Short Communication

Cimetidine Sulfoxidation in Small Intestinal Microsomes

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ABSTRACT:

In previous studies, sulfoxide metabolite was observed in animal and human intestinal perfusions of cimetidine and other H2-antagonists in vivo. L-Methionine, imipramine, and the anionic exchange inhibitor disothiocyanostilbene-2,2'-disulfonic acid reduced metabolite appearance. A sequence of follow-up studies is underway, for the purpose of assessing the contributions of drug metabolism and drug and metabolite transport to variable drug absorption. In this regard, drug-drug and drug-nutrient interactions represent a primary focus of this research. The S-oxidation of cimetidine in mammalian small intestinal microsomes was studied from three different species and two intestinal regions. Based on preparation activity and tissue availability, the relative contributions of flavin-containing monoxygenases and cytochrome P450 enzymes to cimetidine sulfoxidation were evaluated in rabbit jejunal microsomes. Additional inhibitor studies were carried out to evaluate the role of microsomal cimetidine sulfoxidation in the previous in vivo observations.

Cimetidine is an H2-receptor antagonist of therapeutic utility in the treatment of peptic ulcer disease and gastric hypersecretory syndromes (Lipsy et al., 1990). In animals (Dixon et al., 1985; Oldham and Chenery, 1985; Waring and Wood, 1982; Mitchell et al., 1982) and humans (Taylor et al., 1978; Taylor and Cresswell, 1975), cimetidine is metabolized principally to cimetidine S-oxide. In male and female rats, dogs, and humans, 30%, 12%, 15%, and 20% of an oral dose of cimetidine is excreted as the S-oxide in 24 hr (Taylor and Cresswell, 1975). The major monoxygenases that catalyze formation of aliphatic sulfoxides are the cytochromes P-450 (CYP450) (Hunt et al., 1982) and flavin monoxygenases (FMO) (Ziegler, 1980). Previous studies have implicated both of these enzyme systems to a varying extent in the S-oxidation of cimetidine in hepatic microsomes (Dixon et al., 1985; Oldham and Chenery, 1985; Mitchell et al., 1982).

Preliminary studies in our laboratories showed substantial excretion of the S-oxide into the intestinal lumen when cimetidine was perfused through rat jejunum in situ and moderate amounts were excreted in human jejunal perfusions in vivo. Metabolite identification was confirmed in fraction-collected perfusate from both species by mass spectrometry. A regional difference was noted as minimal lumenal appearance of the metabolite was observed in rat ileal perfusions. The appearance of S-oxide in rat jejunum was inhibited by nutrients, methionine and glutathione, the tricyclic antidepressant drug, imipramine, and the anionic-exchange inhibitor, DIDS (disothiocyanostilbene-2,2'-disulfonic acid) (Hui et al., 1994; Piyapolrungroj, 1998).

Studies on cimetidine sulfoxidation by rat, rabbit, and human intestinal microsomes are reported in this communication. Some mechanistic detail is provided in rabbit intestinal microsomes based on tissue availability (animal vs. human) and the sulfoxidation activity of the preparation (rabbit vs. rat). In rabbit jejunum, inhibition studies indicate that FMO contributes about 70%, whereas CYP450 and nonenzymatic oxidation contributes about 30% to cimetidine sulfoxidation. Based on previous in vivo findings, a specific nutrient (methionine) and drug (imipramine) were tested as inhibitors of microsomal cimetidine sulfoxidation to determine drug-nutrient and drug-drug interaction potentials that might influence variable cimetidine elimination by the small intestine. Finally, the capacity for DIDS to inhibit cimetidine sulfoxidation was tested in rabbit and human jejunal microsomes, as this agent almost completely abolished the lumenal appearance of cimetidine sulfoxide in rat jejunal perfusions (Hui et al., 1994).

Materials and Methods

Chemicals. Cimetidine, methimazole, 1-aminobenzotriazole, SKF 525-A, the components of the NADPH-generating system, bovine serum albumin, imipramine, methionine, and DIDS were purchased from Sigma Chemical Co. (St. Louis, MO). SmithKline Beecham Pharmaceuticals (Philadelphia, PA) kindly provided cimetidine sulfoxide. Protein assay reagent was purchased from Bio-Rad Laboratories (Hercules, CA). All other chemicals and solvents used were of the highest purity available from commercial suppliers.

Preparation of Microsomes. Small intestine was excised rapidly from anesthetized rats and rabbits and irrigated with ice-cold 1.14% potassium chloride (Fang and Strobel, 1978; Goon and Klaassen, 1992). The lumen was cut open longitudinally and washed with the potassium chloride solution. The small intestine from the ligament of Treitz to the cecum was divided into the following segments: ileum (in rat, the last 20 cm proximal to the cecum; in rabbit, the last 70 cm proximal to the cecum) and jejunum (in rat and rabbit, to 40 cm and 70 cm, respectively, distal to the ligament of Treitz). Three human jejunal tissue samples of sufficient size to perform cimetidine sulfoxidation activity measurements in microsomes were obtained from the University of Michigan pathology laboratory immediately after jejunal resection of two elderly patients with morbid obesity (one man and one woman) and one elderly female patient with bladder cancer. A pooled sample of human ileal tissue was also tested. However, the individual ileal tissue samples were from...
patients with intestinal disease (subjects with Crohn’s disease and small bowel melanoma.

The upper villus layer of the mucosa was removed with the edge of a glass slide, and the mucosal cells were suspended in a cold homogenization buffer containing 100 mM potassium phosphate, pH 7.4, 1 mM sodium ethylenediamine-

microtetric acid, 150 mM potassium chloride, 0.1 mM dithiothreitol, and 250 mM sucrose to which 0.25 mM phenylmethylsulfonyl fluoride in methanol was added. The mucosal cells were pelleted by spinning at 3000g for 6 min at 4°C using a Beckman J2–21M centrifuge and were washed twice with 100 mM potassium phosphate homogenization buffer. The cell pellets were homoge-

nized in a 4-fold (by pellet weight) volume of homogenization buffer using 10 strokes with a Potter-Elvehjem homogenizer and then sonicated for 30 sec. The homogenate was centrifuged at 12,000g for 35 min to sediment nuclei and mitochondria. The supernatant (S9) was filtered through two layers of cheese-


cloth and centrifuged at 100,000g for 70 min to sediment the microsomal membrane fraction. The microsomes were washed in a minimal volume of buffer containing 100 mM tetrasodium pyrophosphate, pH 7.4, and 1 mM sodium ethylenediaminetetraacetic acid in the homogenizer to remove hemoglo-

bin. The microsomes were resuspended in 100 mM potassium phosphate buffer, pH 7.4, containing 250 mM sucrose and 1 mM sodium ethylenediamine-

itetraacetic acid and stored at −70°C. Protein concentrations were deter-

mined by the Bio-Rad assay using bovine serum albumin as the standard.

Incubation and Metabolite Extraction. The incubation medium contained 50 mM potassium phosphate (pH 7.4), 0.5 mM NADP+, 2.0 mM glucose-6-

phosphate, 2 IU glucose-6-phosphate dehydrogenase, and 1.0 mg rabbit, rat, or human intestinal microsomes in a total volume of 1.0 ml combined and mixed at 4°C. Standard curves were generated from reaction mixtures with 1 mg bovine serum albumin in the absence of microsomes and 0–4 μM authentic cimetidine S-oxide. The reaction mixture was preincubated for 5 min at 37°C, and treatments initiated by the addition of cimetidine (0.1 mM). The reactions were terminated with 200 μl 10% trichloroacetic acid after 30 min at 37°C. Codeine (20 μl of 1 mM) was added as an internal standard. After centrifu-

gation, the supernatant was adjusted to pH 7.0 with 200 μl of 0.5 M sodium phosphate and cimetidine, S-oxide, and codeine in the supernatant were ex-

tracted using solid phase extraction procedures as described previously (Lin et al., 1985). An Altech C18 column was attached to a Vac Elut apparatus and a vacuum pressure of 10 inches of mercury (flow, approximately 1.0 ml/min) was applied.

Cimetidine Sulfoxide Analysis. The methanol elute containing metabolite was evaporated under a stream of nitrogen and the metabolite was reconsti-

uted in 250 μl of mobile phase. The cimetidine and S-oxide (200 μl) were separated by high-performance liquid chromatography using a Nucleosil SA cation exchange column (10 μm, 250 × 4.6 mm; Alltech Associates, Deer-

field, IL) equilibrated with 25% v/v acetonitrile and 75% v/v phosphate buffer (30 mM KH2PO4, 3.7 mM Na2HPO4 and 10 g/liter KCl, adjusted to pH 4 with 85% H3PO4) at a flow rate of 1 ml/min, and identified with UV detection at 228 nm (Lawson et al., 1982). Under these conditions, cimetidine S-oxide and cimetidine, and codeine eluted at 7.7, 10.3, and 18.7 min, respectively. Quan-

tification of the rates of formation of cimetidine S-oxide was performed by comparison with integrated peak area ratios with standard curves. The solid phase extraction modification to this assay improved sensitivity 4-fold. Appropriate controls are run to ensure that nonmicrosomal oxidation was minimal.

Inhibition Studies. For discrimination between FMO and cytochrome P450 enzyme contributions, inhibitors were preincubated for 5 min at 37°C in the presence of intestinal microsomes and the NADPH-regenerating system before addi-

tion of cimetidine. After a preincubation period of 5 min at 37°C, incuba-

tions were performed for up to 30 min and analyzed as described. The concentration of an inhibitor producing a 50% decrease in the rate of cimeti-

dine S-oxide formed (IC50) was determined from plots of the inhibitor con-

centrations vs. percentage of cimetidine S-oxide formed relative to control.

Results and Discussion

Metabolite Production by Microsomal Preparations. It was nec-

essary to use pooled samples from rats to obtain enough tissue for microsomal studies in this species. Cimetidine S-oxide production in this preparation was low and equivalent in both jejunal and ileal

samples and control levels of metabolite in the absence of NaDPh were not statistically distinguishable from levels in the presence of NaDPh. Previous in vivo studies in rats showed that 70% of absorbed cimetidine appeared as the S-oxide in the jejunal lumen from 0.4 mM cimetidine perfusions, whereas S-oxide was not detectable from ileal drug perfusion. Failure to generate anything but nonenzymatic metab-

olite production (without NaDPh = 10.2 ± 5.66; with NaDPh = 13.5 ± 3.62 pmol/min/mg) from rat jejunal microsomes prompted studies in intestinal microsomes from another species.

Biotransformation of cimetidine was substantially greater for rabbit jejunal than ileal microsomes (74.7 ± 25.00 vs. 25.0 ± 2.67 pmol/min/mg), which is consistent with regional difference observations in the rat in vivo study and jejunal cimetidine sulfoxidation showed NADPH dependence (8.1 ± 6.50 pmol/min/mg without NaDPh). The NADPH-dependent cimetidine sulfoxidation by rabbit intestinal microsomes was linear with reaction time (up to 30 min) and protein (0–1.5 mg/ml) concentration, and the data presented represent the 30-min mean ± SD from at least three independent preparations run in duplicate, unless otherwise specified.

Cimetidine sulfoxidation activity was lower in human jejunal microsomes than in rabbit, but sulfoxidation was also NaDPh-dependent (37.2 ± 10.41 vs. 4.4 ± 0.21 pmol/min/mg for two preparations without NaDPh). Normal human ileal tissue could not be obtained, and the single pooled diseased human ileal sample showed very high levels of cimetidine sulfoxidation (241.8 vs. 65.71 pmol/min/mg in the presence and absence of NaDPh, respectively). Although this is not in line with regional observations in vivo (Hui et al., 1994; Piyapolrungroj, 1998), these duplicate enzymatic and nonenzymatic rates of cimetidine S-oxidation in a single pooled human ileal sample may be a function of sample pathology.

Inhibition Studies. The effects of FMO and P450-dependent monoxygenase inhibitors on cimetidine sulfoxidation were studied using rabbit intestinal microsomes. Competitive inhibition of FMO was performed using methimazole (Dixit and Roche, 1984), which decreased the rate of cimetidine S-oxidation to 28% of control values (IC50 value: 9.1 μM). Cytochrome P450 inhibitors SKF-525A (Buening and Franklin, 1976) and aminobenzotriazole (Ziegler, 1980; Mathews et al., 1985) reduced S-oxide formation rate by 20–30% (fig. 1).

Methionine and imipramine were tested for their potential to pro-

vide nutrient and drug interactions with cimetidine sulfoxidation in the upper small intestine based on previous in vivo observations (Hui et al., 1994; Piyapolrungroj, 1998). A total of 5 mM methionine was
observed to reduce the appearance of cimetidine sulfoxide in rat jejunal perfusions of 0.4 mM cimetidine by 25% (Piyapolrungroj, 1998). In a similar perfusion study, the antidepressant drug and FMO-N-oxidation substrate, imipramine (Rouer et al., 1987), reduced luminal cimetidine sulfoxide appearance by 70%, whereas methimazole completely abolished the appearance of cimetidine sulfoxide (Piyapolrungroj, 1998). In rabbit jejunal microsomes, methionine reduced cimetidine sulfoxidation by 20% and imipramine decreased S-oxide formation by 45% at 1 mM inhibitor concentrations (fig. 1). In brush-border membrane vesicle studies, imipramine was observed to significantly reduce the uptake of cimetidine, whereas methionine and methimazole had no effect. Thus, the reduction by imipramine of cimetidine sulfoxide appearance in vivo may be more a function of effects on cimetidine transport (Piyapolrungroj, 1998).

In this rat perfusion model, 0.1 mM DIDS co-perfusion almost totally abolished the luminal appearance of cimetidine sulfoxide in the jejunum (Hui et al., 1994). At 0.1 mM, DIDS reduced cimetidine sulfoxide levels to 25% of control values in rabbit jejunal microsomes and 1 mM DIDS resulted in 80% inhibition of cimetidine sulfoxidation in both rabbit and human jejunal microsomes (fig. 2). As cimetidine uptake in mucosal membrane vesicles was not significantly reduced by the presence of DIDS in the incubation medium (Piyapolrungroj, 1998), inhibition of microsomal enzymes is projected to account for the previous observations in rat perfusion experiments (Hui et al., 1994).

In conclusion, intestinal FMO appears to play a greater role in cimetidine sulfoxidation than do CYP450 enzymes in rabbit jejunal microsomes. Although the strong inhibition of cimetidine sulfoxidation observed for imipramine in vivo may be more a function of cimetidine uptake inhibition, the weaker inhibition in vivo observed for methionine may be related to metabolism. The strong inhibition of in vivo cimetidine sulfoxidation by DIDS, however, is also more likely to be related to an inhibition effect on metabolism.

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