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DETERMINATION OF (*R*)- AND (*S*)-DISOPYRAMIDE IN HUMAN PLASMA USING A CHIRAL α_1-ACID GLYCOPROTEIN COLUMN

JÖRGEN HERMANSSON* and MÄRIT ERIKSSON

Apoteksbolaget AB, Central Laboratory, Box 3045, S-171 03 Solna (Sweden)

and

OLOF NYQUIST

Department of Internal Medicine, Division of Cardiology, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge (Sweden)

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SUMMARY

The direct resolution and quantitation of (*R*)- and (*S*)-disopyramide, isolated from human plasma, was accomplished using a chiral α_1-acid glycoprotein column. A LiChrosorb RP-2 column (50 × 3.0 mm I.D.) was used as a precolumn. Phosphate buffer, pH 6.20, containing 2-propanol and N,N-dimethyloctylamine was used as mobile phase. The precision of the determination of (*R*)- and (*S*)-disopyramide in human plasma, expressed as the relative standard deviation, was 1.8% and 3.3% for (*R*)- and (*S*)-disopyramide, respectively, at a drug level of 0.5 μg/ml. In two subjects who received a single capsule of racemic disopyramide (150 mg), the plasma levels of the (*R*) isomer were about half those of the (*S*) isomer. The half-lives of (*R*)- and (*S*)-disopyramide were similar.

INTRODUCTION

Disopyramide is an antiarrhythmic agent and is marketed as a racemate, i.e. a mixture of two optical isomers, (*R*)- and (*S*)-disopyramide. It has been demonstrated recently that (*R*)- and (*S*)-disopyramide are equipotent with respect to the antiarrhythmic effect [1]. However, the use of disopyramide is limited because the drug also possesses strong anticholinergic properties, resulting, for example, in severe urine retention [2]. Giacomini et al. [3] reported that (*S*)-disopyramide is about three to four times more potent as an anticholinergic

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agent. These results were obtained using longitudinal muscle strips from guinea pig ileum as test model.

Differences in the disposition of the enantiomers of disopyramide have been observed in dogs [4]. The purpose of the present study was to characterize the in vivo disposition of (*R*)- and (*S*)-disopyramide in man, in an attempt to study the prerequisites for obtaining a reduction of the anticholinergic side-effects by the use of only (*R*)-disopyramide.

Separation and quantitation of the disopyramide enantiomers was accomplished in this study using a chiral α_1-AGP column coupled in series with an RP-2 precolumn. The α_1-AGP column was prepared by immobilization of the plasma protein α_1-acid glycoprotein (orosomucoid) on a solid phase. The preparation of an α_1-AGP column has been described previously [5, 6].

EXPERIMENTAL

Chemicals

Racemic disopyramide was obtained from Roussel Labs. (*R*)- and (*S*)-disopyramide, and (*R*)- and (*S*)-monodesisopropyldisopyramide oxalate were kindly supplied by Professor Wendel L. Nelson (School of Pharmacy, Department of Medicinal Chemistry, Seattle, WA, U.S.A.). N,N-Dimethyloctylamine (DMOA) was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and LiChrosorb RP-2 with a mean particle diameter of 5 µm from E. Merck (Darmstadt, F.R.G.). The other chemicals used were of analytical or equivalent grade.

Apparatus

The high-performance liquid chromatographic (HPLC) system consisted of a Waters pump Model M6000 A (Waters Assoc., Milford, MA, U.S.A.), a Waters U6K injector and a Shimadzu SPD-2A ultraviolet (UV) detector with variable wavelength, operating at 261 nm.

Column preparation

The columns were made of precision-bore 316 stainless steel and equipped with modified Swagelok connections and Altex 2-µm frits. The dimensions of the RP-2 column were 50 × 3.0 mm. LiChrosorb RP-2 (0.3 g) was suspended in 3.0 ml of dichloromethane and placed in an ultrasonic bath for 10 sec to be degassed, before being poured into a 10-ml packing column. The packing column was filled with dichloromethane and coupled to a Haskel pump, operating at 300 bars. Acetone (30 ml) was used as the driving liquid. After packing, the column was rinsed with 50 ml methanol and 30 ml of methanol—water (1:1).

The chiral α_1-AGP column (100 × 3.0 mm) was prepared by immobilization of the plasma protein α_1-acid glycoprotein (orosomucoid) on silica microparticles with a mean particle diameter of 13 µm. The preparation of the chiral phase as well as the packing procedure is described elsewhere [6].

Chromatographic technique

A mobile phase of phosphate buffer, pH 6.20, containing 4.3% (v/v) of

2-propanol and 1.95 m*M* DMOA was used. The solvent was degassed in an ultrasonic bath before use.

Extraction procedure

A volume of 100 μl of 2 *M* sodium hydroxide was added to each plasma sample (1.0 ml). Disopyramide was then extracted with 6.0 ml of water-saturated diethyl ether for 15 min. The tubes were centrifuged for 3 min and 5.0 ml of the ether phase were transferred to a conical tube and evaporated to dryness with a stream of dry nitrogen at 40°C. The residue was dissolved in 125 μl of the mobile phase and 50 μl were injected onto the column (the RP-2 column coupled in series with the α_1-AGP column).

Standard curves

Standard curves were prepared by adding 0, 0.3, 1.0, 2.0, 3.0 and 4.0 μg of racemic disopyramide base to 1.0 ml of drug-free human serum. The samples were then handled as described under *Extraction procedure*. Standard curves were constructed by plotting the peak height versus the concentration of (*R*)- and (*S*)-disopyramide.

Reproducibility

Reproducibility studies for the analysis of (*R*)- and (*S*)-disopyramide were performed at two different concentrations. Six 1.0-ml drug-free serum samples were spiked with racemic disopyramide (1.0 μg or 3.0 μg) and the samples were handled as described under *Extraction procedure*. The peak heights of the enantiomers were measured and the relative standard deviations were calculated.

Subjects

Two male patients, 64 and 79 years' old, were given a commercial Durbis® capsule containing 150 mg of racemic disopyramide. At timed intervals venous blood was drawn into heparinized Venoject tubes. The plasma was separated immediately after collection and frozen until analysed.

RESULTS AND DISCUSSION

The enantiomers of disopyramide are extracted into diethyl ether from the alkalinized plasma samples. The extraction time needed to reach equilibrium was studied using four different extraction times: 15, 30, 45 and 60 min. No significant difference in the peak area ratios (*R*/*S*) was obtained when using different extraction times. It was also demonstrated that the peak heights were independent of the extraction time in the above-stated interval and that 15 min is enough to reach equilibrium.

It is demonstrated in this paper and it has also been demonstrated in previous papers from our group [5—7], that (*S*)-disopyramide is bound with higher affinity than the (*R*)-enantiomer to orosomucoid. Therefore, it is of vital importance to determine that both enantiomers are extracted to the same extent into diethyl ether. This study was performed using racemic disopyramide concentrations between 0.30 and 2.5 μg/ml and by use of an extrac-

tion time of 15 min. The areas of the peaks and the R/S area ratios were calculated and it was found that the R/S area ratio was independent of the disopyramide concentration. The mean values of the areas of (*R*)- and (*S*)-disopyramide as a percentage of the total area (the sum of the areas of (*R*)- and (*S*)-disopyramide) are 48.9 and 51.1 with relative standard deviations of 1.83 and 1.95 ($n = 14$), respectively.

Chromatographic studies

It has been previously demonstrated that the disopyramide enantiomers can be separated using a chiral α_1-AGP silica column [5, 6]. However, the plasma

TABLE I

CHROMATOGRAPHIC DATA OBTAINED ON THE α_1-AGP COLUMN

Conditions: column dimensions, 100 × 3.0 mm I.D.; 183 mg α_1-AGP/g solid phase; mobile phase, phosphate buffer, pH 6.20 containing 1.95 m*M* DMOA and 4.3% 2-propanol.

R_s = resolution factor = $\frac{(t_{R(S)} - t_{R(R)}) \cdot 2}{W_{t(R)} + W_{t(S)}}$ where W_t is base width.

	$k'_{(R)}$	$k'_{(S)}$	α	R_s
Disopyramide	1.66	5.70	3.43	3.05
Monodesisopropyldisopyramide	0.72	1.74	2.41	2.11

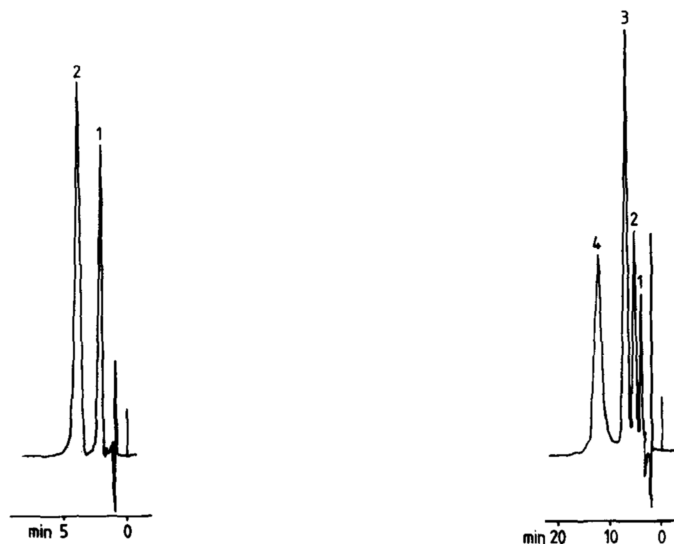

Fig. 1. Separation of disopyramide and monodesisopropyldisopyramide. Column: LiChrosorb RP-2 (50 × 3.0 mm I.D.). Mobile phase: Phosphate buffer pH 6.20 containing 4.3% (v/v) 2-propanol and 1.95 m*M* N,N-dimethyloctylamine. Flow-rate: 0.5 ml/min. UV detection: 261 nm. a.u.f.s. = 0.01. Samples: 1 = monodesisopropyldisopyramide, 2 = disopyramide.

Fig. 2. Resolution of the enantiomers of disopyramide and the enantiomers of monodesisopropyldisopyramide. Columns: LiChrosorb RP-2 (50 × 3.0 mm I.D.) coupled in series with an α_1-AGP column (100 × 3.0 mm I.D.). Other conditions as in Fig. 1. Peaks: 1 = (*R*)-monodesisopropyldisopyramide, 2 = (*S*)-monodesisopropyldisopyramide, 3 = (*R*)-disopyramide, 4 = (*S*)-disopyramide. a.u.f.s. = 0.005.

samples also contain the metabolite, monodesisopropyldisopyramide [8, 9] and it was found that (*S*)-monodesisopropyldisopyramide elutes with approximately the same capacity factor as (*R*)-disopyramide when using the α_1-AGP column alone, despite the fact that many different mobile phase compositions were tested. The chromatographic data using a mobile phase composition of 4.3% (v/v) 2-propanol in phosphate buffer, pH 6.2, and with addition of 1.95 m*M* N,N-dimethyloctylamine are summarized in Table I. It is interesting to note the difference in the separation factors obtained for the enantiomers of disopyramide and the enantiomers of its monodesisopropyl metabolite. Monodesisopropylation of disopyramide decreases the separation factor from 3.43 to 2.41 (cf. Table I). Disopyramide and the monodesisopropyl metabolite can be separated by chromatography on a 50-mm long LiChrosorb RP-2 column using the above-stated mobile phase, as demonstrated in Fig. 1. If the RP-2 column is coupled before the α_1-AGP column as a precolumn, a complete separation of (*R*)- and (*S*)-disopyramide and their metabolites can be obtained, as demonstrated in Fig. 2. This separation system was also used for the determination of (*R*)- and (*S*)-disopyramide in human plasma. The resolution between the disopyramide enantiomers decreases when the precolumn is used. This is probably caused by the fact that the sample zone, transferred to the α_1-AGP column, is broadened by passage through the precolumn.

Standard curve, reproducibility and recovery studies

The reproducibility of the method was determined as described under Experimental. The study was performed at two different concentrations (1.0 and 3.0 μg/ml) of racemic disopyramide and the relative standard deviations found are summarized in Table II.

TABLE II

PRECISION OF PLASMA DETERMINATIONS

Concentration of racemic disopyramide (μg/ml)	Relative S.D.* (%)	
	(*R*)	(*S*)
1.0	1.81	3.32
3.0	1.68	1.66

*Calculated for $n = 6$.

The degree of extraction of (*R*)- and (*S*)-disopyramide into diethyl ether is > 90% with a phase volume ratio, V_{org}/V_{aq}, of 5.45. Standard curves are constructed by plotting the peak height versus the concentration of the disopyramide enantiomers. Linear regression equations of the standard curves were calculated and the equations are as follows: $Y_R = 68.58X - 3.64$, and $Y_S = 32.92X - 1.12$. The standard deviations of the intercept and the slope for (*R*)-disopyramide are 1.52 and 1.24, respectively. The corresponding values for the (*S*) isomer are 0.63 and 0.51. Linear standard curves were obtained in the concentration range studied (0.15—2.0 μg/ml) and the correlation coefficients were in all cases better than 0.999.

Determination of (R)- and (S)-disopyramide in human plasma

The concentrations of (*R*)- and (*S*)-disopyramide in human plasma were determined as described under Experimental. Fig. 3A—C demonstrates chromatograms of blank plasma, of a plasma sample spiked with racemic disopyramide and of a plasma sample obtained from a patient after administration of racemic disopyramide, respectively. The blank plasma chromatogram demonstrates that no interfering peaks are present.

Fig. 4A and B demonstrates the total (free + protein-bound) plasma concentrations of (*R*)- and (*S*)-disopyramide in man in two subjects who received a single oral dose of a 150-mg Durbis® capsule. It can be seen from the plasma concentration—time curves that at all time points there is a higher plasma concentration of (*S*)-disopyramide, i.e. the enantiomer with the strongest anticholinergic potency. It can also be seen that the half-lives of the isomers are similar. The concentration ratio (*S*/*R*) for one subject is about 2 and for the other subject about 1.5.

Kook et al. [10] reported a more than two-fold higher C_{max} and AUC for (*R*)-disopyramide after oral administration of disopyramide to dogs. Obviously, there is a species difference with regard to the disposition of disopyramide between dog and man.

It has been reported that (*S*)- and (*R*)-disopyramide are equipotent with respect to the antiarrhythmic effect [1]. However, it has also been demonstrated that (*S*)-disopyramide is three to four times more potent as an anti-

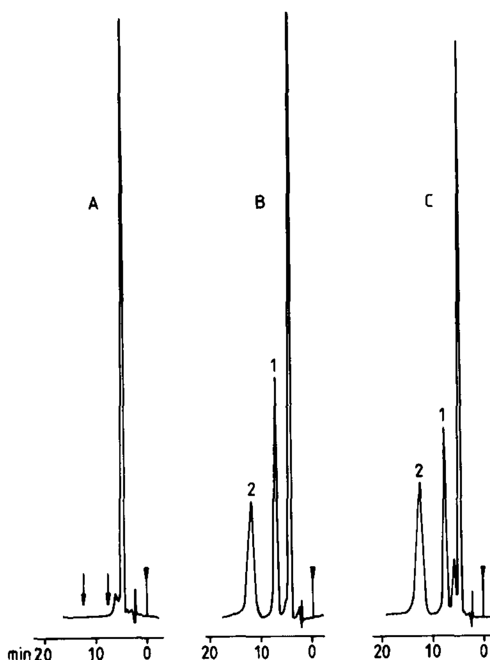

Fig. 3. Separation of (*R*)- and (*S*)-disopyramide isolated from human plasma. (A) Chromatogram of blank plasma. Arrows indicate the retention times of (*R*)- and (*S*)-disopyramide. (B) Blank plasma spiked with racemic disopyramide (1.5 μg/ml). Peaks: 1 = (*R*)-disopyramide, 2 = (*S*)-disopyramide. (C) Plasma sample from a patient obtained 1 h after administration of a 150-mg Durbis capsule. Conditions as in Fig. 2. a.u.f.s. = 0.04.

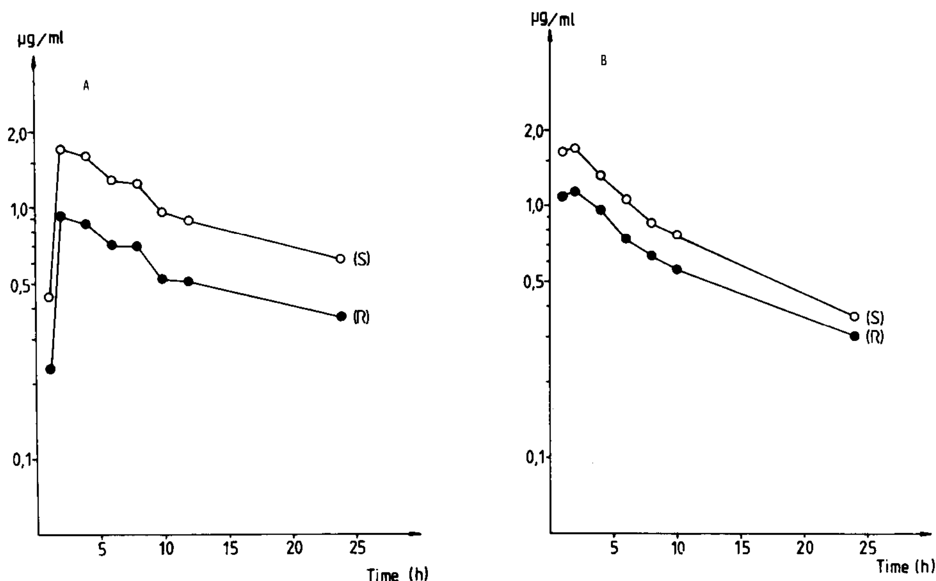

Fig. 4. Plasma concentrations of (*R*)- and (*S*)-disopyramide in two subjects (A and B) each receiving 150-mg commercial capsules containing racemic disopyramide.

cholinergic agent [3]. It was demonstrated in Fig. 4A and B that the concentration of (*S*)-disopyramide was about two times as high as that of the (*R*)-isomer. It is thus reasonable to assume that the anticholinergic side-effects associated with the use of racemic disopyramide can be reduced if only (*R*)-disopyramide is administered. To assess the possibility of a therapeutic improvement by using only the (*R*)-isomer, the free levels of (*R*)- and (*S*)-disopyramide in plasma must be determined. A prerequisite for obtaining this improvement is that the concentration of free (*S*)-disopyramide is high when compared with the free plasma concentration of (*R*)-disoyramide, i.e. a situation comparable with that obtained when using the total (free + protein-bound) plasma concentrations of the enantiomers (Fig. 4A and B).

(*S*)-disopyramide is bound to human α_1-acid glycoprotein (orosomucoid) with higher affinity than the (*R*) form [5—7]. Studies are now in progress to examine the influence of the stereoselective protein binding on the pharmacokinetics and the pharmacodynamics of (*R*)- and (*S*)-disopyramide and to study if any therapeutic benefits can be obtained by using only (*R*)-disopyramide.

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