Biochemical Pharmacology, Vol. 40, No 4, pp. 893–895, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0 00

SHORT COMMUNICATIONS

Enantioselective metabolism of encainide by rat liver microsomes

(*Received* 19 *May* 1989; *accepted* 16 *January* 1990)

Encainide [**1**, Fig. 1; 4-methoxy-2′,2-(1-methyl-2-piperidyl)ethyl benzanilide] is an antiarrhythmic drug that is effective for suppression of complex ventricular arrhythmias [1]. Metabolism of encainide in human subjects occurs under genetic control of the debrisoquine type [2]. Extensive metabolizers convert encainide to ODE* (**2**: Fig. 1) and MODE [2, 3]. In poor metabolizers, small amounts of ODE and NDE (**3**; Fig. 1) have been detected; however, no MODE is formed in these subjects. ODE and MODE are potent antiarrhythmic agents in their own right [4]. It has been suggested that they contribute to the efficacy of encainide during long-term therapy in the extensive metabolizer phenotype [5].

Encainide possesses a single chiral center adjacent to the piperidine nitrogen and, like many other cardiovascular drugs, it is normally administered to human subjects as a racemate [6–8]. There are numerous examples of enantioselective metabolism of chiral drugs [9]. However, little is known about enantioselectivity in the metabolism of encainide. Examination of this aspect of encainide metabolism was of particular interest since its metabolites are potent antiarrhythmic agents in their own right [4, 5]. Recently, we developed a method that allows efficient separation of the enantiomers of encainide and its metabolites [10]. The method is based on chiral derivatization with (−)-menthyl chloroformate [(−)-MCF] followed by normal phase HPLC separation of the diastereomers that are formed. The availability of this methodology has allowed enantioselectivity in the metabolism of encainide to be examined *in vitro* in rat liver microsomes.

Materials and Methods

Chemicals. Racemic [^{14}C]encainide (0.95 μCi/mg, ^{14}C was present at the benzanilide carbonyl), (+)- and (−)-encainide, MODE, ODE and NDE were provided by Bristol-Myers (Wallingford, CT). Triethylamine, *N,N′*-diisopropylethylamine and (−)-MCF were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Diazomethane was generated just prior to use from *N*-nitrosomethyl urea (ICN, Plainview, NY). HPLC grade solvents were purchased from American Scientific Products (Atlanta, GA). All other chemicals were of reagent grade and were used without purification.

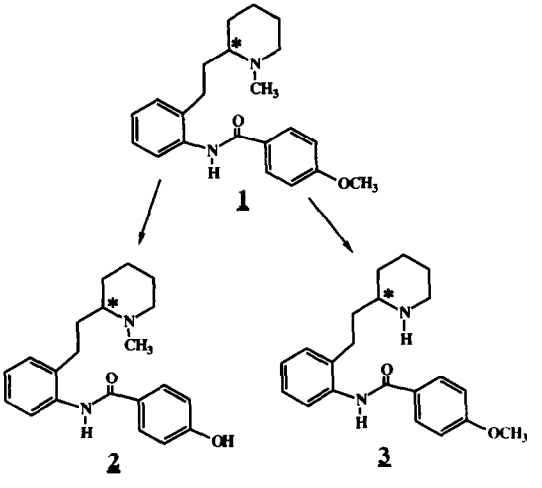

Fig. 1. Scheme for metabolism of encainide (**1**) by rat liver microsomes. Encainide was metabolized to *O*-demethyl-encainide (ODE) (**2**) and *N*-demethyl-encainide (NDE) (**3**). The asterisk indicates the position of the chiral center in the molecule.

HPLC. Chromatography was carried out as described previously [10]. System A used a semi-preparative Ultrasphere silica column (10 μm, 10 mm × 250 mm; Altex) with a mobile phase of methanol/0.01 M aqueous ammonium acetate, pH 5 (60:40, v/v). The flow rate was maintained at 2 mL/min. System B consisted of an Ultrasphere (5 μm, 4.6 mm × 250 mm; Altex) silica column. The mobile phase was isopropanol/hexane/triethylamine (2.0:97.9:0.1, by vol.) at a flow rate of 1 mL/min.

Incubation of encainide with rat liver microsomes. Rat liver microsomes were isolated as reported elsewhere [11]. Incubations of [^{14}C]encainide (0.95 μCi/mg) were carried out at 37° with a protein concentration of 1 mg/mL. They were initiated by the addition of 1 mg/mL of NADPH. The reaction was stopped by addition of ethyl acetate (2 mL) at appropriate times as noted below.

Separation, purification and quantification of metabolites. The incubation mixture was evaporated to dryness and dissolved in 10 mL of water. The aqueous solution was applied to a C-18 Sep-Pak, which was prewashed with 5 mL of methanol followed by 5 mL of water. Metabolites were eluted from the Sep-Pak by washing with 5 mL of methanol. The methanol was evaporated to dryness, and metabolites were dissolved in 0.5 mL methanol/water (50:50; v/v) and chromatographed on HPLC system A. Individual peaks corresponding to ODE and NDE were collected and a portion (10%) was used for liquid scintillation counting to determine the amount of radioactivity. Quantification was based on recovered radioactivity. The remainder was derivatized with (−)-MCF for chiral analysis.

Identification of metabolites. Encainide (250 nmol, 0.95 μCi/mg) was incubated with rat liver microsomes (5 mL) for 60 min. Metabolites were chromatographed on HPLC system A. Two metabolites (**2** and **3**) and unchanged drug (**1**) were collected as individual peaks and were rechromatographed on HPLC system A to remove minor impurities. Metabolites were identified by co-elution on HPLC with authentic standards. Structural assignments were confirmed by GC/EIMS as described previously [3].

Preparation of menthyl carbamate derivatives. NDE and encainide (0.2 to 5 μg) in acetonitrile/*N,N′*-diisopropylethylamine (50 μL, 90:10, v/v) were heated at 60° with a 50-μL solution of (−)-MCF in acetonitrile (10:90, v/v). After heating for 2 hr, the acetonitrile was evaporated and the resulting carbamate derivatives were dissolved in

* Abbreviations: ODE, *O*-demethyl-encainide; NDE, *N*-demethyl-encainide; MODE, 3-methoxy-*O*-demethyl-encainide; TMS, trimethylsilyl; GC/EIMS, combined gas chromatography/electron impact mass spectrometry; BSTFA, bis(trimethylsilyl)-trifluoroacetamide; EI, electron impact; and (−)-MCF, (−)-menthyl chloroformate.

hexane (150 μL) ready for HPLC analysis as described below. ODE was treated first with diazomethane to form the aromatic methyl ether and then derivatized in the same way [10].

Chiral analysis of metabolites. Menthyl carbamate diastereomers of NDE and ODE were resolved on HPLC system B. Areas under individual peaks in the UV chromatogram were used to quantify the relative amounts of (−)- and (+)-enantiomers.

Results and Discussion

It was shown previously that encainide was metabolized by rat liver microsomes to give ODE and a small amount of NDE [10]. MODE, a major *in vivo* metabolite in rats (unpublished) and humans [3], was not observed as an *in vitro* metabolite. The mechanism for MODE formation has not yet been delineated. However, it is evident that the microsomal enzyme system cannot carry out the multiple biotransformations that are required. The metabolism of encainide by rat liver microsomes was NADPH dependent. It was inhibited completely by metyrapone (1 mM) and by pre-incubation of microsomes with SKF-525A (1 mM). These observations indicated that metabolism of encainide was cytochrome P450 dependent. Incubations of encainide (50 μM) were carried out with rat liver microsomes for 5, 15, 30, 45 and 60 min. Isolation of ODE and quantification by radioactivity counting revealed that the linearity in formation of ODE fell markedly after 15 min (Fig. 2). Analysis of the ODE revealed that the (−)-enantiomer was always in excess over the (+)-enantiomer and that their relative amounts were constant at all time points (67:33). The metabolism of encainide was studied at concentrations in the range 10–2000 μM for 15 min. Chiral analysis of ODE isolated from the incubate revealed that the rate of formation of the (−)-enantiomer was faster than that of the corresponding (+)-enantiomer until saturation was reached (Fig. 3). In a previous study, it was shown that at low substrate concentrations (+)-encainide was in excess of (−)-encainide in the residual drug [10]. At higher concentrations, the proportion of encainide metabolized was too small to detect any enantiomeric excess in the residual encainide.

Lineweaver–Burk plots for the formation of the two ODE-enantiomers were linear (Fig. 4). From these plots the apparent K_m values for the formation of (−)- and (+)-ODE were calculated to be 16 and 51 μM respectively. The

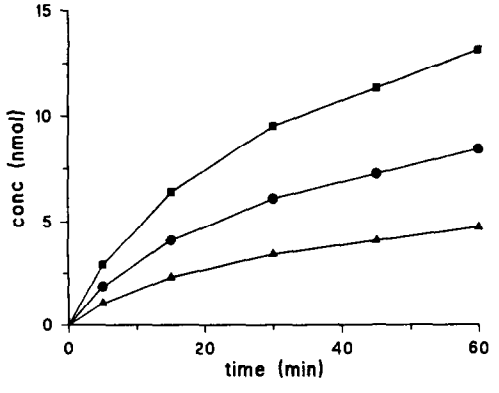

Fig. 2. Formation of (−) + (+)-ODE (■), (−)-ODE (●) and (+)-ODE (▲) from encainide by rat liver microsomes as a function of time. Experiments were carried out in duplicate. Data are presented from a single experiment. Concentrations of (−) + (+)-ODE were the sum of the individual values for the two enantiomers.

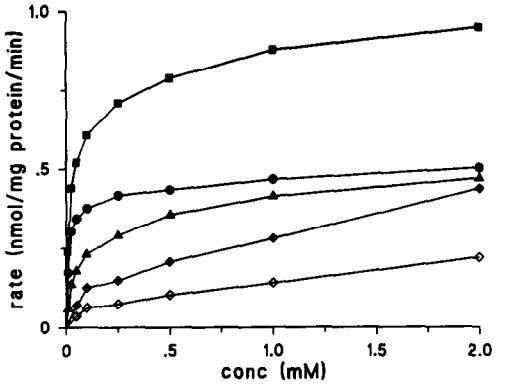

Fig. 3. Rate of formation of ODE and NDE enantiomers from encainide by rat liver microsomes as a function of concentration. Key: (−) + (+)-ODE (■), (−)-ODE (●), (+)-ODE (▲), (−) + (+)-NDE (◆), and (−)- or (+)-NDE (◇). Experiments were carried out in duplicate. Data are presented from a single experiment. Concentrations of (−) + (+)-ODE were the sum of the individual values for the two enantiomers.

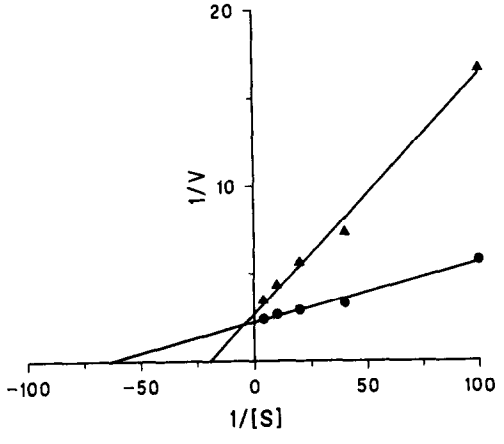

Fig. 4. Lineweaver–Burk plot for the formation of ODE by rat liver microsomes. Key: (−)-ODE (●), and (+)-ODE (▲). Experiments were carried out in duplicate. Data are presented from a single experiment.

V_{max} for the formation of (−)-ODE (0.46 nmol/mg protein/min) was similar to that for the (+)-enantiomer (0.37 nmol/mg protein/min).

NDE was formed in small amounts at low substrate concentrations but became a much more abundant metabolite at higher concentrations (Fig. 3). Chiral analysis of the purified NDE indicated that two enantiomers were present in equal amounts at all substrate concentrations (Fig. 3). Thus, there was no enantioselectivity in the formation of NDE.

In summary, these data suggest that the enantioselective metabolism of encainide is a consequence of the lower K_m of (−)-encainide, compared with (+)-encainide for the cytochrome P450 enzyme responsible for O-demethylation.

Alternatively, it is known that there are at least two cytochrome P450 enzymes responsible for the conversion of encainide to ODE in human liver microsomes [12]. Should similar enzymes be present in the rat, it is possible that one of them may have been selective for the metabolism of (−)-encainide. Studies are now in progress to distinguish these possibilities.

Acknowledgement—This work was supported by NIH Grants GM 15431 and ES 00267 and by Bristol-Myers.

Departments of Pharmacology and Chemistry
Vanderbilt University
Nashville, TN 37232; and
** Pharmaceutical Research and Development Division*
Bristol-Myers Co.
Wallingford, CT 06492
U.S.A.

HEMANT K. JAJOO
CHANDRA PRAKASH
ROBERT F. MAYOL*
IAN A. BLAIR†

REFERENCES

1. Brogden RN and Todd PA, Encainide: A review of the pharmacological properties and therapeutic efficacy. *Drugs* **34**: 519–538, 1987.
2. Wang T, Roden DM, Wolfenden HT, Woosley RL, Wood AJJ and Wilkinson GR, Influence of genetic polymorphism on the metabolism and disposition of encainide in man. *J Pharmacol Exp Ther* **228**: 605–611, 1984.
3. Jajoo HK, Mayol RF, LaBudde JA and Blair IA, Structural characterization of urinary metabolites of the antiarrhythmic drug encainide in human subjects. *Drug Metab Dispos* **18**: 28–37, 1990.
4. Barbey JT, Thompson KA, Echt DS, Woosley RL and Roden DM, Antiarrhythmic activity, electrocardiographic effects and pharmacokinetics of the encainide metabolites *O*-desmethyl encainide and 3-methoxy-*O*-desmethyl encainide in man. *Circulation* **77**: 380–98, 1988.
5. Winkle RA, Peters F, Kates RE and Harrison DC, Possible contribution of encainide metabolites to the long term antiarrhythmic efficacy of encainide. *Am J Cardiol* **51**: 1182–1188, 1983.
6. Williams KW and Lee E, The importance of drug enantiomers in clinical pharmacology. *Drugs* **30**: 333–354, 1985.
7. Caldwell J, Winter SM and Hutt AJ, The pharmacological and toxicological significance of stereochemistry of drug disposition. *Xenobiotica* **18** (Suppl 1): 59–70, 1988.
8. Ariens EJ, Stereoselectivity of bioactive agents. In: *Stereochemistry and Biological Activity of Drugs* (Eds. Ariens EJ, Soudijn W and Timmermans PB), pp. 11–32. Blackwell, New York, 1983.
9. Low LK and Castagnoli N, Enantioselectivity in drug metabolism. *Annu Rep Med Chem* **13**: 304–315, 1978.
10. Prakash C, Jajoo HK, Mayol RF and Blair IA, Resolution of enantiomers of the antiarrhythmic drug encainide and its major metabolites by chiral derivatization and HPLC. *J Chromatogr* **493**: 325–335, 1989.
11. Remmer H, Griem H, Schenkman JB and Estabrook RW, Methods for the elevation of hepatic microsomal mixed function oxidase levels and cytochrome P-450. *Methods Enzymol* **10**: 703–708, 1967.
12. Guengerich FP, Distlerath LM, Reilly PEB, Wolff T, Shimada T, Umbenhauer DR and Martin MV, Human liver cytochrome P-450 involved in polymorphisms of drug oxidation. *Xenobiotica* **16**: 367–378, 1986.

† Correspondence: Ian A. Blair, Ph.D., Department of Pharmacology, Vanderbilt University, Nashville, TN 37232.

Biochemical Pharmacology, Vol. 40, No 4, pp. 895–898, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Binding of retelliptine, a new antitumoral agent, to serum proteins and erythrocytes

(*Received* 11 *December* 1989; *accepted* 21 *March* 1990)

Retelliptine hydrochloride (Fig. 1) is a new antitumoral derivative in the ellipticine series exhibiting high cytotoxicity against several experimental tumor models in mouse and rat without serious side effects [1]. Its antitumor efficiency may be due to intercalation into DNA which could alter secondly topoisomerase II activity and involve single and/or double strand breaks [2, 3].

At physiological serum pH, this drug is a lipophilic, weak basic molecule ($pK'_a = 7.84$) and it is predominantly in an unionized form.

No previous assays about serum protein binding of this intercalant class has been published. So, before beginning phase I trial and in order to better understand their binding and blood distribution, we have investigated the binding of retilliptine to blood components.

Materials and Methods

Chemicals. Radiolabelled retelliptine hydrochloride ([^{14}C]3′propyl; 11.34 mCi/mmol) was gently provided by SANOFI Recherche (Montpellier, France). Purity checked by mass spectrometry was above 98%. Stock solution was prepared in distilled water (0.5 mM). Stability in water has been established previously. At pH < 8, aqueous solubility was excellent up to 4 mM.

Blood fractions. All the proteins used were from human origin. They included human serum albumin (HSA, Sigma 1887), α_1-acid glycoprotein (AAG, Behring), polyvalent γ-globulins (Sigma, G 2388) and lipoproteins (HDL, LDL, VLDL: Sigma, L 2014, L 2139, L 2264). Erythrocytes were prepared from fresh human blood samples. Heparin was added to blood prior to centrifugation. Red cells were washed out twice by large volumes of isotonic saline solution to eliminate residual plasma.

Binding assay. Methods usually used for measuring protein binding as equilibrium dialysis or ultrafiltration were not appropriate for retilliptine because of very important non-specific adsorption on cellulose dialysis membranes. So, the binding of retilliptine to proteins and erythrocytes