1% agarose gel. Bands of approximately 872 bp were cut from the gel, electroeluted, phenol extracted twice, phenol/chloroform extracted once and ethanol precipitated.

A $CsCl_2$ purified penetration of pcDNA3 was restriction digested with EcoRI and Xho I and the fragments separated on a 1% agarose gel. A 5.4 kb fragment was cut from the gel, electroeluted, phenol extracted twice, chloroform extracted once and ethanol precipitated. The fragments from the phagemids were ligated into the pcDNA3 vector at a 1:1 and 3:1 ratio using T_4 DNA ligase (Promega). SCS-1 competent cells (Stratagene, LaJolla, CA) were transformed according to the manufacturer's protocol and 100 ml of transformed cells were plated on LB-ampicillin plates. Resistant colonies were picked and inoculated into 5 ml of LB-ampicillin overnight at 37 °C. Plasmids were purified from the Wizard Minipreps DNA Purification System. The presence of NAT inserts in the plasmids was confirmed by obtaining appropriate size fragments upon restriction digestion. DNA sequences of the inserts were confirmed by cycle sequencing with the SequiTherm™ Cycle Sequencing Kit (Promega). One clone containing each plasmid was grown in large quantity. Plasmids were isolated from the large-scale preparation by $CsCl_2$ gradient centrifugation for transfection experiments. Plasmids containing the pcDNA3 vector and the NAT1 or NAT2 coding regions were named pCEN100 and pCEN200 respectively.

Transient transfection of COS-1 cells with NAT1

All transfections were performed in 12.5 cm^2 flasks which had been seeded with 3.0×10^5 cells and then grown overnight. The transfection mixture was prepared by mixing 6.3 μg pCEN100, 26 ml lipofectin (Gibco/BRL), and 258 μl Optimem™ media (Gibco/BRL) in a polystyrene tube and incubating for 15 min at room

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temperature. After the incubation, 2.1 ml of Optimem™ was added to the tube and 1.3 ml was added to each of two flasks. The flasks were incubated for 5 h at 37 °C in 5% CO_2, the transfection medium was removed and fresh growth medium was added. The cells were treated with drug either 24 or 4 h after transfection, as indicated in the figure legend.

Stable transfection of HepG2 cells

HepG2 cells were grown to 50% confluency in 25 cm^2 flasks. The plasmid pCEN 100 was prepared for transfection by mixing 6.3 μg DNA, 26 μl lipofectAMINE™ and 258 ml Optimem™ in a polystyrene tube and incubating for 15 min at room temperature. After the incubation, 2.1 ml Optimem™ was added to each tube and the contents were added to the 25 cm^2 flask. Transfection with pCEN200 was performed the same as the pCEN100 transfection except that 5.2 μg of DNA was used. Strains of cells containing the integrated DNA were selected by the addition of geneticin at 800 μg ml^{-1}. Clones of cells grown in 96-well plates were screened for activity as described above. The clones showing the highest activity towards the NAT substrates *p*-aminobenzoic acid (PABA) for NAT1 and SMZ for NAT2 were propogated for use in the cytoxicity assay.

N-acetyltransferase activity in isolated MNL and cell lines

NAT activity in cytosolic or S9 (supernatant obtained after centrifugation of cellular homogenate at 9000 × *g* for 20 min at 4 °C) fractions of cells was measured as described previously using an acetyl coenzyme A (acetylCoA) concentration of 100 μM and substrate concentrations of 100 μM (PABA) or 500 μM (SMZ) (Cribb *et al.* 1991b). MNL were isolated from heparinized blood and soluble fractions prepared as described previously (Cribb *et al* 1991b). Cell lines were harvested by scraping with a rubber policeman, washed with phosphate-buffered saline, and re-suspended in homogenization buffer (10 mM triethanolamine–HCl, 1 mM EDTA, 1 mM dithiothreitol, 50 mM KCl, pH 7.0) and disrupted by sonication on ice (2 × 30 s). In experiments to screen transfected cells for activity, cells were washed to remove cell culture medium and the drug was added directly to the cells in a HEPES-buffered medium (15 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid; 125 mM magnesium sulphate; 1 mM sodium phosphate, 1 mM calcium chloride, and 10 mM glucose) for 2 h. The media were harvested, protein precipitated by the addition of 1/10 volume of 15% perchloric acid, and drug analysed by HPLC. Quantitation of SMX metabolites (Cribb *et al.* 1992) and acetylated metabolites of PABA and SMZ were performed as described previously (Cribb *et al.* 1991b).

Patient population

MNL were isolated for measurement of NAT1 activity from 11 normal, healthy volunteers ('controls') and five patients with a history of SMX HR. Subjects were enrolled after informed written consent and in accordance with the guidelines of, and after approval by, the Hospital for Sick Children Human Ethics Committee, Toronto, Canada. Healthy volunteers had no history of a previous sulphonamide hypersensitivity reaction. Five had a known history of exposure to sulphamethoxazole-containing medications with no adverse reactions. Control subjects tested 'negative' in the *in vitro* microsomal toxicity assay with sulphamethoxazole (see below) (Riley *et al.* 1991). As we have previously found no difference between exposed individuals with no history of adverse reactions and unexposed subjects and as the two control groups were similar in the current study, they are treated as a single group in presentation of data. The five patients with a known history of SMX HR (fever, skin rash, and multiorgan toxicity 7–14 days after starting therapy) had a 'positive' *in vitro* microsomal toxicity assay. As described in the introduction, the *in vitro* microsomal toxicity assay measures the increase in cell death in isolated MNL exposed to hepatic microsome-generated sulphamethoxazole reactive metabolites (i.e. SMX-HA) compared with a control incubation with no drug (Shear *et al.* 1986, Riley *et al.* 1991). Cell death greater than three SD above the mean increase in cell death observed in MNL isolated from control individuals is considered a 'positive' result in this type of assay (Shear *et al* 1986, Shear and Spielberg 1988, Riley *et al.* 1991). In our laboratory, an increase in cell death greater than 7% is considered 'positive' with sulphamethoxazole. The control group had a mean increase in cell death of 2 ± 1.4 % compared with 18.5 ± 3.7% in the patient group.

Results

Toxicity of synthetic acetoxySMX

The cytotoxicity of synthetic acetoxySMX was compared with that of SMX-HA in two cells lines: RPMI 1788, a human lymphoblastoid cell line, and HepG2, a human hepatoma cell line. In both cell lines, acetoxySMX was non-toxic over the concentration range tested, while SMX-HA showed dose-dependent toxicity (Figure 2).

Effect of expression of NAT1 and NAT2 on toxicity of SMX-HA

Non-transfected COS-1 cells had very low NAT activity in S9 fractions as measured with PABA (ranging from not detectable to 0.36 nmol mg^{-1} min^{-1} depending on the culture) or SMZ (not detectable). Transient transfection with pCEN 100 resulted in an activity of 7.1 ± 2 nmol PABA acetylated min^{-1}

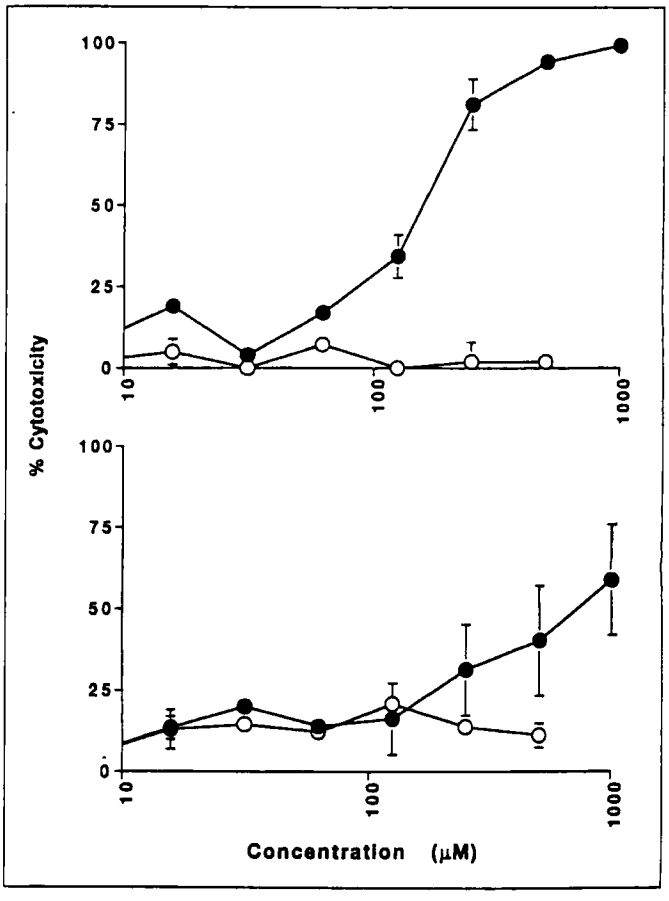

Figure 2. Toxicity of SMX-HA (●) and acetoxySMX (○) was assessed in a human lymphoblastoid cell line (RPMI 1788, top) and a human hepatoma cell line (HepG2, bottom) as described in Materials and Methods. Mean ± SE (n = 3) are shown for each point.

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mg^{-1} cellular protein. Expression of NAT1 in COS-1 cells had no effect on the toxicity of SMX-HA towards this cell line (Figure 3).

Transient transfection only results in expression of the enzyme in a fraction of the cell population and this could have decreased the ability to detect modulation of toxicity by NAT1. Therefore, stable transfectants in HepG2 cells were developed. In contrast to COS-1 cells, cytosolic fractions of HepG2 cells displayed endogenous NAT1 activity (2 nmol PABA acetylated mg^{-1} min^{-1}) but essentially no NAT2 activity (less than 0.02 nmol SMZ acetylated mg^{-1} min^{-1}). The stable transfection with pCEN100 resulted in a marked increase in activity towards PABA (28 nmol min^{-1} mg^{-1} cytosol) and to SMZ (0.27 nmol min^{-1} mg^{-1}). Transfection with pCEN200 increased acetylation towards SMZ (0.59 nmol min^{-1} mg^{-1}), a substrate for NAT2, but only slightly increased PABA acetylation (2.2 nmol min^{-1} mg^{-1}). The activity in the HepG2-NAT1 cell line is approximately 15–30 times that observed in human liver and 5-10 times that observed in human mononuclear leucocytes. The activity in the HepG2-NAT2 cell line is approximately equal to that observed in human liver from fast acetylators.

The cytotoxicity of SMX-HA towards HepG2 cell lines was not altered by the presence of either transfected enzyme (Figure 3).

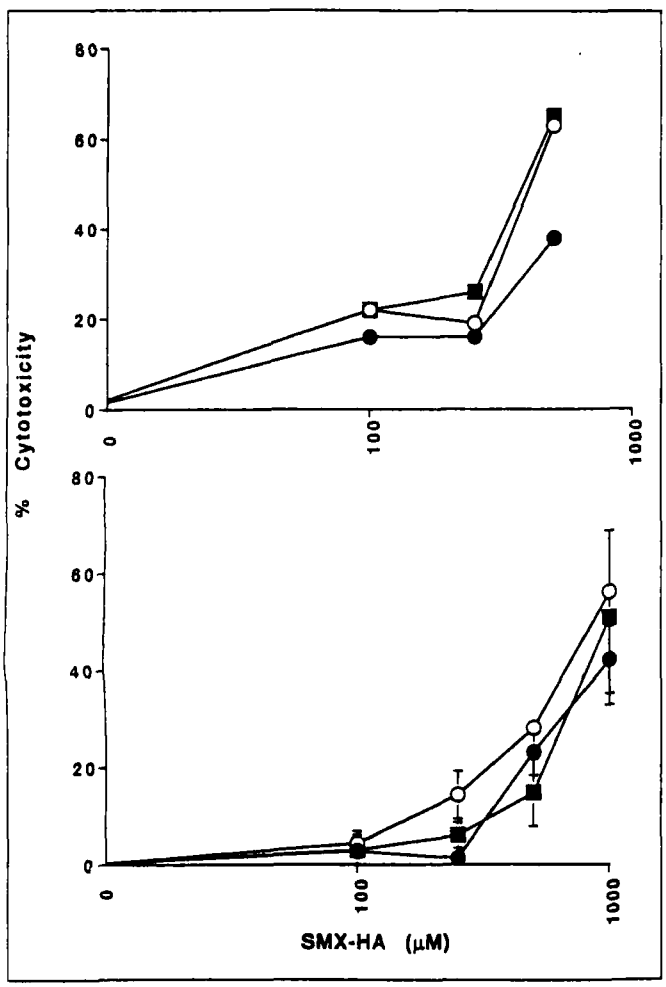

Figure 3. Top: Toxicity of SMX-HA towards COS-1 cells transiently transfected with either the control plasmid pcDNA3 (○) or a plasmid containing the coding region for NAT1 (■) is compared with toxicity in non-transfected COS-1 cells (●). A representative experiment of three replicate experiments with similar results is shown. See Materials and Methods for details. Bottom: Toxicity of SMX-HA towards cell lines stably expressing NAT1 (○) or NAT2 (■) compared with the parental HepG2 cell line (●). Mean ± SE of three experiments is shown. See Materials and Methods for details.

Activity of NAT1 in MNL of patients

The activity of NAT1 in MNL isolated from patients with a history of SMX HR (n = 5) was compared with the activity in MNL from healthy controls (n = 11). With 100 μM PABA as a substrate, NAT1 activity was not deficient in the MNL of patients compare with MNL from controls (5.7 ± 1.4 nmol mg^{-1} min^{-1} vs 4.1 ± 1.2 nmol min^{-1} mg^{-1}; mean ± SD). Similar results were obtained using p-aminosalicylate, SMX, and SMX-HA as substrates (data not shown).

Discussion

The data obtained in these experiments do not support the hypothesis that acetylation of SMX-HA to form acetoxySMX contributes to its over-all bioactivation. Synthetic acetoxySMX was not directly toxic to either RPMI 17/88 or HepG2 cell lines over the concentration range tested. Failure to observe toxicity could however have been related to an inability of acetoxySMX to penetrate the cell. Therefore, we determined the effect of elevated expression of NAT1 and NAT2 on the *in vitro* toxicity of SMX-HA using transient and stable expression systems. SMX-HA was toxic to COS-1 cells but this toxicity was not altered by the transient expression of NAT1. Expression of recombinant NAT2 was low in this system and so was not investigated. Transient expression may only involve a percentage of the cells, therefore we produced stable cell lines expressing NAT1 and NAT2 using HepG2 cells. Over expression of NAT1 (approximately 15 times that observed in human liver) and expression of NAT2 (approximately equal to NAT2 expression in human liver) did not alter the toxicity of SMX-HA towards the HepG2 cells. Therefore, expression of NAT1 and NAT2 did not directly affect the toxicity of SMX-HA even under conditions in which intracellular formation of acetoxySMX would be expected to occur. These results are consistent with the previous observations that acetoxySMX does not spontaneously react with glutathione (Nakamura *et al.* 1995) and is not highly reactive with protein (Cribb *et al.* 1996b), properties often associated with reactive intermediates.

Since acetoxySMX does not appear to be more toxic than SMX-HA, it is unlikely that high levels of NAT1 expression would be associated with susceptibility to SMX HR. However, a deficiency in NAT1 activity could still predispose to SMX HR by decreasing the clearance of SMX and allowing more to be metabolized to SMX-HA. We therefore evaluated the expression of NAT1 in the MNL of patients with SMX HR as a marker of systemic NAT1 activity (Crill *et al.* 1991b). The results clearly demonstrated that a constitutive NAT1 deficiency was not present in the MNL of any of the five patients susceptible to SMX HR studied nor was the mean activity significantly decreased compared with the control

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population. Hence, genetic variation in NAT1 does not appear to be a common risk factor for SMX HR nor does it explain the observed increased susceptibility of MNL isolated from patients to the toxicity of SMX reactive metabolites. These results do not however rule out a constitutive or environmentally-induced loss of NAT1 as a risk factor in some patients.

While we have observed NAT1 activity within the normal range in the patients studied here, previous studies have shown an association between a systemic deficiency in NAT2 activity ('slow acetylator phenotype') and SMX HR (Rieder *et al.* 1991, Wolkenstein *et al.* 1995). While we did not observe an effect of NAT activity on SMX-HA toxicity in the current experiments, we have previously shown that the combination of NAT and acetylcoenzyme A significantly reduces covalent binding associated with SMX-HA *in vitro* (Cribb *et al.* 1996b). Conceivably, altering the extent of covalent binding might influence an immunologically-mediated toxicity without affecting *in vitro* cytotoxicity. The formation of acetoxySMX cannot be accurately measured directly in subcellular fractions because it is rapidly metabolized back to SMX-HA and to SMX (see Figure 1), however, we have measured the acetylCoA-dependent metabolism of SMX-HA (100 μM) to SMX in liver cytosol fractions as an indirect marker of acetylation of SMX-HA. In the absence of acetylCoA as a cofactor, no metabolism of SMX-HA to SMX was observed; in the presence of acetylCoA, metabolism of SMX-HA to SMX occurred and was higher in 'fast acetylator' livers (0.245 ± 0.022 nmol mg^{-1} 30 min^{-1}; mean ± SD; (n = 3)) compared with 'slow acetylator' livers (0.145 ± 0.05 nmol mg^{-1} 30 min^{-1}; n = 2) (livers phenotyped by SMZ acetylation (Cribb, unpublished data)). Formation of SMX did not correlate with NAT1 activity as measured by PABA acetylation (r = 0.04; p = 0.99). The apparently greater contribution of NAT2 to the acetylation of SMX-HA in human liver is consistent with the previously reported *in vitro* studies on the acetylation of SMX-HA by NAT1 and NAT2 (Nakamura *et al.* 1995). Thus, NAT2 deficiency may be associated with SMX HR through an effect on the covalent binding and relase of SMX-HA from the liver and not just an effect on clearance of the parent compound or on the cytotoxicity of SMX-HA. These preliminary observations require further investigation to understand the apparently complex relationship between arylamine/hydroxylamine toxicity and acetylation activity *in vivo*.

In summary, acetoxySMX is not highly toxic and is unlikely to play a major role in SMX HR. A constitutive deficiency of NAT1 was not associated with susceptibility to HR in the five patients studied and does not explain the increased susceptibility of MNL isolated from patients to the toxicity of SMX-HA *in vitro*. We cannot rule out that a deficiency in NAT1 may contribute to susceptibility in rare individuals but it does not appear to be a common factor. This is consistent with the observation that acetylation of SMX does not appear to be altered in HIV-seropositive patients (van der Ven *et al.* 1995) or patients with AIDS (Lee *et al.* 1994) despite the high incidence of sulphonamide ADR. We are continuing to investigate other metabolic and cellular defence pathways which might play a role in susceptibility to SMX HR *in vivo* and explain the increased susceptibility of isolated MNL from patients to the toxicity of SMX-HA *in vitro*. This information will ultimately help us to understand the pathogenesis of these drug toxicities.

References

Chao, E. S., Dunbar, D. and Kaminsky, L. S. (1988) Intracellular lactate dehydrogenase concentration as an index of cytotoxicity in rat hepatocyte primary culture. *Cell Biology and Toxicology*, **4**, 1–11.

Cribb, A. E., Miller, M., Leeder, J. S., Hill, J. and Spielberg, S. P. (1991a) Reactions of the nitroso and hydroxylamine metabolites of sulphamethoxazole with reduced glutathione. *Drug Metabolism and Disposition*, **19**, 900–906.

Cribb, A. E., Grant, D. M., Miller, M. and Spielberg, S. P. (1991b) Expression of monomorphic arylamine *N*-acetyltransferase (NAT1) in human leukocytes. *Journal of Pharmacology and Experimental Therapeutics*, **251**, 1241–1247.

Cribb, A. E., Nakamura, H., Grant, D. M., Miller, M. A. and Spielberg, S. P. (1992) Role of polymorphic and monomorphic human arylamine *N*-acetyltransferases in determining suphamethoxazole metabolism. *Biochemica, Pharmacology*, **45**, 1277–122.

Cribb, A. E., Lee, B. L., Trepanier, L. A. and Spielberg, S. P. (1996a). Adverse reactions to sulphonamide and sulphonamide-trimethoprim antimicrobials: clinical syndromes and pathogenesis. *Adverse Drug Reactions and Toxicological Reviews*, **15**, 9–50.

Cribb, A. E., Nuss, C. E., Alberts, D. W., Lamphere, D. B., Grant, D. M., Grossman, S. J. and Spielberg, S. P. (1996b) Covalent binding of sulphamethoxazole reactive metabolites to human and rat liver subcellular fractions assessed by immunochemical detection. *Chemical Research in Toxicology*, **9**, 500–507.

Dupret, J.-M. and Grant, D. M. (1992) Site-directed mutagenesis of recombinant human arylamine *N*-acetyltransferase expressed in *Escherichia coli*. *Journal of Biological Chemistry*, **267**, 7381–7385.

Kearns, G. L., Wheeler, J. G., Childdress, S. H. and Letzig, L. G. (1994) Serum sickness-like reactions to cefaclor—role of hepatic-metabolism and individual susceptibility. *Journal of Pediatrics*, **125**, 805–811.

Lee, B. L., Delahunty, T. and Safrin, S. (1994) The hydroxylamine of sulphamethoxazole and adverse reactions in patients with acquired immunodeficiency syndrome. *Clinical Pharmacology and Therapeutics*, **56**, 184–189.

Nakamura, H., Cribb, A. E., Utrecht, J., Nasser, Z., Miller, M., Grant, D. M. and Spielberg, S. P. (1995) Metabolism and toxicity of *N*-acetoxysulphamethoxazole: a potential candidate for the ultimate toxin in sulphonamide hypersensitivity reactions. *Journal of Pharmacology and Experimental Therapeutics*, **274**, 1099–1104.

Ponsoda, X., Rover, J., Castell, J. V. and Gonez-Lechon, M. J. (1991) Measurement of intracellular LDH activity in 96-well cultures: a rapid and automated assay for cytotoxicity studies. *Journal of Tissue Culture Methods*, **13**, 21–24.

Rieder, M. J., Uetrecht, J., Shear, N. H., Cannon, M., Miller, M. and Spielberg, S. P. (1989). Diagnosis of sulphonamide hypersensitivity reactions by in-vitro 'rechallenge' with hydroxylamine metabolites. *Annals of Internal Medicine*, **110**, 286–289.

Rieder, M. J., Shear, N. H., Kanee, A., Tang, B. K. and Spielberg, S. P. (1991) Prominence of slow acetylator phenotype among patients with sulphonamide hypersensitivity reactions. *Clinical Pharmacology and Therapeutics*, **49**, 13–17.

Riley, R. J., Cribb, A. E. and Spielberg, S. P. (1991) Glutathione transferase μ deficiency is not a marker for predisposition to sulphonamide toxicity. *Biochemical Pharmacology*, **42**, 696–698.

Shear, N. H. and Spielberg, S. P. (1988) Anticonvulsant hypersensitivity syndrome: in vitro assessment of risk. *Journal of Clinical Investigation*, **82**, 1826–1832.

Shear, N. H., Spielberg, S. P., Grant, D. M., Tang, B. K. and Kalow, W. (1986) Differences in metabolism of sulphonamides predisposing to idiosyncratic toxicity. *Annals of Internal Medicine*, **105**, 179–84.

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VAN DER VEN, A. J. A. M., VREE, T. B., KOLMER, E. W. J. V., KOPPMANS, P. P AND VAN DER MEER, J. W. M. (1995) Urinary recovery and kinetics of sulphamethoxazole and its metabolites in HIV-seropositive patients and healty volunteers after a single oral dose of sulphamethoxazole. *British Journal of Clinical Pharmacology*, **38**, 147–150.

WOLKENSTEIN, P., CARRIERE, V., CHARUE, D., BASTUJIGARIN, S., REVUZ, J. AND ROUJEAU, J. C. (1995) A slow acetylator phenotype is a risk factor for sulphonamide-induced toxic epidermal necrolysis and Stevens–Johnson syndrome. *Pharmacogenetics*, **5**, 255–258.

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