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INHIBITION OF HEPATIC MICROSOMAL DRUG METABOLISM BY THE STEROID HYDROXYLASE INHIBITOR SU-10'603

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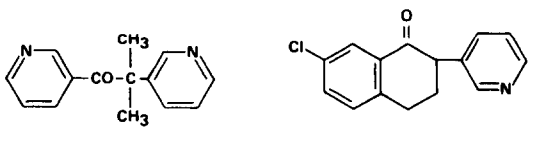
Abstract—SU-10'603 is a pyridine derivative that has been widely used as a steroid 17-hydroxylase inhibitor. Studies were done to compare the effects of SU-10'603 with those of the structurally related compound, metyrapone, on hepatic microsomal drug metabolism *in vitro* in rats and guinea pigs. In rat liver microsomes, SU-10'603 produced a concentration-dependent (0.01 to 1.0 mM) inhibition of ethylmorphine demethylation, aniline hydroxylation, and benzo[*a*]pyrene hydroxylation. A concentration of 0.1 to 0.2 mM decreased the metabolism of all three substrates by approximately 50%. SU-10'603 was a more potent inhibitor of ethylmorphine metabolism than metyrapone, and its relative potency was even greater with respect to aniline and benzo[*a*]pyrene metabolism. Similar results were obtained with guinea pig liver microsomes. SU-10'603 and metyrapone produced type II spectral changes in hepatic microsomes, but the apparent affinity of SU-10'603 for cytochrome(s) P-450 was greater than that of metyrapone. Both compounds inhibited the binding of type I substrates to microsomal cytochromes P-450; SU-10'603 was the more potent inhibitor. The results indicate that SU-10'603 is a potent inhibitor of hepatic microsomal monooxygenases whose mechanism of action is similar to that of metyrapone.

A wide variety of steroid hydroxylase inhibitors have been described, many of which have found experimental and/or clinical applications [1–3]. Clinically such compounds are used in the diagnosis and treatment of disorders associated with abnormal steroidogenesis. Steroid hydroxylase inhibitors have also been of considerable research value for characterizing steroidogenic enzymes and defining their roles in different metabolic pathways. Since all of the steroid hydroxylases are cytochrome P-450-dependent monooxygenases [4–7], it is not surprising that cytochromes P-450 are the target sites for many of these enzyme inhibitors. Accordingly, the specificity of each inhibitor resides in its capacity for interacting with different cytochromes P-450.

In addition to their roles in steroidogenesis, cytochromes P-450 catalyze numerous drug-metabolizing reactions in the liver and in extra-hepatic tissues [8, 9]. In general, different cytochromes P-450 are involved in steroidogenesis and drug metabolism, and most monooxygenase inhibitors are relatively specific for one type of reaction or the other [1–3, 10, 11]. However, metyrapone (2-methyl-1,2-bis[3-pyridyl]-1-propanone), a potent steroid 11β-hydroxylase inhibitor, also inhibits a variety of drug-metabolizing enzymes and has been quite useful in drug metabolism research [10, 12, 13]. The steroid 17α-hydroxylase inhibitor SU-10'603 (7-chloro-3,4-dihydro-2-[3-pyridyl]-naphthalene-1-[2H]-one) is structurally related to metyrapone (Fig. 1), but to our knowledge, effects of this compound on drug-metabolizing reactions have not been examined previously [1–3]. In the course of studies on adrenal microsomal metabolism, we noted that SU-10'603 seemed to inhibit xenobiotic as well as steroid metabolism. Accordingly, the studies described in this paper were initiated to evaluate the effects of SU-10'603 on hepatic drug metabolism.

METHODS

Male Sprague–Dawley rats (250–300 g), obtained from Zivic–Miller Laboratories, Pittsburgh, PA, and English Short Hair guinea pigs (800–1000 g), obtained from Camm Research Institute, Wayne, NJ, were housed under standardized conditions of light (6:00 a.m. to 6:00 p.m.) and temperature (22°). All animals were untreated and allowed at least 7 days to become acclimated to the housing conditions prior to use in experiments. Animals were killed by decapitation between 9:00 and 10:00 a.m. Livers were quickly removed and homogenized in cold



Metyrapone SU-10'603

Fig. 1. Structures of metyrapone and SU-10'603.

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1.15% KCl. All steps in the preparation of microsomal fractions were carried out at 0–4°. Homogenates were centrifuged at 9000 *g* for 20 min, and the supernatant fraction was centrifuged in a Beckman preparative ultracentrifuge at 105,000 *g* for 60 min to obtain the microsomes. Microsomal pellets were resuspended in 1.15% KCl containing 0.05 M Tris–HCl buffer (pH 7.4) at a concentration of 4–5 mg protein/ml.

Aniline hydroxylase activity was assayed as the rate of *p*-aminophenol production by hepatic microsomes as described previously [14, 15]. Benzo[*a*]pyrene hydroxylation was determined by the fluorometric method of Nebert and Gelboin [16]. Quinine sulfate was calibrated against authentic 3-OH-benzo[*a*]pyrene and routinely used as the fluorescence standard. Ethylmorphine N-demethylation was assayed as the amount of formaldehyde formed using the method of Nash [17], as previously described [18]. For all enzyme assays, conditions were established to ensure linearity of product formation with respect to protein concentrations and incubation times.

Cytochrome P-450 was measured as the dithionite-reduced CO complex as described by Omura and Sato [19] with an Aminco DW-2a recording spectrophotometer. NADPH–cytochrome *c* reductase activity was assayed by the method of Phillips and Langdon [20]. NADPH–cytochrome P-450 reductase activity was determined with an Aminco DW-2a recording spectrophotometer in the dual wavelength (450–490 nm) mode, essentially as described by Gigon *et al.* [21] and modified by Jansson and Schenkman [22]. The reaction was run at 22°. Metyrapone- and SU-10'603-induced spectral changes in hepatic microsomes were obtained with an Aminco DW-2a recording spectrophotometer. Spectra resulting from inhibitor interactions with reduced cytochromes P-450 were obtained following the addition of sodium dithionite to the microsomal suspensions. Microsomal protein concentrations were determined by the method of Lowry *et al.* [23].

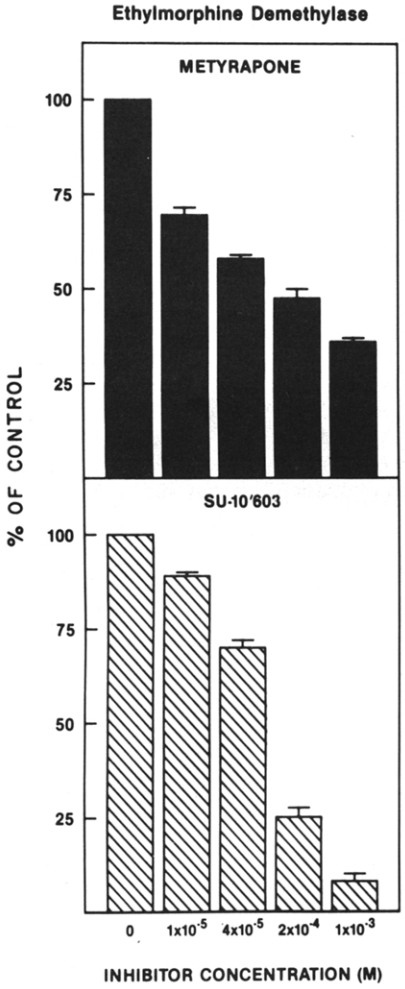

Fig. 2. Dose-dependent effects of metyrapone and SU-10'603 on ethylmorphine demethylase activity in rat liver microsomes. Values are expressed as mean percent of enzyme activity in the absence of inhibitors ± SE of five experiments. Control (100%) enzyme activity was 12.3 nmol · min^{-1} · (mg protein)$^{-1}$.

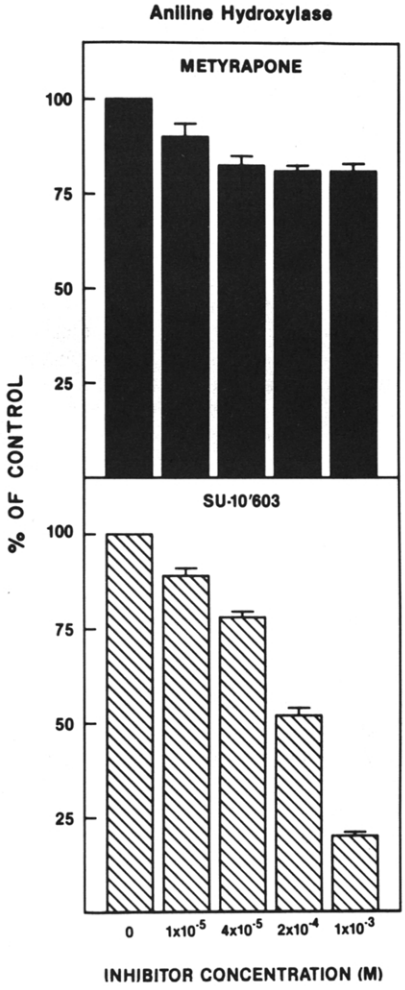

Fig. 3. Dose-dependent effects of metyrapone and SU-10'603 on aniline hydroxylase activity in rat liver microsomes. Values are expressed as mean percent of enzyme activity in the absence of inhibitors ± SE of five experiments. Control (100%) enzyme activity was 1.2 nmol · min^{-1} · (mg protein)$^{-1}$.

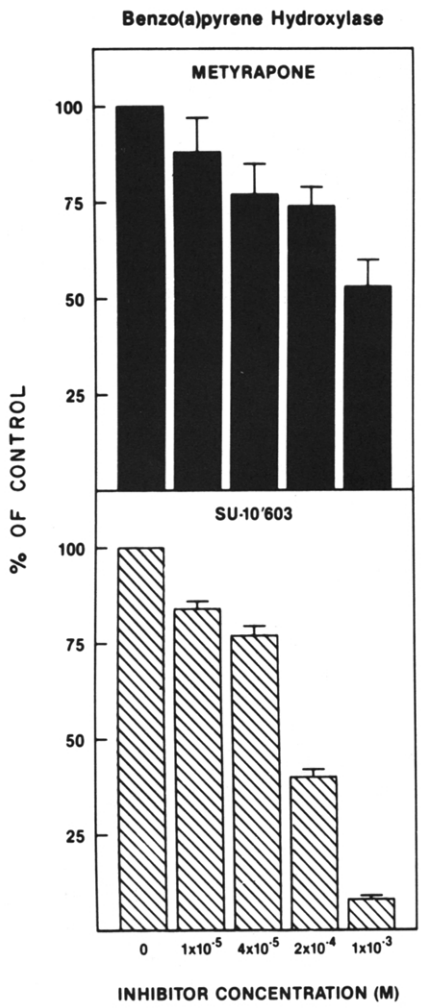

Fig. 4. Dose-dependent effects of metyrapone and SU-10'603 on benzo[*a*]pyrene hydroxylase activity in rat liver microsomes. Values are expressed as mean percent of enzyme activity in the absence of inhibitors ± SE of five experiments. Control (100%) enzyme activity was 0.62 nmol · min^{-1} · (mg protein)$^{-1}$.

RESULTS AND DISCUSSION

Addition of SU-10'603 to suspensions of rat liver microsomes produced a concentration-dependent inhibition of ethylmorphine demethylation (Fig. 2). SU-10'603 was a more potent inhibitor of enzyme activity than metyrapone, particularly at higher concentrations of the two compounds (Fig. 2). The potency of SU-10'603 relative to metyrapone was even greater with respect to inhibition of aniline hydroxylase and benzo[*a*]pyrene hydroxylase activities (Figs. 3 and 4). Metyrapone was a poor inhibitor of aniline hydroxylation in rat liver microsomes, decreasing activity by only 15–20% at concentrations as high as 1.0 mM (Fig. 3). By contrast, 0.2 mM SU-10'603 decreased aniline hydroxylase activity by approximately 50%. At a concentration of 1.0 mM, metyrapone inhibited benzo[*a*]pyrene hydroxylase activity by approximately 50% (Fig. 4). The same concentration of SU-10'603 decreased the rate of benzo[*a*]pyrene hydroxylation in rat hepatic microsomes by 90–95%. Results similar to those described above for the effects of metyrapone and SU-10'603 on drug-metabolizing enzymes in rat liver microsomes were also obtained with guinea pig hepatic microsomal preparations (data not shown).

Upon addition to rat liver microsomes, SU-10'603, like metyrapone, produced a typical type II spectral change (data not shown), suggesting interactions at or near the heme component of cytochromes P-450 [10, 24]. Both compounds also produced difference spectra in dithionite-reduced hepatic microsomes. The maximal spectral changes produced were greater for SU-10'603 than for metyrapone and the apparent affinity of SU-10'603 for cytochromes P-450 (based upon the spectral dissociation constant) was also greater than that of metyrapone (data not shown). Both compounds blocked the binding of type I substrates to cytochromes P-450 in an apparently competitive manner (Fig. 5); SU-10'603 was a more potent blocker than metyrapone. Neither compound affected NADPH–cytochrome *c* reductase activity in rat hepatic microsomes (Table 1), but both seemed to decrease the rate of reduction of cytochromes P-450. The latter is probably attributable to interference with the binding of CO to reduced cytochromes P-450, the parameter monitored in the NADPH–cytochrome P-450 reductase assay.

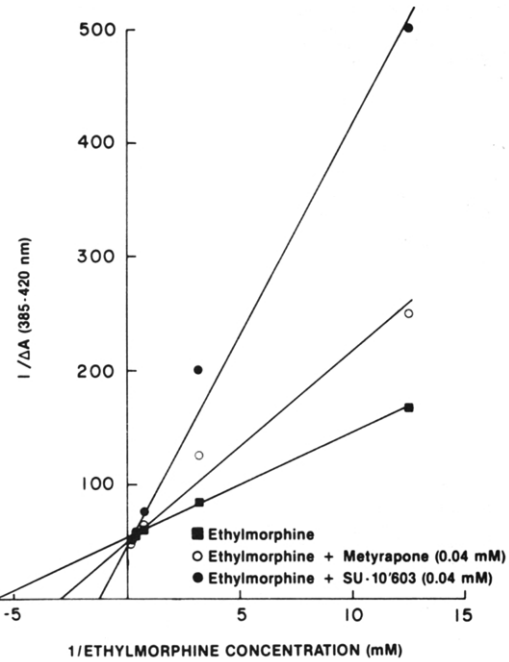

Fig. 5. Double-reciprocal plot illustrating the effects of SU-10'603 and metyrapone on the ethylmorphine-induced type I spectral change ($\Delta A_{385-420\,nm}$) in rat hepatic microsomes. Spectra were obtained at room temperature and were corrected for the baseline of equal light absorbance. Metyrapone and SU-10'603 were added to the microsomal suspensions (2.1 mg protein/ml) in 10 μl ethanol and ethylmorphine in a maximum of 100 μl of 1.15% KCl–0.05 M Tris–HCl (pH 7.4). Reference cuvettes were balanced with corresponding volumes of the appropriate vehicle.

Table 1. Effects of SU-10'603 and metyrapone on NADPH–cytochrome P-450 and NADPH–cytochrome *c* reductase activities in rat liver microsomes*

Inhibitor concn (mM)	NADPH–cytochrome P-450 reductase (% of control)	NADPH–cytochrome *c* reductase (% of control)
Metyrapone		
0	100†	100‡
0.2	72 ± 8	103 ± 4
1.0	37 ± 3	102 ± 6
SU-10'603		
0	100†	100‡
0.2	63 ± 4	105 ± 6
1.0	30 ± 3	104 ± 7

* Values are expressed as mean percent of control (0 inhibitor) ± SEM of four experiments.

† One hundred percent is equivalent to 4.6 nmol·min^{-1}·(mg protein)$^{-1}$.

‡ One hundred percent is equivalent to 86.7 nmol·min^{-1}·(mg protein)$^{-1}$.

The results indicate that, contrary to prior assumptions, the inhibitory effects of SU-10'603 are not limited to steroid hydroxylation reactions. SU-10'603 is a potent and relatively non-specific inhibitor of hepatic microsomal drug metabolism whose mechanism of action appears to be similar to that of metyrapone. The compound is a more potent inhibitor of xenobiotic metabolism than metyrapone and may, therefore, be useful in investigations on the involvement of cytochromes P-450 in hepatic drug metabolism. However, the non-specificity of SU-10'603 as a monooxygenase inhibitor must be considered when it is used with preparations containing multiple cytochrome P-450 isozymes.

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