Identification of a Pyridinium Metabolite in Human Urine following a Single Oral Dose of 1-[2-[Bis[4-(trifluoromethyl)phenyl]methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic Acid Monohydrochloride, a γ-Aminobutyric Acid Uptake Inhibitor

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Single-dose administration of 50 mg of 1-[2-[bis[4-(trifluoromethyl)phenyl]methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid monohydrochloride resulted in temporary neurological and psychological symptoms in two subjects. Because of the nature of adverse effects, urine from a subject who received CI-966 orally was extracted to investigate the metabolism of CI-966 in man. An unknown urinary component was identified as a pyridinium metabolite of CI-966 based on HPLC-MS and 1H and 13C NMR. Structural confirmation was achieved by chromatographic and spectroscopic comparisons to a reference standard. In several in vitro screens and preclinical studies, the pyridinium metabolite appears to possess minimal pharmacological activity.

Introduction

CI-966 is a novel anticonvulsant which differs structurally and mechanistically from currently marketed anticonvulsants (1). CI-966 acts by inhibiting the synaptic uptake of GABA, which is a major inhibitory neurotransmitter in the mammalian central nervous system (2). Inadequate GABAergic inhibition may be the underlying mechanism of some seizure disorders in man (3–5). Proposed indications were for treatment of partial seizures and generalized tonic–clonic seizures.

A randomized, double-blind, escalating single-dose-tolerance, and pharmacokinetic study comparing the effects of CI-966 capsules with those of placebo was conducted in healthy volunteers (6, 7). The study was terminated at the 50-mg dose due to adverse effects experienced by two female subjects. Physical signs included unresponsiveness to nonpainful sensory stimuli, tremor, myoclonus, increased muscle rigidity, and cogwheeling. Short- and long-term memory impairment was also observed. Severity of symptoms appeared to correlate with plasma CI-966 concentration and all physical signs had resolved within 24 h following dosing. However, psychiatric symptoms resembling those observed in manic and schizophrenic patients lasted for 5 to 6 days in both subjects, suggesting that CI-966 elimination from brain may be slower than that from plasma (harmonic mean t1/2 of 16.8 h) or biotransformation of CI-966 to one or more active metabolites occurs. It is possible that some of the neurological and psychological symptoms observed following administration of 50 mg of CI-966 may be unrelated to inhibition of GABA uptake and could be attributed to an unknown pharmacological response to CI-966 or a metabolite (8). In fact, CI-966 undergoes reductive and oxidative metabolism in rats (9). A piperidine and pyridinium metabolite of CI-966 were identified in pooled rat urine.

Because of the severity of adverse events and our knowledge of the metabolism of CI-966 in rats, it was deemed important to examine the metabolic fate of CI-966 in man.

Materials and Methods

Isolation Procedure. Approximately 1 L of human urine (0–6 and 24–48 h collections) from a single male subject given 25 mg of CI-966 was processed by solid-phase extraction using Analytchem (Harbor City, CA) 3-mL CN end-capped cartridges. Urine was adjusted to pH 9 with base and aliquots (6 mL) were applied to individual CN cartridges, previously conditioned with 3 × 2 mL of acetonitrile and 3 × 2 mL of 50 mM ammonium phosphate dibasic (pH 9) (buffer A). Each cartridge was sequentially washed with 2 × 2 mL of buffer A, 2 mL of 20% acetonitrile in buffer A, 3 × 2 mL of 50 mM ammonium acetate (pH 2.1) (buffer B), and 2 × 1 mL of 5% acetonitrile in buffer B. Eluates eluted with 2 × 750 μL of 60% acetonitrile in buffer B. The eluent was evaporated to dryness and the residue was reconstituted in a mobile phase/95% ethanol (95:5) mixture. Analytes were resolved using a LC-18-DB 5-μm semipreparative HPLC column (10 mm i.d. × 25 cm) by Supelco, Inc. (Bellefonte, PA), maintained at 40 °C. Mobile phase consisted of 30% acetonitrile in 50 mM ammonium phosphate (pH 2.1). Flow rate was 4.5 mL/min. Absorbance was monitored at 225 nm. An ISCO Foxy fraction collector (Isco, Inc., Lincoln, NE) with peak detection capability was used to collect the peaks of interest. Isolates were desalted using the same 3-mL CN end-capped cartridges according to the following procedure. Samples were...
diluted to 10% acetonitrile in buffer A and applied to cartridges previously conditioned with 3 × 2 mL of acetonitrile and 3 × 2 mL of buffer A. Cartridges were washed with 3 × 2 mL of buffer A and 3 × 2 mL of water (HPLC grade). Analytes were eluted with 2 × 750 μL of methanol and evaporated to dryness prior to mass spectroscopy.

HPLC–MS Analysis. For thermospray ionization analysis, a Waters 600MS multisolvent delivery system was interfaced to a VG Trio-2 mass spectrometer using a plasma spray/thermospray probe assembly and associated ion source. Separation was achieved using a 3-μm Pecosphere C18CR column (4.6 mm i.d. × 3.3 cm) by Perkin-Elmer (Norwalk, CT) and a mobile phase consisting of 50% acetonitrile in 100 mM ammonium acetate (pH 2) at a flow rate of 0.7 mL/min. The mass spectrometer was operated in the plasma spray mode with a source temperature of 200 °C, capillary temperature of 190 °C, and discharge current of 300 μA. Full scans (700–150 D) were taken repetitively at a scan rate of 1 s decade⁻¹.

NMR Analyses. ¹H and ¹⁹F NMR analyses were performed in deuterated methanol on a Varian XL300 NMR equipped with a M68000 computer.

Reference Standards. Details of the chemical synthesis of the pyridinium metabolite and CI-966 reference standards were previously reported (9).

Results and Discussion

HPLC–UV analysis of urine from a male subject who received a single 25-mg dose revealed the presence of a major unknown UV-absorbing peak (peak A of Figure 1). Positive ion HPLC–MS analysis of the isolated unknown gave a mass spectrum with a presumed molecular ion at m/z 470, three mass units less than that of CI-966, and a major fragment ion at m/z 426 (base peak) corresponding to loss of 44 mass units (-CO₂) (Figure 2). A single fluorine resonance with the same chemical shift as the bis(trifluoromethyl) groups of CI-966 was observed in the NMR spectrum (Figure 3), suggesting that 1-[2-[bis[4-(trifluoromethyl)phenyl]methoxy]ethyl] group remains unchanged in the metabolite. The ¹H NMR spectrum of CI-966 (Figure 4B) shows a resonance assigned to the vinyl proton at 7.2 ppm. This signal is not present in the ¹H NMR spectrum of the metabolite, but four new peaks appeared between 8.0 and 9.5 ppm (Figure 4A). These observations are consistent with a 3-substituted pyridinium moiety. Structural confirmation of the metabolite as a pyridinium derivative of CI-966 was made by chromatographic and spectroscopic comparisons to synthetic reference material. Presence of unchanged CI-966 in human urine was confirmed by coinjection with reference standard on HPLC–MS.
Metabolism of CI-966 to a pyridinium metabolite represents an overall four-electron oxidation. A proposed metabolic scheme is shown in Figure 5. Recently, haloperidol was found to undergo a similar four-electron oxidation to the putative neurotoxin HPP+ (10). The similarity in acute neurological side-effects, including Parkinsonism, of haloperidol and CI-966 raised concern whether the conversion of CI-966, a 1,3-disubstituted tetrahydropyridine analog, to a pyridinium species may lead to neurotoxicity.

Preclinical toxicological studies conducted in rhesus monkeys with a structurally similar compound, 1-(4,4-bis(4-fluorophenyl)butyl)-4-(4-fluorophenoxy)-1,2,3,6-tetrahydropyridine, resulted in nigrostriatal abnormalities (11) similar to those seen with MPTP (12). The authors proposed a possible structure–activity relationship of tetrahydropyridine analogs with neurologic and associated neuropathologic manifestations. Formation of a pyridinium species a priori may not be sufficient to elicit neurotoxicity. In fact, this has been shown with two nonneurotoxic MPTP analogs, 4’-methyl-MPTP and 4’-fluoro-MPTP (13). Further studies are necessary to elucidate the structure–activity relationships of the conversion of 1,3-disubstituted tetrahydropyridine compounds, such as CI-966, to pyridinium species and potential neurotoxicity.

Of the two forms of monoamine oxidase (A and B), MAO B appears to be primarily responsible for the in vivo bioactivation of MPTP to MPP+ for the following reasons. In vitro catalytic activity of MAO B toward MPTP is approximately 14 times that of MAO A (14), and both oxidation products, MPDP+ and MPP+, are more inhibitory to MAO A than B. Ki values of 2.4 μM and 3.0 μM were reported for MPDP+ and MPP+, respectively, with MAO A compared to 230 μM (MPDP+) and 300 μM (MPP+) with MAO B (15, 16). Unlike MPTP, MAO B-mediated biotransformation of CI-966 to the pyridinium metabolite seems highly unlikely. MAO B structure–activity relationships with MPTP derivatives suggest a requirement for small substituents (i.e., methyl-, ethyl-, propyl-) on the tertiary nitrogen for conversion to a dihydropyridinium species (17, 18). Furthermore, compounds bearing substituents on the 3-position as opposed to the 4-position of the tetrahydropyridine moiety are not good substrates for oxidation by MAO B (19). Therefore, oxidation of CI-966 to the dihydropyridine derivative most likely occurs through a non-MAO B-mediated oxidation. CI-966 may be metabolized by the cytochrome P-450 system which has been shown to catalyze the ring α-carbon oxidation of various cyclic tertiary amines (20–24), including MPTP (25), to iminium species.

Subsequent oxidation of the CI-966 dihydropyridine derivative to the pyridinium metabolite may be enzymatic, i.e. catalyzed by the cytochrome P-450 system as is haloperidol (26), or nonenzymatic via bimolecular redox disproportionation in a manner similar to that observed for the conversion of MPDP+ to MPP+ (27). Recently, Gerlach et al. (13) have proposed a direct hydride transfer from MPDP+ to a cellular two-electron oxidant. Additionally, a sequence of two one-electron oxidations of MPDP+ via a MPP+ intermediate is feasible under physiological condition, with further oxidation to MPP+ occurring within the dopaminergic neuron (13).

Chronic administration of high doses of CI-966 to beagle dogs in a 13-week toxicity study did not cause protracted movement disorders nor did it result in nigrostriatal lesions as determined by histology or enzymatic assays for tyrosine hydroxylase or glutamic acid decarboxylase activity of brain tissue. Furthermore, the pyridinium metabolite of CI-966 was only active as an inhibitor of in vitro dopamine uptake into rat neocortical slices at high concentrations (IC50 = 100 μM) (8) unlike the MPTP metabolite, MPP+ (IC50 = 0.14 μM) (28). The pyridinium metabolite of CI-966 was not active in a battery of 35 receptor binding assays including subtypes of dopamine, glutamate, and GABA receptors (8). Administration of the pyridinium metabolite to mice did not cause any behavioral effects at intravenous doses up to 30 mg/kg (8). Finally, an explanation for the neurological and psychological symptoms seen in healthy subjects receiving CI-966 is that they result from the effects of increased GABA concentration in many areas of the brain (8).

Although available data with CI-966 do not suggest any neurotoxic action of CI-966 or its metabolites, additional studies are needed to more thoroughly explore the possibility. Appropriate studies to assess neurotoxic potential of the pyridinium metabolite of CI-966 would include mitochondrial respiration inhibition, intracerebral microdialysis, and neuronal toxicity in cell culture.

Conclusions

CI-966, which contains a tetrahydropyridine moiety with a bulky substituent on the tertiary nitrogen, undergoes biotransformation to a pyridinium derivative in man. To the best of our knowledge, this is the first report of a 1,3-disubstituted tetrahydropyridine which is metabolized to a pyridinium metabolite in man. Investigators working with such tetrahydropyridine analogs should be cognizant of potential bioactivation of these compounds.

References


