Alteration of drug metabolizing enzymes in sulphite oxidase deficiency

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The aim of this study was to investigate the possible effects of sulphite oxidase (SOX, E.C. 1.8.3.1) deficiency on xenobiotic metabolism. For this purpose, SOX deficiency was produced in rats by the administration of a low molybdenum diet with concurrent addition of 200 ppm tungsten to their drinking water. First, hepatic SOX activity in deficient groups was measured to confirm SOX deficiency. Then, aminopyrine N-demethylase, aniline 4-hydroxylase, aromatase, caffeine N-demethylase, cytochrome b5 reductase, erythromycin N-demethylase, ethoxyresorufin O-deethylation, glutathione S-transferase, N-nitrosodimethylamine N-demethylation and pentoxysresorufin O-deethylation activities were determined to follow changes in the activity of drug metabolizing enzymes in SOX-deficient rats. Our results clearly demonstrated that SOX deficiency significantly elevated A4H, caffeine N-demethylase, erythromycin N-demethylase and N-nitrosodimethylamine N-demethylation activities while decreasing ethoxyresorufin O-deethylation and aromatase activities. These alterations in drug metabolizing enzymes can contribute to the varying susceptibility and response of sulphite-sensitive individuals to different drugs and/or therapeutics used for treatments.

Key Words: sulphite oxidase deficiency, sulphite sensitivity, drug metabolizing enzymes, cytochrome P450s, rat

Sulphite is generated endogenously in mammalian tissues from the normal processing of sulphur-containing amino acids. “Sulphites” is a generic term for a group of compounds including sulphur dioxide, sodium sulphite, sodium and potassium bisulphite and sodium and potassium metabisulphite. They are antioxidants useful for their antimicrobial action and prevention of enzymatic and nonenzymatic discoloration (browning) of foods.(1) Sulphites are used in vinegar, pickles, relishes, olives and sauerkraut and in concentrates of bulk juices and purees such as tomato. They are used in the processing of many food ingredients such as gelatin, beet sugar, corn sweeteners and food starches. In addition, they may be found in a number of parenteral medications including sulphur dioxide, sodium sulphite, sodium and potassium metabisulphite. In this paper, we have investigated the effects of SOX deficiency on GST and on the most common CYPs known to metabolize a variety of drugs. In addition to CYPs, phase II enzymes, such as glutathione S-transferase (GST), are important in drug metabolism. GST represents a complex monoglyc family of cytosolic enzymes(7) that are widely distributed in the animal kingdom. GSTs play an important role in detoxification by conjugating reduced glutathione to a large number of electrophilic metabolites derived from a variety of xenobiotics, including carcinogens, toxins and drugs.

Deficiency of SOX in humans leads to progressive cerebral degeneration, major neurological abnormalities, dislocated ocular lenses, mental retardation, severe seizures, and early death, usually between 2 and 6 years of age.(8) SOX deficiency can occur for two reasons. The first is a defect in the synthesis of its molybdenum cofactor, which also affects xanthine dehydrogenase and aldehyde oxidase. The second is a specific sulphite oxidase defect due to mutations in the gene encoding SOX on chromosome 12q.(9)

Partial SOX deficiency is a possible mechanism involved in sulphite sensitivity. Adverse reactions, including anaphylactic reactions, dermatitis, urticaria, flushing, hypotension, abdominal pain and diarrhea, have been reported in sulphite-sensitive individuals. Despite numerous studies addressing adverse responses in sulphite sensitivity, the clinical importance of changes in drug metabolizing enzymes in sulphite-sensitive individuals due to SOX deficiency remains to be elucidated. For example, there are no reports concerning sulphite-related changes of drug metabolizing enzymes ingested by SOX-deficient rats. It was demonstrated that a molybdenum deficient diet would result in a sulfur handling defect at the level of transformation of sulfite to sulfate and SOX-deficiency could be induced in rats by supplying low molybdenum diet and concomitantly administrating tungsten, which had been shown to be competitive antagonist of molybdenum utilization.(10–12)

In this paper, we have investigated the in vivo effects of SOX deficiency on GST and on the most common CYPs known to metabolize drugs and many other xenobiotics in the liver, lung, kidney and small intestine of rats.

Materials and Methods

Chemicals. The following chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO); p-amino-phenol, aniline, bovine serum albumin (BSA), butylated hydroxy-
toluene (BHT), CaCl₂, 1-chloro-2,4-dinitrobenzene (CDNB), cholate, ethylene diamine tetra acetic acid disodium salt (EDTA), Folin phenol reagent, glycerol, glycine, D-glucose-6-phosphate monosodium salt, D-glucose-6-phosphate dehydrogenase, reduced glutathione (GSH), N-2-hydroxyethylpiperazine-N-2, ethane sulfonic acid (HEPES), β-nicotinamide adenine dinucleotide phosphate (NADPH), NAPDH, N-nitrosodimethylamine (NDMA), TPA, trans-2-(2-hydroxymethyl)1,3-propanediol (TRIS), phenylhydroxylsulphonyllfluoride (PMSF), polyethylene sorbitan monolaurate (TWEEN 20), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium potassium tartarate. The reagents used for hepatic SOX activity assay were obtained from Merck (Darmstadt, Germany). All other chemicals and solvents were obtained from commercial sources at the highest grade of purity available.

Animals and treatment. Healthy male Wistar rats, about 3 months old, weighing 200–250 g, were obtained from University Animal House. They were housed in small cages at standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12 h light-dark cycle and were fed ad libitum with standard rat chow and tap water. All experimental procedures in animals were performed under appropriate regimes with veterinary services and licensed projects. Rats were divided into two groups: a control group (C) and a SOX-deficient group (D). SOX deficiency was produced in rats by the administration of a low molybdenum diet (AIN 76, and a SOX-deficient group (D). SOX deficiency was produced in projects. Rats were divided into two groups: a control group (C) and a SOX-deficient group (D). SOX deficiency was produced in rats by the administration of a low molybdenum diet (AIN 76, and a SOX-deficient group (D). SOX deficiency was produced in rats by the administration of a low molybdenum diet (AIN 76, and a SOX-deficient group (D). SOX deficiency was produced in rats by the administration of a low molybdenum diet (AIN 76, and a SOX-deficient group (D). SOX deficiency was produced in rats by the administration of a low molybdenum diet (AIN 76, and a SOX-deficient group (D).
Effects on EROD and CN3D activities in the liver, kidney and small intestines of rats (Table 1). Although EROD activities were significantly depressed to some extent in liver, kidney and intestine of SOX-deficient animals, controversial CN3D activities were found in the liver and kidney in SOX-deficient rats. EROD and CN3D activities are known to be quite specific probes for the CYP1A family, which often mediates the metabolic bioactivation of carcinogens and procarcinogens, such as PAHs, polycyclic aromatics and aromatic and heterocyclic amines.26 On the other hand, special precautions should be taken while treating sulphite-sensitive individuals with tricyclic antidepressants, such as amitriptyline and imipramine, because CYP1A2 is one of the down-modulation of CYP activities, activities of the hepatic conjugation enzyme, cytosolic GST, remained unchanged in a SOX-deficient state. Observed activation of catalytic activities was generally consistent with the protein levels of related CYP isofoms in rat liver microsomes prepared from control and SOX-deficient rats (Fig. 1). The densitometric scanning of Western blot results revealed that hepatic CYP2E1 protein level was increased significantly around 1.42-fold in the SOX-deficient rats relative to the control. Similarly, CYP2C6 and CYP3A1 protein level was increased (1.60 and 3.45-fold). Moreover, SOX-deficiency caused depression of aromatase activity only in liver confirm the accuracy of the results because it is known to be associated with CYP2B and expressed primarily in liver.31 Although it is involved in the metabolism of small number of drugs, it drives attention due to its role in metabolism of drugs, such as cyclophosphamide, used to treat various types of cancer and some autoimmune disorders.32 Therefore, sulphite sensitive individuals taking cyclophosphamide may encounter adverse side effects. In contrast with the up- and down-modulation of CYP activities, activities of the hepatic conjugation enzyme, cytosolic GST, remained unchanged in a SOX-deficient state. Sulphite and sulfiting agents, such as sulfur dioxide and the salts of bisulfite and of metabisulphite, are currently used for a variety of preservative properties that include controlling microbial growth, preventing browning and spoilage, and bleaching some foods.33 It is estimated that up to 500,000 sulphite-sensitive individuals live in the United States.34 Although sulphites are apparently safe for consumption by most subjects, numerous studies have described individuals with sulphite sensitivity that experience adverse reactions on ingestion of sulfiting agents.34,35 In this study, we examined the in vivo effects of sulphite oxidase deficiency on the catalytic activities of major phase I drug metabolizing enzymes in rat livers. We found that sulphite oxidase deficiency had statistically significant effects on many of the cytochrome P450-dependent monoxygenase systems examined in the liver, lung, kidney and small intestine of rats. The activity of CYP1A, 2E1 and 3A appeared to be particularly vulnerable to the effect of SOX deficiency while CYP19, 2B and 2C are less affected. Knowledge of the impact and nature of these alterations associated with SOX deficiency may help to advance the individualization of medication management in this population.

Table 1. The effect of sulphite oxidase deficiency on the activity of xenobiometric metabolizing enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Liver</th>
<th>Lung</th>
<th>Kidney</th>
<th>Small Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX (Units/mg protein)</td>
<td>2.22 ± 0.32</td>
<td>0.0035 ± 0.0001***</td>
<td>nm</td>
<td>nm</td>
</tr>
<tr>
<td>Control</td>
<td>SOX-Deficient</td>
<td>SOX-Deficient</td>
<td>SOX-Deficient</td>
<td>SOX-Deficient</td>
</tr>
<tr>
<td>b5 RED (nmol/min/mg protein)</td>
<td>0.252 ± 0.01</td>
<td>0.194 ± 0.008</td>
<td>nm</td>
<td>nm</td>
</tr>
<tr>
<td>NDMA-ND (nmol/min/mg protein)</td>
<td>0.377 ± 0.007</td>
<td>0.456 ± 0.011**</td>
<td>0.071 ± 0.006</td>
<td>0.097 ± 0.009</td>
</tr>
<tr>
<td>A4H (nmol/min/mg protein)</td>
<td>0.306 ± 0.028</td>
<td>0.79 ± 0.023***</td>
<td>0.051 ± 0.006</td>
<td>0.054 ± 0.004</td>
</tr>
<tr>
<td>EROD (pmol/min/mg protein)</td>
<td>12.8 ± 3.5</td>
<td>92.8 ± 1.8*</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>CN3D (nmol/min/mg protein)</td>
<td>0.29 ± 0.066</td>
<td>0.657 ± 0.003**</td>
<td>0.129 ± 0.008</td>
<td>0.098 ± 0.004</td>
</tr>
<tr>
<td>ERND (nmol/min/mg protein)</td>
<td>0.159 ± 0.02</td>
<td>0.535 ± 0.016**</td>
<td>0.052 ± 0.006</td>
<td>0.051 ± 0.006</td>
</tr>
<tr>
<td>APND (nmol/min/mg protein)</td>
<td>0.773 ± 0.093</td>
<td>0.948 ± 0.041*</td>
<td>0.233 ± 0.021</td>
<td>0.223 ± 0.009</td>
</tr>
<tr>
<td>Aromatic (pmol/min/mg protein)</td>
<td>4.02 ± 0.058</td>
<td>2.93 ± 0.061</td>
<td>nm</td>
<td>nm</td>
</tr>
<tr>
<td>PROD (pmol/min/mg protein)</td>
<td>21.0 ± 1.66</td>
<td>13.5 ± 0.65**</td>
<td>23.3 ± 1.89</td>
<td>22.2 ± 1.72</td>
</tr>
<tr>
<td>GST (nmol/min/mg protein)</td>
<td>140 ± 11.4</td>
<td>128 ± 22.1</td>
<td>nm</td>
<td>nm</td>
</tr>
</tbody>
</table>

1 Data are presented as the mean ± SD at least four sets of triplicate determinations. *p<0.05, **p<0.01, ***p<0.001 vs Control. nd: not detectable, nm: not measured.
Acknowledgments

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Abbreviations

A4H aniline 4-hydroxylase
BHT butylated hydroxytoluene
BSA bovine serum albumin
CDNB 1-chloro-2,4-dinitrobenzene
CN3D caffeine N-demethylase
CYP cytochrome P450
EDTA ethylene diamine tetra acetic acid disodium salt
ERND erythromycin N-demethylase
EROD ethoxyresorufin O-deethylase
GSH reduced glutathione
GST glutathione S-transferase
HEPES N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid
NADPH β-nicotinamide adenine dinucleotide phosphate
NDMA N-nitrosodimethylamine
NDMA-ND N-nitrosodimethylamine N-demethylase
PMSF phenylmethylsulphonylfluoride
SOX sulphite oxidase deficiency
TRIS 2-amino-2-(hydroxymethyl)-1,3-propanediol
TWEEN 20 polyoxyethylene sorbitan monolaurate

References

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Fig. 1. The expression level of CYP1A1, CYP2B1, CYP2C6, CYP2E1 and CYP3A1 proteins in control rats and SOX-deficient rats. Treatments were carried out as described in Materials and Methods (a–e). Representative immunoblot analysis of liver microsomal CYP1A1, CYP2B1, CYP2C6, CYP2E1 and CYP3A1 proteins in experimental groups with rabbit anti-rat CYP1A1, CYP2B1, CYP2C6, CYP2E1 and CYP3A1 IgG. Lane 1–2, control; lane 3–5, SOX-deficiency. (f) Comparison of the CYPs protein levels among experimental groups. The bar graphs represent the mean intensity of the bands obtained from Western blot results. Experiments were repeated at least 3 times. Results are presented as the mean ± SD. *p<0.05, **p<0.01, ***p<0.001.


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