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Journal of Chromatography B, 697 (1997) 67–75

JOURNAL OF CHROMATOGRAPHY B

Review

Capillary electrophoresis for pharmacokinetic studies

D. Levêque*, C. Gallion-Renault, H. Monteil, F. Jehl

Laboratory of Pharmacokinetics, Institute of Bacteriology, 3 Rue Koeberlé, 67000 Strasbourg, France

Abstract

Different analytical techniques involving capillary electrophoresis for the determination of drugs and metabolites in biological fluids are described. Pharmacokinetic studies carried out using capillary electrophoresis are presented, as well as the in vitro metabolism investigations. The advantages and the limitations of capillary electrophoresis for pharmacokinetic studies are discussed. © 1997 Elsevier Science B.V.

Keywords: Reviews; Capillary electrophoresis; Pharmacokinetics

Contents

1. Introduction

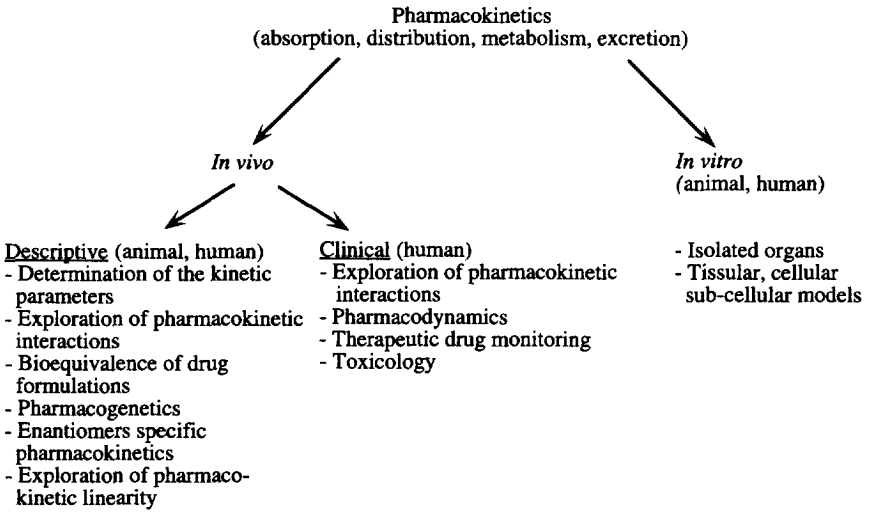
A prerequisite for the study of the pharmacokinetic profile of a drug is the development and the validation of an analytical method enabling the accurate determination of concentrations of the parent compound and possible metabolites in various biological fluids (serum, urine, bile, tissue extracts, cerebrospinal fluid, aqueous humor, prostatic fluid, etc). The quality of the pharmacokinetic analysis relies on the validation of the assay but also on the biological fluid sampling design, e.g., the number, the timing, the volume of the specimens. This point is a critical factor in descriptive pharmacokinetics. For instance, the number of experimental points must be sufficient for accurate determination of the pharmacokinetic parameters (bioavailability, absorption rate, elimination half-life, volume of distribution). Hence the number of sampling specimens is generally high (10–20 per day). However, in clinical pharmacokinetics, the number of specimens is reduced since often single concentrations (i.e., no pharmacokinetic parameters) are needed. The different aspects of pharmacokinetics are presented in Table 1.

Most kinetic investigations (experimental and clinical) are performed using high-performance liquid chromatography (HPLC) since this methodology

*Corresponding author.

0378-4347/97/$17.00
PII S0378-4347(97)00335-6

Table 1
The different aspects of pharmacokinetics



is selective, adaptable, automatable and the analysis times are short. Gas chromatography is seldom used. Radioactive assays (radiolabelled drugs, radioimmunoassays) are frequently performed during the preliminary steps of the development of a drug and particularly in metabolic studies. Immunoassays are used for drug monitoring and toxicology since they are simple and fast.

Capillary electrophoresis (CE) is a relatively new technique of separation and quantitation regrouping different modes of separation [1]. This methodology has raised interest during recent years and many applications in the separation of nucleic acids, amino acids, peptides, proteins, ions, carbohydrates have been described [2,3]. Furthermore, numerous techniques have described the determination of drugs in pharmaceutical forms [4,5]. Capillary electrophoresis provides complementary approaches for identification and analysis, and it has been used for chiral separation formulation analysis or purity confirmation, the latter being the primary application in pharmaceutical analysis [4,5]. On the other side, relatively few techniques have reported the determination of drugs in biological fluids and very few kinetic studies have been performed using this methodology. Theoretically, capillary electrophoresis presents numerous advantages for application in pharmacokinetics: low sample volume injected, high separation efficiency and low consumable expense.

Generally the position of capillary electrophoresis with regard to HPLC has been largely debated. Capillary electrophoresis is complementary to HPLC in qualitative or semi-quantitative terms. Numerous reviews have recently been published on the various applications of capillary electrophoresis [1–5]. This one briefly outlines the use of capillary electrophoresis for the determination of drugs in biological fluids and consequently the specific interesting aspects for pharmacokinetic studies.

2. Determination of drugs in biological fluids by capillary electrophoresis

The different analytical techniques involving CE for the determination of drugs in biological fluids are presented in Table 2 [6–79]. These methods have been classified on a qualitative/quantitative basis. A quantitative determination includes a validation procedure (accuracy, precision, linearity, robustness, limit of detection/quantitation). Qualitative determination is concerned with the separation of compounds in biological fluids. A semi-quantitative determination means that the validation study has

Table 2
Analytical techniques involving capillary electrophoresis for the determination of drugs in biological fluids

Pharmacological or chemical class	Drug	Mode of CE	Mode of detection	Biological fluid	Type of determination	Ref.
Barbiturates	thiopental	MECC	UV	plasma	semi-quantitative	[6]
	various	MECC	UV	serum	qualitative	[7]
	various	MECC	UV	serum	qualitative	[8]
	phenobarbital	MECC	UV	dialysates	semi-quantitative	[9]
	hexobarbital	MECC	UV	plasma	qualitative	[10]
	various	MECC	UV	various	quantitative	[11]
Benzodiazepines	various	MECC	UV	serum	qualitative	[8]
	nitrazepam and metabolites	MECC	UV	urine	quantitative	[12]
	various	MECC	UV	urine	qualitative	[13]
Methylxanthines	theophylline and metabolites	MECC	UV	plasma	quantitative	[14]
		MECC	UV	serum	qualitative	[8]
		MECC	UV	various	semi-quantitative	[15]
		MECC	UV	ultrafiltrate	semi-quantitative	[16]
		MECC	UV	urine, hepatic microsomes	quantitative	[17]
	caffeine	MECC	UV	various	qualitative	[15]
		MECC	UV	serum	qualitative	[8]
Drugs of abuse	various	MECC	UV	serum, urine	qualitative	[18]
Supplementary drugs	ferulic acid	CZE	UV	plasma	quantitative	[19]
Probe drugs used for metabolic studies	antipyrine	MECC	UV	serum	quantitative	[20]
		MECC	UV	plasma	quantitative	[21]
	dextromethorphan	CZE	fluorescence	urine	quantitative	[22]
	racemetorphan and metabolites	CZE	UV	urine	quantitative	[23]
	various	MECC	UV	urine	qualitative	[24]
	mephenytoin and metabolites	MECC	UV	urine	quantitative	[25]
Antihistamines	dimethindene and metabolite	MECC	UV	urine	quantitative	[26]
Histamine H2 receptor antagonists	cimetidine	MECC	UV	serum	quantitative	[27]
		CZE		urine	qualitative	[28]
	cimetidine and metabolites	CZE	UV	plasma	quantitative	[29]
Anesthetics	bupivacaine	MECC	UV	serum	quantitative	[30]
	enantiomers	CZE	UV	pleural drain fluid	quantitative	[21]
Psychotropic drugs	lithium	CZE	conductivity	serum, plasma	quantitative	[31]
	haloperidol and metabolites	CZE	UV	microsomal incubates	qualitative	[32]
		CZE	ESI-MS	microsomal incubates	qualitative	[33]
		CZE	MS	urine	qualitative	[34]
	zopiclone enantiomers and metabolites	CZE	LIF	urine	quantitative	[35]
Vitamins	vitamin B6 and metabolites	MECC	LIF	urine	quantitative	[36]
	vitamin C	CZE	UV	plasma	quantitative	[37]
	vitamin A	CZE	LIF	blood	quantitative	[38]

(*Contd.*)

Table 2. *Continued.*

Pharmacological or chemical class	Drug	Mode of CE	Mode of detection	Biological fluid	Type of determination	Ref.
Contrast medium	iohexol	CZE	UV	serum	quantitative	[39]
	iopamidol	CZE	UV	serum	quantitative	[40]
Anticonvulsants	various	MECC	UV	serum	quantitative	[41]
	various	MECC	UV	serum	qualitative	[8]
	various	MECC	UV	serum	quantitative	[42]
	various	MECC, CZE	UV	serum	qualitative	[43]
	felbamate	MECC	UV	serum	semi-quantitative	[44]
	gabapentin	CZE	UV	serum	quantitative	[45]
	lamotrigine	CZE	UV	serum	quantitative	[46]
Drugs used for the treatment of Parkinson's disease	L-DOPA	CZE	amperometric	extracellular fluid	quantitative	[47]
Diuretics	various	MECC	UV	serum	qualitative	[8]
Drugs affecting cardiovascular function	cicletanine enantiomers	MECC	UV	plasma	quantitative	[48]
	verapamil enantiomers	MECC	UV	plasma	quantitative	[49]
		CZE	UV	albumin solution	quantitative	[50]
	carvedilol enantiomers	MECC	UV	serum	quantitative	[51]
	diltiazem and metabolite	CZE	UV	plasma	quantitative	[52]
	amiodarone and metabolite	CZE	UV	serum	quantitative	[53]
Anticoagulants	warfarin enantiomers	MECC		plasma	quantitative	[54]
Analgesics and antiinflammatory drugs	paracetamol	MECC	UV	plasma	qualitative	[55]
		CZE, CITP	UV	serum	qualitative	[43]
	ibuprofen	CZE	UV	serum	quantitative	[56]
	naproxen	CZE	UV, LIF	serum	quantitative	[57]
		MECC	UV	serum	quantitative	[58]
Mucolytic agents	*S*-carboxy-methyl-L-cystein and metabolites	CZE, CITP	UV	urine	semi-quantitative	[59]
Drugs used for the treatment of cancer	methotrexate and metabolite	CZE	LIF	serum	quantitative	[60]
	cytosine arabinoside	CZE	UV	serum	quantitative	[61]
	doxorubicin, epirubicin, daunorubicin	CZE	LIF	plasma	quantitative	[62]
	suramin	CZE	UV	serum	quantitative	[63]
	coumarin and metabolite	CZE	UV	serum, urine	quantitative	[64]
	buthionine sulfoximine diastereosisomers	MECC	UV	plasma	quantitative	[65]
	prospidin	CZE	UV	tissue extract	quantitative	[66]
	paclitaxel	MECC	UV	plasma, urine	quantitative	[67]
Antimicrobial agents	cefpiramide	MECC	UV	plasma	quantitative	[68]
	aspoxicillin	MECC	UV	plasma	quantitative	[69]
	penicillin G	CZE	UV	gastric fluid	qualitative	[70]
	cefixime and metabolites	CZE	UV	urine	quantitative	[71]

Table 2. *Continued.*

Pharmacological or chemical class	Drug	Mode of CE	Mode of detection	Biological fluid	Type of determination	Ref.
	fosfomycin	CZE	indirect UV	serum	quantitative	[72]
		CZE	indirect UV	serum, aqueous humor, cerebrospinal fluid	quantitative	[73]
	amikacin	MECC	fluorescence	plasma	quantitative	[74]
	5-fluorocytosine	MECC	UV	serum	quantitative	[75]
	fluconazole	MECC	UV	plasma	quantitative	[76]
Drugs used in the treatment of asthma	bambuterol	CZE	UV	plasma	quantitative	[77]
	terbutaline enantiomers	CZE	MS, UV	urine	qualitative	[78]
		CZE	UV	plasma	qualitative	[79]

MECC, micellar electrokinetic chromatography; CZE, capillary zone electrophoresis; CITP, capillary isotachophoresis; LIF, laser-induced fluorescence; MS, mass spectrometry.

partially been carried out. Roughly, capillary electrophoresis is concerned with all pharmacological/chemical classes. If the number of validated techniques (e.g., applicable for kinetic investigations) tends to increase, it has to be stressed that they primarily concern 'old' drugs for which the kinetic properties are well characterised or for drugs seldom used in clinical practice. The most recent drugs concerned by capillary electrophoresis are the antimicrobial agents cefixime [71] and aspoxicillin [69] (in development), the antihypertensive agent cicletanine [48], the recent anticonvulsants felbamate [44], gabapentin [45], and lamotrigine [46], and the anticancer drugs suramin [63], SR 4233 [80] (both in investigation) and paclitaxel [67]. Among the different modes of CE, micellar electrokinetic chromatography (MECC) is the most commonly used, followed by capillary zone electrophoresis (CZE). Another mode of separation, capillary isotacophoresis (CITP), has been tested for the determination of paracetamol [43] and the mucolytic agent *S*-carboxymethyl-L-cysteine [59]. The potential applications of these methods evaluated by the authors are therapeutic drug monitoring, toxicology and forensic science, resolution of isomers (reviewed in Ref. [81]) and metabolites, and descriptive pharmacokinetics.

Besides the general advantages of CE (low volume sample required, low consumption, high separation efficiency) this methodology presents specific interesting features for the determination of drugs in biological fluids.

First, MECC has the capability of solubilizing disturbing proteins and other endogenous materials (use of sodium dodecylsulfate in the working buffer) allowing direct injection of the serum sample. This pretreatment phase often represents a time-consuming step in the HPLC methodology, although an extraction procedure has the advantage of decreasing the limit of detection by concentrating the sample. Second, UV absorbance detection coupled with CE permits the analysis of drugs with poor chromophores by working at low wavelengths (190–200 nm) or by using indirect detection (introduction of a chromophore in the working buffer). This simple approach can avoid derivatization. Hence, it has successfully been used for the determination of fosfomycin in various human biological fluids [72,73]. Thirdly, as stated above, CE requires a low sample volume (nl, μl) when compared with HPLC. This point is particularly interesting, especially in experimental pharmacokinetics where sampling specimens of sufficient volume and number are difficult to obtain or need the sacrifice of the animal, the ideal situation being to perform continuous sampling on the same subject; iterative blood sampling is difficult on a small rodent.

On the other hand, the main potential limitations of CE in pharmacokinetic studies could be the poor sensitivity due to the small injection volume used. Hence, the limits of detection/quantitation could be too high to accurately determine the elimination phase of drugs and hence the terminal half-life. The

quantitative capabilities of CE can be enhanced by concentrating the sample (stacking or a classical extraction) and/or by using a highly sensitive detection means such as laser-induced fluorescence (for fluorescent compounds). Nevertheless, as will be seen below, it appears to be too early to draw the definitive limits of this methodology for kinetic studies since it has rarely been used.

3. Applications to pharmacokinetic studies

If increased attention is paid to the quantitative determination of drugs in biological fluids by CE, very few techniques have been used for pharmacokinetic investigations. To our knowledge, only six studies have been published as full papers [9,16,47,80,82,83] (Table 3).

MECC coupled with microdialysis has been used to determine the pharmacokinetic properties of phenobarbital in rats after intraperitoneal injection (40 mg/kg) [9]. Iterative sampling could be performed in conscious animals by means of microdialysis probes set in the jugular vein and in the brain. Blood and brain dialysates could be obtained every 10 min during a 4-h period and were analyzed by MECC. The dialysate concentration–time curves were reported, but the kinetic parameters of unbound phenobarbital were not calculated.

O'Shea et al. [47] studied the disposition of L-DOPA given in rats by i.v. bolus injection (25 mg/kg). Blood was continuously sampled by microdialysis every 5 min for a period of 3 h in awake animals. The volumes of the samples were 5 μl. L-DOPA was assayed in the dialysates, on line, by CZE with amperometric detection. Although the duration of the study was also short (3 h) it was sufficient to evaluate the elimination parameters. The terminal half-life was 10.3 min (S.D., 1.1) and was found to be shorter than that obtained in anesthetized rats.

MECC associated with capillary ultrafiltration probes has been used to determine the pharmacokinetic properties of theophylline in rats after intraperitoneal injection (15 mg/kg) [16]. The capillaries were implanted in subcutaneous tissue, and allowed the iterative sampling of ultrafiltered extracellular liquid specimens in awake animals (rate, 1–3 μl/ml). These specimen (free of proteins) were obtained every 15 min over a 6-h period and were assayed both by MECC and HPLC. The kinetic parameters were very similar when estimated by both methodologies. Hence, the terminal half-lives were 3.1 h (standard error of the mean (S.E.M.), 0.4) and 3.2 h (S.E.M., 0.4) and the clearances were 1.5 (S.E.M., 0.2) and 1.5 ml/min (S.E.M., 0.1) using MECC and HPLC, respectively. Nevertheless, separation times were shorter by HPLC (4 vs. 6–10 min). The total analysis time was even longer for MECC (25 min) due to rinsing and equilibrating between each injection. The kinetic parameters, in particular the elimination half-life and the clearance, were similar when estimated by both methodologies.

Hogan et al. [80] investigated the fate of an investigational anticancer drug SR 4233 and its main metabolite SR 4317 in the rat by means of capillary electrophoresis coupled with microdialysis sampling. Blood dialysates were obtained every 2 min for a period of 90 min after i.v. injection of 4 mg/kg. The interval sampling (90–120 s) was very short and

Table 3
Pharmacokinetic studies performed by capillary electrophoresis techniques

Drug	Species	Dosage	Biological fluid	Type of sampling	Duration of sampling (h)	Mode of CE	Mode of detection	Ref.
Phenobarbital	rat	40 mg/kg	blood and brain dialysates	microdialysis	4	MECC	UV	[9]
L-DOPA	rat	25 mg/kg	blood dialysate	microdialysis	3	CZE	amperometric	[47]
Theophylline	rat	15 mg/kg	extracellular fluid	capillary ultrafiltration	5	MECC	UV	[16]
SR 4233	rat	4 mg/kg	blood dialysate	microdialysis	1.5	MECC	LIF	[80]
Antipyrine	rat	20 mg/kg	serum	conventional	6	MECC	UV	[82]
Fosfomycin	human	4 g	serum and aqueous humor	conventional	12	CZE	indirect UV	[83]
Nicotine	rat		skin dialysate	microdialysis	24	CZE	electrochemical	[84]

permitted a good estimation of the distribution and elimination half-lives. The latter was 15.3 min and hence the duration of the study (90 min), although short, appeared sufficient.

Brunner et al. [82] quantitated serum antipyrine concentrations by MECC in rats after intravenous bolus injection (20 mg/kg). Ten blood samples (100 μl) could be drawn on each animal over a 6-h period. Serum was deproteinized with acetonitrile before injection. The reasons for the choice of CE were not exposed. It is likely that CE allowed the determination of antipyrine in low volumes of serum and hence permitted iterative blood sampling on the same animal.

The sole human investigation concerns the penetration of the antibacterial agent fosfomycin in aqueous humor [83]. Twenty-one patients undergoing cataract surgery received fosfomycin (4 g) given by a 1-h infusion. Blood and aqueous humor samples were obtained over a 0–12-h period. With regard to aqueous humor specimens, the patients were only sampled once, each point corresponding to the mean of four patients. As exposed above, fosfomycin is a non-chromophoric drug whose assay by CE and indirect UV absorbance detection represents the only accurate and rapid analytical method. Other alternatives are the time-consuming gas chromatography and the non-specific microbiological assay. The other advantage of CE in this study was the possibility to quantitate fosfomycin in aqueous humor samples, the volumes of which are limited (100 μl). The aqueous humor concentrations were measurable over the study period. The ocular penetration of fosfomycin was judged to be excellent since the concentrations were above the minimal inhibitory concentrations for usually susceptible strains.

Recently, Zhou et al. [84] measured nicotine concentrations by capillary electrophoresis coupled with microdialysis in the skin of rats after application of a transdermal system. In this work, reported in an abstract form, nicotine levels could be determined continuously up to 24 h.

Hence, CE coupled with iterative microsampling strategies (capillary ultrafiltration, microdialysis) offers a great potential for the rapid and accurate characterization of preclinical pharmacokinetic profiles particularly in small and awake (i.e., not anesthetized) rodents. Furthermore, it appears a promising approach for investigating drugs with short elimination half-lives (<1 h) and it allows the rapid and direct determination of the unbound drug fraction (e.g., active, not bound to proteins).

With regard to therapeutic drug monitoring, some authors have claimed that their methods were already applied in routine practice, concerning the antimicrobial agents 5-fluorocytosine [75] and fosfomycin [73].

Besides in vivo pharmacokinetics, capillary electrophoresis has begun to be used for in vitro drug metabolism studies and particularly for the separation and the structural characterization of metabolites (Table 4). Tomlinson et al. [85] evaluated the in vitro metabolism of the histamine H_2 receptor antagonist mifentidine in animal hepatic microsomes by means of on-line CZE–MS. Five metabolites could be analyzed in the incubates.

The same authors used the same approach to investigate the metabolic fate of the neuroleptic drug haloperidol in microsomal incubates (reviewed in Ref. [86]). Haloperidol and six metabolites were first resolved by CE with diode-array detection. More information concerning the chemical structure of the compounds was obtained using CE coupled with MS.

Bogan et al. [87] studied interspecies differences in the metabolism of coumarin in liver microsomes

Table 4
In vitro drug metabolism studies performed by capillary electrophoresis

Drug	Species	Material	Mode of CE	Mode of detection	Ref.
Mifentidine and metabolites	guinea pig	liver microsome	CZE	MS	[85]
Haloperidol and metabolites	guinea pig	liver microsome	CZE	Diode array detection	[86]
	human	urine		MS	
Coumarin and metabolites	various	liver microsome preparations	CZE	UV	[87]

preparations by CE with UV absorbance detection. The product of coumarin biotransformation, 7-OH coumarin was separated and quantitated from 100 μl incubate samples. When compared to HPLC, capillary electrophoresis was judged more rapid (shorter analysis time, no sample clean up). Based on the comments of the authors [86,87], CE appears to be a very promising method for drug metabolism investigations due to the high separation efficiency, the rapidity of the procedure and the possibility of coupling with mass spectrometry. The main limitation is the habitual lack of sensitivity which can be overcome by using preconcentration of the sample.

4. Conclusions

Even if the number of published quantitative CE methods increases, it will still represent a minor analytical methodology for pharmacokinetic investigations. The theoretical advantages for kinetic studies are the very low sample volume required (when compared to HPLC), the possibility of direct injection of biological fluids, the high separation efficiency for the identification and separation of metabolites or stereoisomers, and the capability of quantitating drugs with poor chromophores by indirect UV absorbance detection or by using low wavelengths. Furthermore, capillary electrophoresis coupled with on-line microsampling strategies (ultrafiltration probes, microdialysis) represents a very attractive technique for studying preclinical kinetic profiles of drugs, particularly with rapid elimination. The main limitation of CE could be the high limits of detection/quantitation obtained which may hinder the accurate determination of concentrations in the late phase of elimination due to the small volume injected. If CE already seems to be appropriate for particular kinetic investigations ('first-line' use), a widespread use in the future will probably define its specific interest in pharmacokinetics.

Acknowledgments

The authors acknowledge the excellent secretarial work of Mrs M. Hoehn.

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