ELSEVIER

Journal of Chromatography B, 682 (1996) 349–357

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Comparison of the Hummel–Dreyer method in high-performance liquid chromatography and capillary electrophoresis conditions for study of the interaction of (*RS*)-, (*R*)- and (*S*)-carvedilol with isolated plasma proteins

Jana Oravcova[a], Dagmar Sojkova[a], Wolfgang Lindner[b,*]

[a]*Institute of Preventive and Clinical Medicine, Limbova 14, 833 01 Bratislava, Slovak Republic*
[b]*Institute of Pharmaceutical Chemistry, Karl-Franzens-University of Graz, Schubertstrasse 1, A-8010 Graz, Austria*

Received 7 November 1995; revised 5 February 1996; accepted 19 February 1996

Abstract

The Hummel–Dreyer method in capillary zone electrophoresis was compared with the corresponding high-performance liquid chromatographic (HPLC) variant in order to study the interaction of racemic carvedilol and its individual enantiomers with isolated human plasma proteins [α_1-acid glycoprotein (AGP) and human serum albumin (HSA)]. The binding parameters characterizing the high-affinity binding site of AGP evaluated by using capillary electrophoresis [$K_{a(RS)}$= $(3.01\pm1.15)\cdot10^6$ l/mol; $K_{a(S)}=(2.13\pm0.53)\cdot10^6$ l/mol; $K_{a(R)}=(4.88\pm1.57)\cdot10^6$ l/mol] were in good accordance with those obtained by HPLC [$K_{a(RS)}=(3.88\pm1.74)\cdot10^6$ l/mol; $K_{a(S)}=(1.80\pm0.53)\times10^6$ l/mol; $K_{a(R)}=(5.43\pm2.53)\cdot10^6$ l/mol]. Relatively small quantitative differences have been observed considering the attachment of (*R*)-carvedilol to the secondary low-affinity binding sites on α_1-acid glycoprotein by comparing these two methods. In general, the Hummel–Dreyer method applied to capillary zone electrophoresis conditions was verified to be an efficient and fast technique for reliable description of quantitative binding parameters of hydrophobic drugs.

Keywords: Hummel–Dreyer method; Enantiomer separation

1. Introduction

As recently summarized [1], the introduction of new analytical methodologies has a remarkable impact on the knowledge and understanding of the complex mechanisms involved in the process of ligand attachment to different protein binding sites. It is quite clear that only by adoption of sensitive and specific experimental methods is it possible to follow and describe the sometimes very small differences in binding to relevant protein binding sites (e.g. if interactions of enantiomers of highly hydrophobic drugs are considered). Capillary electrophoresis (CE) offers in this respect an attractive methodological tool, since besides its speed and flexibility: (a) all interacting components can be studied in free buffer solution at so-called physiological conditions, (b) simultaneous measurement of binding constants of multiple binding interactions with protein (and complex protein mixtures) is possible and (c) in principle, only nanogram quantities of protein and ligand are required. Furthermore, just like in chromatography,

*Corresponding author.

0378-4347/96/$15.00
PII S0378-4347(96)00092-8

quantitative (frontal analysis, vacancy peak method, Hummel–Dreyer method, etc. [2–4]) as well as qualitative (affinity CE [5–7]) approaches could be adopted. The latter approach is attractive particularly for mimicking of (stereoselective) displacing phenomena at relevant protein binding sites. Most recently, Nakagawa and co-workers have introduced a high-performance frontal analysis method in CE conditions (HPFA-CE) for estimation of binding parameters [3] and have expanded further its use for the direct determination of enantioselective protein binding of verapamil (with *S/R* ratio ~1.7) after injection of a (*RS*)-verapamil (200 or 300 μmol/l)–HSA (550 μmol/l) mixture [4].

Carvedilol, (*RS*)-(±)-1-(carbazolyl-4-oxy)-3-[(2-(*o*-methoxyphenyloxy)ethyl)amino]-2-propanol (Fig. 1), is a newer chiral β-blocking agent with vasodilating properties (related mainly to its α_1-blocking activity) demonstrating far more antioxidant activity than other commonly used β-blockers [8–11]. Since the pharmacodynamic profile of the two enantiomers of carvedilol is markedly different [(*S*)-enantiomer is approx. 160 times more active than (*R*)-isomer in the β-adrenergic receptor blocking activity], it is important to consider also various enantiospecific pharmacokinetic aspects [12] including protein binding characteristics. As it has been shown previously in rat [13,14], (*R*)-carvedilol was preferentially bound to plasma [with an unbound fraction (*S/R*)-enantioselectivity ratio, of $f_{u(S/R)}=1.53$]. Hereby, rat albumin was regarded to be the main transport protein exhibiting inverse enantioselectivity as compared to rat plasma ($f_{u(S/R)}=0.79$; recalculated from [14]).

The main purpose of the present study was to investigate and to describe in detail the CE method as a potentially useful approach for monitoring quantitative drug–protein binding aspects and to compare it to the established high-performance liquid chromatography (HPLC) approach of the Hummel–Dreyer method [15,16]. Inter alia it has already been applied to study protein interactions of other lipophilic drugs, such as (*R*)- and (*S*)-propafenone [17] or (*R*)- and (*S*)-isradipine [18]. In the present contribution the Hummel–Dreyer method adopted for HPLC as well as for CE conditions was applied to examine the binding characteristics of (*RS*)-, (*R*)- and (*S*)-carvedilol interaction with isolated human plasma proteins (α_1-acid glycoprotein, AGP and human serum albumin, HSA).

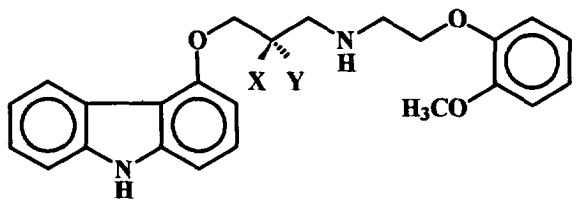

Fig. 1. Structural formula of carvedilol. (+)-(*R*)-Carvedilol: X = OH, Y = H; (−)-(*S*)-carvedilol: X = H, Y = OH.

2. Experimental

2.1. Drugs and chemicals

(*RS*)-(±)-Carvedilol (Batch 441822-00), (*R*)-(+)-carvedilol (Batch 90014-94), and (*S*)-(−)-carvedilol (Batch 90024-94) were kindly provided by Boehringer Mannheim (Mannheim, Germany); the optical purity of (*R*)- and (*S*)-carvedilol was checked by HPLC and was in both cases >99%. In order to improve the solubility in aqueous media, hydrochlorides of (*RS*)-, (*R*)- and (*S*)-carvedilol were prepared and used in all experiments. HSA free of fatty acids (A-1887, Lot 118F9311) and human AGP (G-9885, Lot 13H9336) were obtained from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade. Buffer solutions used particularly in the CE study were prepared daily using water from a Milli-Q water purification system (Millipore, Milford, MA, USA) and filtered through 0.2-μm Nalgene nylon filter membranes (Nalge, Rochester, NY, USA).

2.2. High-performance liquid chromatography

The liquid chromatographic system consisted of a pump module (Waters 510, Waters, Milford, MA, USA), a sample injector (Type 7125, Rheodyne, Cotati, CA, USA) equipped with a 50-μl sample loop and a variable-wavelength UV–Vis detector (Spectro-Monitor 3200, LDC Analytical, Riviera Beach, FL, USA) operating at 245 (for drug concentration in the mobile phase of 1–20 μmol/l) and 210 nm (≤1 μmol/l). The measurements were performed on 15 cm×3.3 mm I.D. and 3 cm×3.3

mm I.D. columns packed with 5 μm LiChrosorb Diol (Cat. No. 15660 and 15651, respectively; Merck, Darmstadt, Germany) and quantitated by using integration software package APEX (ECOM, Prague, Czech Republic). A flow-rate of 1.2 ml/min was used throughout the binding experiments which were carried out at ambient temperature.

2.3. Capillary electrophoresis

CE measurements were made with a Model 270 A capillary electrophoresis system (Applied Biosystems, a division of Perkin-Elmer, Foster City, CA, USA) equipped with Version 3.50 of Turbochrom software. CE conditions: untreated fused-silica 75 μm I.D. capillary or 50 μm I.D. capillary with a high sensitivity optical cell (LC Packings, Amsterdam, Netherlands; [18]). Effective and total length of capillaries used were approx. 50 and 65 cm, respectively. Polyacrylamide-coated capillary (75 μm I.D.) was prepared with slight modifications according to Hjertèn [20] and PVA-coated capillary (75 μm I.D.) was obtained from Hewlett-Packard (Vienna, Austria). Further experimental conditions: hydrodynamic injection (15 or 20 s, 20 mBar), applied voltage +15–20 kV (running current values were in the range 55–65 μA), ambient temperature, UV detection at 245 nm (at drug concentration in the running buffer >5 μmol/l) or 210 nm (at drug concentration of ≤5 μmol/l).

2.4. Determination of the protein binding

The individual experimental steps of the Hummel–Dreyer method (I–IV) and of the experimental design applied are summarized in Fig. 2. The mechanisms taking place in step II at HPLC and CE conditions are further highlighted in Fig. 3a and b, respectively.

Step I. AGP and HSA were diluted in 0.067 mol/l phosphate buffer (pH 7.40) and mixed with drugs at various drug/protein molar ratios achieving the final concentration of 10 μmol/l and 30 μmol/l, respectively.

Step II. Phosphate buffer solutions of (*RS*)-, (*R*)- and (*S*)-carvedilol in the range 0.125–20 μmol/l (i.e. at expected therapeutic concentration range) were used as mobile phase/running buffer additives in order to preequilibrate the column/capillary (Fig. 3, step 1). After injection of the protein–drug mixture, the second equilibrium was rapidly reestablished in accordance with the free drug concentration in the mobile phase (HPLC) or running buffer (CE) (Fig. 3a and b, step 2). In principle, by injecting a sample in which the concentration of drug is identical with the concentration of drug equilibrating the column or capillary, a negative peak could be observed, corresponding to the amount of drug bound to the protein (i.e. equivalent to "bound" drug concentration) (step 3.1.). By increasing the concentration of drug in the sample mixture (the concentration of protein remain thereby constant), it is possible to overwhelm this binding-induced concentration deficiency and "saturate" the system. According to this, the peaks became gradually less negative or even positive (step 3.2.). Generally, the separation by using HPLC operates by employing a size-exclusion principle as well as drug partitioning to the stationary phase. In CE, the separation is based on the measurement of differences in electrophoretic mobilities of interacting species. Therefore, in CE it is possible to evaluate also interaction between molecules of similar size (but with different electrophoretic mobility). Representative electropherograms corresponding to the situation presented schematically in Fig. 3 (3.1.) and (3.2.) are shown in Fig. 4a (negative peak) and Fig. 4b (positive peak), respectively.

Step III. For the exact quantification of drug bound at given mobile phase/running buffer drug concentration, the following calibration procedure was adopted: a set of 5–7 samples with different drug concentration and constant protein concentration was evaluated by interpolating (or extrapolating, if only negative peaks have been detected) the respective calibration curve (peak height versus drug concentration in the sample). The correlation coefficients were usually in the range 0.999–0.975.

Step IV. Binding parameters (n=number of binding sites, K_a=association constant) of the reversible drug(D)–protein(P) interaction ($[D]+[P] \rightleftarrows [DP]$) were determined by employing the scientific graph system SigmaPlot (Version 1.02 for Windows; Jandel Scientific, Erkrath, Germany). In the case of AGP, the best fit of (*RS*)-carvedilol as well as of its

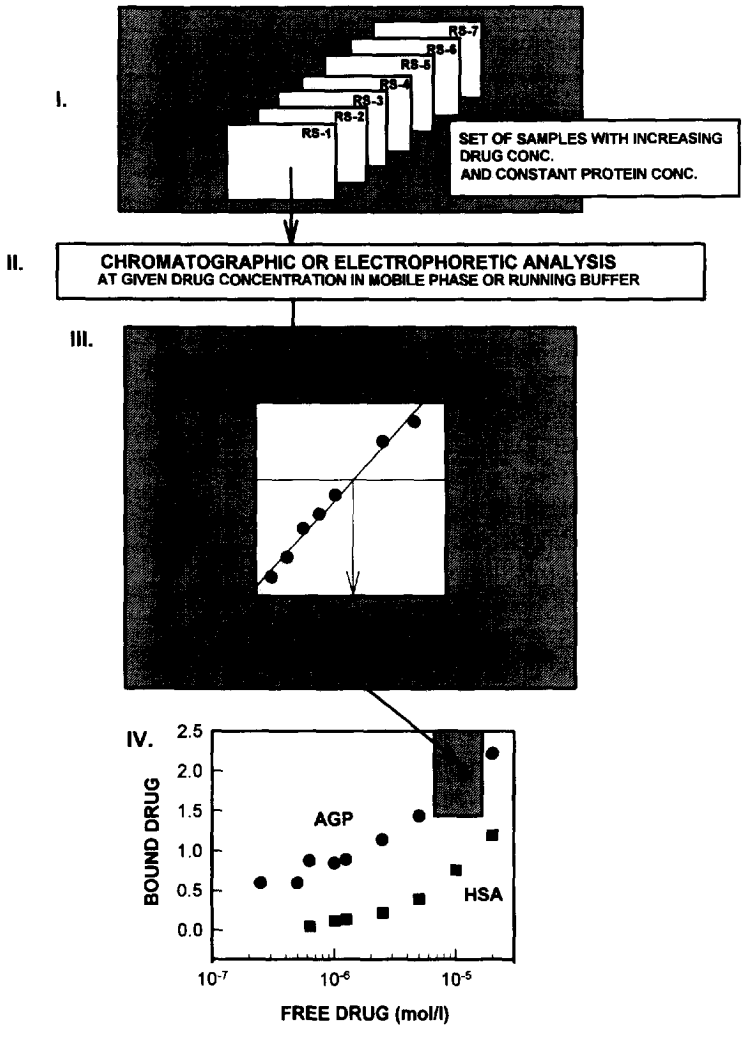

Fig. 2. Individual steps of the experimental design applied. I: set of samples with varying drug/protein molar ratio (at constant protein concentration); II: HPLC or CE analysis of sample set at given drug concentration (drug is used as mobile phase or running buffer additive); III: calculation of bound drug concentration by internal calibration procedure at given drug concentration; IV: resulting data point in binding isotherm ("bound drug" is the molar concentration of bound drug divided by molar concentration of the protein, i.e. AGP or HSA).

individual enantiomers was obtained by non-linear least-squares curve fitting to Eq. (1):

$$B = \sum_{i=1}^{z} \frac{n_i K_{ai} F}{1 + K_{ai} F} + n_i' K_{ai}' F \qquad (1)$$

where B is the concentration of drug bound per mole of the protein, F is the free drug concentration, z is the number of classes of specific binding sites and $n_i' K_{ai}' F$ is the nonspecific binding component (describing the binding to secondary low-affinity binding sites). The interaction of (*RS*)-, (*R*)- and (*S*)-carvedilol with HSA was characterized by significantly lower affinity as opposed to AGP and it was described by using nonspecific binding component ($B = n_i K_{ai} F$).

3. Results and discussion

The binding of (*RS*)-carvedilol to human AGP, as studied by the HPLC approach could be character-

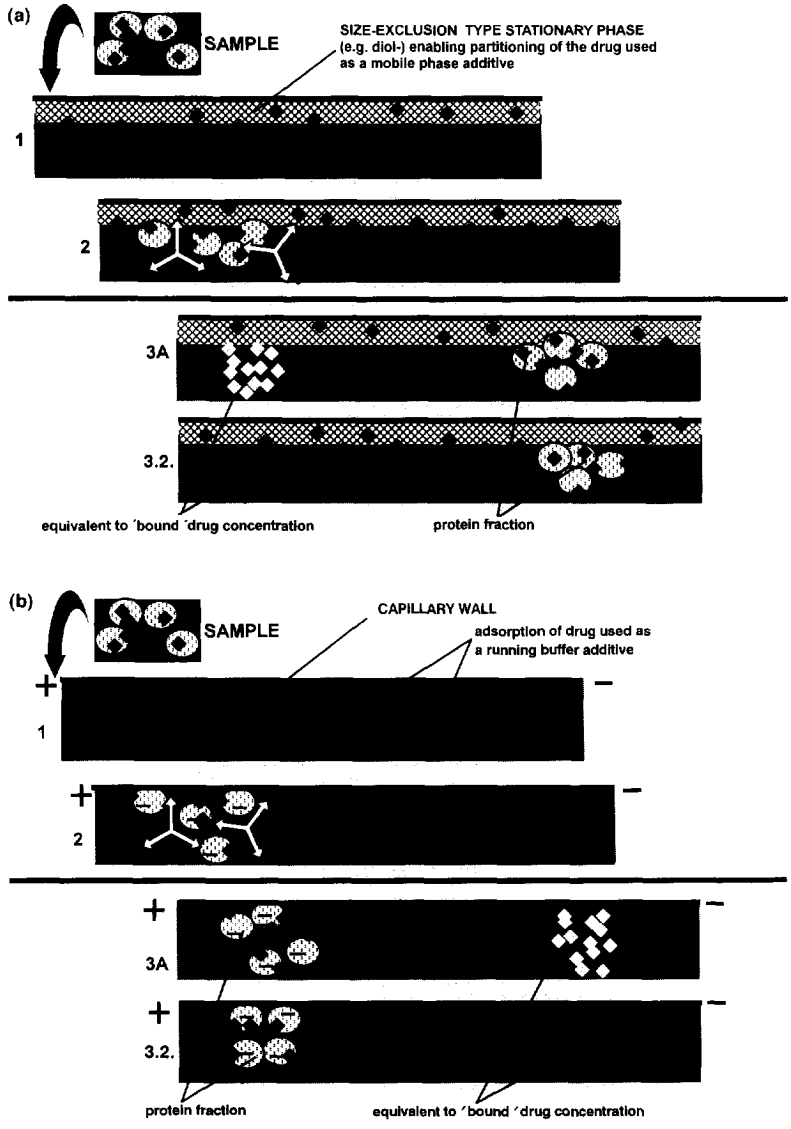

Fig. 3. Schematic diagram of Hummel–Dreyer method in (a) HLPC and (b) CE conditions. The signs + and − in Fig. 3b denote the anodic and cathodic end of the capillary, respectively. (1) Sample injection after achieving the "first" equilibrium of the separation system (using free drug in mobile phase (Fig. 3a) or running buffer (Fig. 3b); (2) reestablishing of "second" equilibrium in the interstices of the stationary phase/mobile phase in HPLC or inside the CE capillary according to the concentration of drug in sample vs. mobile phase/running buffer concentration; Steps 3.1. and 3.2. illustrate two of the possibilities achievable after second equilibrium: (3.1.) separation of protein fraction (complexed, resp. uncomplexed protein molecules) and "bound" drug by injecting the same concentration as was used for column/capillary equilibration (step 1). The empty symbols illustrate the concentration deficiency caused by protein binding, i.e. negative peak on chromatogram or electropherogram, which could be detected this way. (3.2.) Separation of protein fraction (complexed, resp. uncomplexed protein molecules) and "bound" drug by saturating the system, i.e. by injecting an excess of drug in the sample as compared to the concentration of drug in mobile phase or running buffer.

ized by the binding model [Eq. (1)] with a high-affinity binding component [$K_a = (3.88 \pm 1.74) \cdot 10^6$ 1/mol] as well as nonspecific binding [$n_1 K_{a1} = (6.88 \pm 1.64) \cdot 10^4$ 1/mol] (Table 1). As opposed to this model, the attachment of (*RS*)-carvedilol to HSA was nonspecific and of low affinity [$n_2 K_{a2} =$

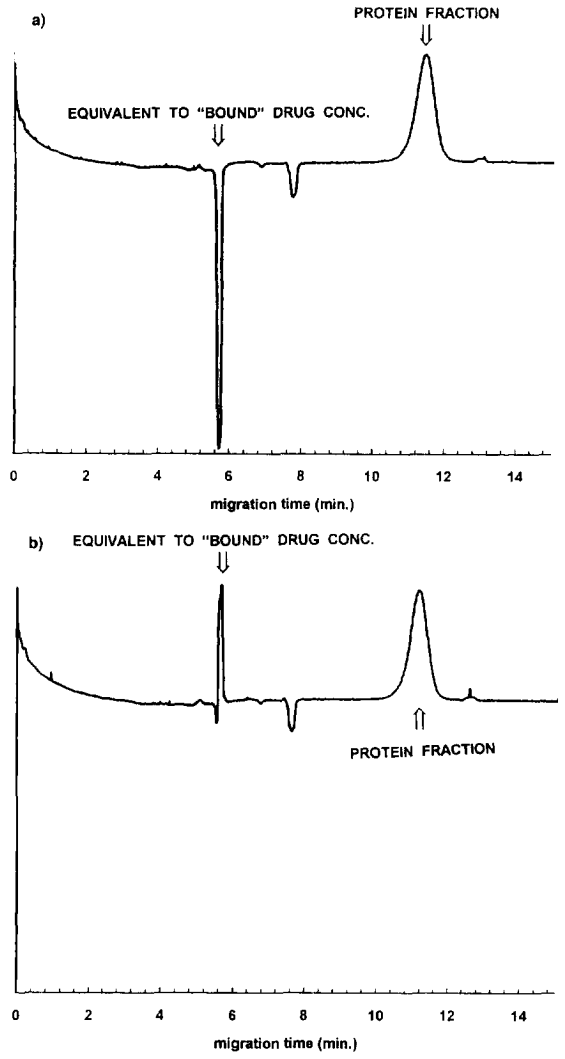

Fig. 4. Representative electropherogram of (*RS*)-carvedilol interaction with human AGP (10 μmol/l). CE conditions: concentration of (*RS*)-carvedilol·HCl in running buffer: 20 μmol/l; 75 μm untreated fused-silica capillary (effective length approx. 50 cm); hydrodynamic injection (20 s, 20 mbar), applied voltage: 20 kV; 25°C, detection at 210 nm. Injected sample: mixed solution of AGP and (*RS*)-carvedilol with drug/protein molar ratio, [D/P] = 1 (a) and [D/P] = 2.5 (b).

$(6.42\pm0.33)\cdot10^4$ l/mol] (Table 1). The interaction of carvedilol enantiomers with AGP was slighly stereoselective with preferential binding of (*R*)-enantiomer [$K_{a(R)} = (5.43\pm2.53)\cdot10^6$ l/mol] as compared to its antipode [$K_{a(S)} = (1.80\pm0.53)\cdot10^6$ l/mol] (Table 1). On the other hand, there were no discriminative binding properties of HSA for individual carvedilol enantiomers. The CE binding study with (*RS*)-carvedilol and its individual enantiomers (Fig. 5) confirmed the quantitative results obtained by HPLC approach: the resulting binding parameters evaluated by CE measurements (Table 2) were in good accordance with binding parameters obtained by HPLC (Table 1). The quantitative differences observed for nonspecific binding in case of (*R*)-carvedilol-AGP interaction by adopting CE vs. HPLC (i.e. n_1K_{a1} in Table 1 and $n_1'K_{a1}'$ in Table 2) could be related, at least in part, to the various conformational adaptations of the protein molecule. On the one hand, the changes could be evoked by "detergent effects" of high concentration of carvedilol on the protein molecule [21]. On the other hand, the interactions of protein with HPLC stationary phase used may exhibit some differences as opposed to the capillary wall (either untreated or coated). It has been suggested recently that in affinity CE irreversible adsorption of HSA, used as a running buffer additive, to the surface of capillary may also occur, corresponding to a coverage of about 0.72 monolayer and an apparent concentration of adsorbed HSA of approximately 1–2.7 μmol/l [5,22]. As commonly known, AGP is an acidic protein (isoelectric point 2.7) consisting of a single peptide chain with five carbohydrate units which are linked to the peptide chain via the asparagine residues. It contains negatively charged aspartic acid residues and terminal serine group as well as positively charged groups stemming from the arginine, lysine and histidine residues. Consequently, the protein is negatively charged (similarly as the capillary wall at physiological pH). The presented CE measurements exhibited good reproducibility and no significant changes in electrophoretic mobility of the components studied could be detected. This speaks in favour of the fact that during this examination the protein–wall adsorption phenomena were not significant. Nevertheless, we have investigated also the alternative use of polyacrylamide [20] and PVA-coated capillaries. Unfortunately, both types of coatings were seen to be unsuitable – mainly due to strong adsorption of the drugs – to be used as a running buffer additive thus giving rise to an additional, but unspecific secondary equilibrium via the "dynamic drug-coating procedure". Although the

Table 1
Binding parameters of (*RS*)-, (*R*)- and (*S*)-carvedilol interaction with human AGP (10 μmol/l) and HSA (30 μmol/l) as determined by Hummel–Dreyer method for HPLC conditions

Ligand	AGP			HSA
	n	K_a (l/mol)	$n_1K_{a_1}$ (l/mol)	$n_2K_{a_2}$ (l/mol)
(*RS*)-Carvedilol	1.02±0.14	(3.88±1.74)×10^6	(6.88±1.64)×10^4	(6.42±0.33)×10^4
(*S*)-Carvedilol	1.11±0.16	(1.80±0.53)×10^6	(3.15±1.43)×10^4	(6.31±0.42)×10^4
(*R*)-Carvedilol	0.69±0.12	(5.43±2.53)×10^5	(1.27±0.23)×10^5	(5.95±0.35)×10^4

n=number of binding sites per molecule of protein; K_a=association constant; $n_1K_{a_1}$, $n_2K_{a_2}$=nonspecific binding components. The parameters were calculated as given in Section 2.4 by considering the presence of one class of high-affinity binding sites and nonspecific binding in case of AGP [Eq. (1)], resp. nonspecific binding component which became evident for the interaction with HSA.

HPLC as well as CE part of our comparative study was performed at room temperature (i.e. at approximately 25°C), attention should be paid also to the heating effects generated inside the capillary at given experimental conditions (the temperature of buffer inside the capillary could be 10°C above the oven temperature [5]). Briefly summarizing, the CE approach used was judged as a highly efficient separation technique (total analysis time per run and datapoint, respectively by employing CE was 12 min vs. ≥40 min using HPLC) for studying the binding interactions of hydrophobic, highly protein-bound drugs (also in form of their individual optical isomers).

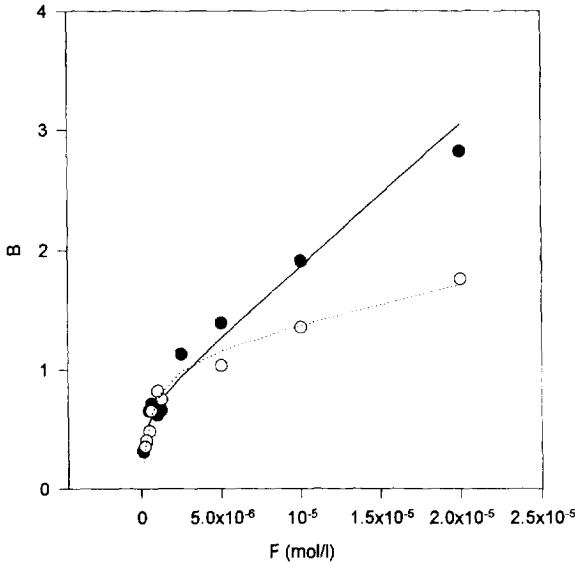

Fig. 5. Experimental data describing the interaction of (*R*)-carvedilol (●) and (*S*)-carvedilol (○) with human AGP (10 μmol/l) as studied by Hummel–Dreyer method adopted for CE (conditions are given in Section 2.3). Each point was determined using multilevel calibration procedure as it is schematically presented in Fig. 2. The lines denote the non-linear curve fitting of the data to Eq. (1).

Our results on the absence of chiral discriminative properties of HSA for carvedilol enantiomers are contradictory to the results reported previously with rat albumin in ex vivo and in vitro experiments using equilibrium dialysis [14]. Stereoselective binding differences between albumins of different species could present one possible explanation of these discrepancies as was demonstrated e.g. in vitro for (*RS*)-ofloxacine [7] and is consistent with its stereoselective species-dependent protein binding in vivo [23]. Nevertheless, it is necessary to point out that the methodological approach applied may also significantly influence the generation and interpretation of binding data. The potential shortcomings of the use of equilibrium dialysis for evaluation of protein binding profiles of highly hydrophobic drugs have been recently discussed in detail elsewhere [1].

Kraak et al. [2] first used the Hummel–Dreyer method, frontal analysis method and vacancy peak method for capillary zone electrophoresis conditions for the determination of (*RS*)-warfarin (3–900 μmol/l) binding to HSA. For practical purposes, the simplified experimental approach of Hummel–Dreyer method described by Pinkerton and Koeplinger [16] was adopted. However, as already presented previously [24] and further confirmed also in our experiments, the binding data obtained at low drug concentration were somewhat scattered, thus disabling the generation of "smooth" and reliable binding isotherms. In order to minimise the experimental error (reported to be >10% of duplicate

Table 2
Binding parameters of (*RS*)-, (*R*)- and (*S*)-carvedilol interaction with human AGP (10 μmol/l) as determined by Hummel–Dreyer method in capillary zone electrophoresis conditions

Ligand	AGP		
	n	K_a (l/mol)	$n_1'K_{a1}'$ (l/mol)
(*RS*)-Carvedilol	1.05±0.05	(3.01±1.15)×10^6	(9.48±1.64)×10^4
(*S*)-Carvedilol	1.06±0.15	(2.13±0.53)×10^6	(8.94±1.68)×10^4
(*R*)-Carvedilol	0.70±0.12	(4.88±1.57)×10^6	(1.01±0.17)×10^5

n = number of binding sites per molecule of protein; K_a = association constant; $n_1'K_{a1}'$ = nonspecific binding component. The parameters were calculated as given in Section 2.4 by considering the presence of one class of high-affinity binding sites and nonspecific binding in case of AGP [Eq. (1)].

measurements [2]), we have decided to use the "conventional" experimental procedure consisting of multilevel calibration at each drug concentration studied [25]. The second major methodological problem in the conventional CE experiments was related to insufficient UV-detection sensitivity at lower carvedilol concentrations (≤2.5 μmol/l). This limitation could be overcome to a certain extent by the use of a commercially available high sensitivity optical cell with Z-shaped capillary [19], improving the detection limit by factor of approximately 12. This enabled us to determine the binding isotherm of carvedilol and its enantiomers at therapeutically relevant concentrations.

At present, there is a growing number of papers describing various forms and modifications of CE for the characterization of many important aspects of noncovalent binding interactions (e.g. Refs. [2–7,22,26–29]). Recently, Ohara et al. [4] developed the HPCE–FA method, which allowed direct enantioselective determination of the unbound concentration of a basic chiral drug [(*RS*)-verapamil] using a chiral selector (trimethyl-β-cyclodextrin) as a running buffer additive. Apart from many important advantages of this method (sample size of approx. 200 nl or possibility to determine the unbound concentration of individual enantiomer by injecting "racemic" sample), its limitation is given at present by poor detectability, i.e. resulting in the necessity to use supratherapeutical drug concentrations (200–300 μmol/l).

In general, Heegard and Robey [29] have already summarized the usefulness of CE operating at fully physiological, nondenaturing conditions and offering fast, reproducible and easily automated quantitative analysis of reversible binding interactions unchallenged by any other present method. In specific cases, however, the applicability of CE could be complicated by the solubility, detectability and recovery of analytes studied. The Hummel–Dreyer method in CE could be in this respect a method of choice if description of binding constants of highly protein-bound drugs is needed and if only trace amounts of drugs (particularly in their enantiomer form) and/or proteins are available. As recently stressed by Hage et al. [30], the consideration of individual enantiomer binding characteristics is important in developing an accurate picture of drug–protein binding, especially if more complex interactions or displacing phenomena at target binding sites are taken into account.

Acknowledgments

Perkin-Elmer (Perkin-Elmer, Vienna, Austria) supported this work by providing us access to a 270 A CE system with HS optical cell. The stay of Jana Oravcova at University of Graz was financially supported by Fonds zur Förderung der wissenschaftlichen Forschung (FWF, Vienna, Austria; Project No. M00147-CHE).

References

[1] J. Oravcová, B. Böhs and W. Lindner, J. Chromatogr. B, 677 (1996) 1.

[2] J.C. Kraak, S. Busch and H. Poppe, J. Chromatogr., 608 (1992) 257.
[3] A. Shibukawa, Y. Yoshimoto, T. Ohara and T. Nakagawa, J. Pharm. Sci., 83 (1994) 616.
[4] T. Ohara, A. Shibukawa and T. Nakagawa, Anal. Chem., 67 (1995) 3520.
[5] D.K. Lloyd, S. Li and P. Ryan, Chirality, 6 (1994) 230.
[6] T. Arai, M. Ichinose, H. Kuroda, N. Nimura and T. Kinoshita, Anal. Biochem., 217 (1994) 7.
[7] T. Arai, N. Nimura and T. Kinoshita, Biomed. Chromatogr., 9 (1995) 68.
[8] A.J. Nichols, A.C. Sulpizio, D.J. Ashton, J.P. Hieble and R.R. Ruffolo, Chirality, 1 (1989) 265.
[9] T. Morgan, Clin. Pharmacokin., 26 (1994) 335.
[10] T.-L. Yue, H.-Y. Cheng, P.G. Lysko, P.J. McKenna, R. Feuerstein, J.-L. Gu, K.A. Lysko, L.L. Davis and G. Feuerstein, J. Pharm. Exp. Ther., 263 (1992) 92.
[11] T.-L. Yue, P.J. McKenna, J.-L. Gu, H.-Y. Cheng, R.E. Ruffolo and G.Z. Feuerstein, Cardiovasc. Res., 28 (1994) 400.
[12] C. de Mey, K. Breithaupt, J. Schloos, G. Neugebauer, D. Palm and G.G. Belz, Clin. Pharmacol. Ther., 55 (1994) 329.
[13] M. Fujimaki, Chirality, 4 (1992) 148.
[14] E. Stahl, E. Mutschler, U. Baumgartner and H. Spahn-Langguth, Arch. Pharm. (Weinheim), 326 (1993) 529.
[15] J.P. Hummel and W.J. Dreyer, Biochim. Biophys. Acta, 63 (1962) 530.
[16] T.C. Pinkerton and K.A. Koeplinger, Anal. Chem., 62 (1990) 2114.
[17] J. Oravcová, W. Lindner, P. Szalay, L. Bohácik and T. Trnovec, Chirality, 3 (1991) 30.
[18] J. Oravcová, D. Sojková, E. Bencsíková, P. Bohov and T. Trnovec, Chirality, 7 (1995) 167–172.
[19] S.E. Moring, C. Pairaud, M. Albin, S. Locke, P. Thibault and G.W. Tindall, Amer. Lab., 7/1993.
[20] S. Hjertèn, J. Chromatogr., 347 (1985) 191.
[21] B. Honoré, Pharmacol. Toxicol., 66 (Suppl. II) (1990) 1.
[22] J. Yang and D.S. Hage, Anal. Chem., 66 (1994) 2719.
[23] O. Okazaki, T. Kurata, H. Hakusui and H. Tachizawa, Xenobiotica, 22 (1992) 439.
[24] M.G. Quaglia, E. Bossu and C. Dell'Aquila, Proceedings of the 19th International Symposium on Column Liquid Chromatography and Related Techniques (Abstract Book II), Innsbruck, 1995, p. 199.
[25] B. Sebille, N. Thuaud and J.P. Tillement, J. Chromatogr., 167 (1978) 159.
[26] Y.-H. Chu, L.Z. Avila, H.A. Biebuyck and G.M. Whitesides, J. Med. Chem., 35 (1992) 2915.
[27] Y.-H. Chu, J.K. Chen and G.M. Whitesides, Anal. Chem., 65 (1993) 1314.
[28] N.H.H. Heegard and F.A. Robey, J. Immunol. Methods, 166 (1993) 103.
[29] N.H.H. Heegard and F.A. Robey, Int. Chromatogr. Lab., 21 (1994) 2.
[30] D.S. Hage, T.A.G. Noctor and I.W. Wainer, J. Chromatogr. A, 693 (1995) 23.