ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 881–888

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Influence of a polymeric formulation of ketoprofen on its diffusion into cerebrospinal fluid in rats

M. Matoga [a], F. Péhourcq [a], F. Lagrange [a], F. Fawaz [b], B. Bannwarth [a,*]

[a] *Departments of Pharmacology and Therapeutics, EA 525, Université Victor Segalen, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France*

[b] *Laboratory of Galenic Pharmacy and Biopharmacy, Université Victor Segalen, 33076 Bordeaux Cedex, France*

Received 2 April 2001; received in revised form 12 July 2001; accepted 19 July 2001

Abstract

Poly(D,L)lactide nanocapsules (NCs) have been proposed as an alternative carrier for many drugs. We investigated the influence of this formulation on the pharmacokinetics of ketoprofen in the plasma and cerebrospinal fluid (CSF). Male Wistar rats were given intraperitoneal dose of ketoprofen (5 mg/kg) in a suspension of NCs or in a carboxymethylcellulose (CMC) solution (reference preparation). Blood and CSF samples were collected at different times up to 24 h after dosing. The unbound fraction of ketoprofen in plasma (f_u) was determined using ultrafiltration. The total (C_T) and free (C_F) concentrations of ketoprofen in plasma and the simultaneous CSF concentrations (C_{CSF}) were measured by a HPLC method and the areas under the curve (AUC_T, AUC_F, AUC_{CSF}) were calculated. AUC_T of ketoprofen-loaded NCs in plasma was similar to that of the reference solution, while AUC_F of the former (5.41 mg/l × h) was higher than that produced by the latter (4.03 mg/l × h). Accordingly, the unbound fraction (f_u) was higher after administration of NCs than that of the solution (2.5 and 1.8%, respectively). Finally, AUC_{CSF} were identical for both formulations. These findings suggest that the binding of ketoprofen to plasma proteins is not the major factor that governs its blood-to-CSF exchanges. © 2002 Published by Elsevier Science B.V.

Keywords: Non steroidal anti-inflammatory drugs; Arylpropionic acids; Lipophilicity; CSF; Nanocapsules

1. Introduction

Various drug polymeric colloidal carriers, particularly nanocapsules (NCs), have been developed in order to ensure a controlled release of pharmaceutical agents and/or exert a preventive effect against the harmful side-effects induced by a direct contact of tissues with a strong amount of drug [1–3].

To act on the central nervous system, drugs have to cross the blood–brain barrier, which separates the brain and the cerebrospinal fluid (CSF) from bloodstream [4]. Non steroidal anti-inflammatory drugs (NSAIDs), apart from their classical peripheral site of action, were shown to display a central analgesic effect [5,6]. Moreover, various central nervous system adverse reactions

* Corresponding author. Tel.: +33-5-57-571590; fax: +33-5-56-930407.

E-mail address: bernard.bannwarth@thera.u-bordeaux2.fr (B. Bannwarth).

0731-7085/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0731-7085(01)00585-4

have been ascribed to NSAID therapy [7]. All these features justify the penetration of NSAIDs through the blood–brain barrier to be studied. An earlier investigation of our group has shown that the diffusion of a series of arylpropionic NSAIDs into the CSF in rats depends primarily on their molecular lipophilicity [8]. According to Bonati et al. [4], the pharmacokinetics of a drug in the plasma may influence its pharmacokinetic in CSF. Since the polymeric formulation of an NSAID would result in changes in its plasma pharmacokinetic profile, we conducted a study investigating the plasma and CSF pharmacokinetics of a new polymeric formulation of ketoprofen in rats. These pharmacokinetic characteristics were compared with those of ketoprofen in solution.

2. Materials and methods

2.1. Chemicals

Racemic ketoprofen was purchased from Sigma (St. Quentin Fallavier, France). Benzyl benzoate was supplied by Sigma, poly(D,L-lactide) polymer (mol. wt. 110 000) was obtained from Boehringer Ingelheim (Germany). Phospholipids (Epikuron®200) and poloxamer 188 (Synperonic®F68) were furnished by Lucas Meyer (Hamburg, Germany) and ICI (Clamart, France), respectively. All other chemicals and solvents were of analytical or HPLC grade. Water was deionized and doubly-glass distilled.

2.2. Nanocapsules

2.2.1. Preparation

We used a suspension of poly(D,L)lactide NCs, which is a biodegradable and biocompatible drug carrier of lactid acid polymers [9]. NCs containing ketoprofen were prepared by interfacial deposition of a preformed poly (D,L-lactide) polymer, as described by Fessi et al. [10]. Briefly, the poly(D,L-lactide) polymer (250 mg), ketoprofen (25 mg) and phospholipids (250 mg) were dissolved in 25 ml of acetone. The mixture was dissolved in 1 ml of an oily phase (benzyl benzoate) at 40 °C. The resulting organic solution was poured, with moderate magnetic stirring (400 rpm), into 100 ml of water containing poloxamer 188 (250 mg) used as non-ionic surfactant. This operation was repeated six times. These suspensions were mixed and filtered through a sintered glass funnel (grade 4). Acetone and water were removed under reduced pressure at 38 °C to obtain the final desired concentration [11,12].

2.2.2. Colloidal system characteristics

2.2.2.1. Particle size. The NC size presented a mean value of 416 ± 110 nm. These measurements were carried out with laser-light scattering using a Coulter N4 MD Super-nanosizer Coultronics (Electronics, Margency, France) calibrated with a standard latex nanoparticule suspension (300 nm).

2.2.2.2. Particle stability. After 3 months of storage at $+4$ °C, several stability tests, including size measurements, drug loading, drug loss and morphological examination were performed. No changes in the content and size of particles were observed during the storage, indicating that the nanoparticle suspensions remained stable over at least 3 months.

2.2.2.3. Determination of ketoprofen concentrations in nanoparticle suspensions. Non-incorporated ketoprofen was isolated by the combination of different ultrafiltration and centrifugation procedures, and measured in the ultrafiltrate. After a complete dissolution of nanoparticles in acetone and precipitation of polymer by methanol, the mixture was centrifuged at 12 000 rpm for 20 min. Total concentrations of ketoprofen in the nanoparticle suspensions were measured in the supernatant. The concentrations of ketoprofen were determined by a RP-HPLC method [13]. Indomethacin was used as an internal standard. The compounds were chromatographed on a C_{18} reversed-phase column by a mobile phase consisting of a mixture of acetonitrile and phosphate buffer 0.06 M at pH 7.4 (30/70, v/v) after a direct injection of samples. The concentration of ketoprofen incorporated into nanoparticles was calculated by the difference between the total and

non-incorporated concentrations. NCs showed a high ketoprofen-entrapment efficiency (98%,), with a final concentration of 2 mg/ml.

2.3. Pharmacokinetic study

2.3.1. CSF and blood sampling

The present research adhered to the Principles of Laboratory Animal Care (NIH publication # 85–23, revised 1985). Experiments were carried out on male Wistar rats (Iffa Credo, Arbresle, France), weighing approximately 200 g. The rats had free access to tap water and food pellets. Rats were randomly divided into two groups. All received a single intraperitoneal (i.p.) dose of ketoprofen (5 mg/kg) either in carboxymethylcellulose (CMC) 5% solution or in NC suspensions. In both cases, ketoprofen was at a concentration of 2 mg/ml.

The rats were anesthetized with pentobarbital (5%). A single blood and CSF sample was drawn concomitantly from each animal at 0.5, 1, 3, 6, 10 and 24 h after drug administration. Four animals were used per sampling time for the two different formulations. CSF was drawn according to the method described by Nohjoh et al. [14]. The heads of animals were fixed on a stereotaxic apparatus and a 3 in. needle attached to a short length of plastic tubing was inserted into the cisterna magma.

The CSF samples were collected in a polypropylene microtube and frozen at -80 °C until analysis. The blood samples were obtained by cardiac puncture that was performed immediately after CSF withdrawal. They were collected in heparinized tubes and centrifuged at $2500 \times g$ for 10 min. After centrifugation, the plasma was frozen at -80 °C until analysis.

2.3.2. Determination of the unbound fraction of ketoprofen in the plasma

The fraction of ketoprofen unbound to plasma proteins was determined by ultrafiltration (Centrifree, Amicon, USA). Ultracentrifugation was performed at 37 °C during 1 h at $3000 \times g$. The ultrafiltrates were collected and frozen at -80 °C until analysis.

2.3.3. Determination of ketoprofen concentrations in biological samples

The free (ultrafiltrate) and total concentrations of ketoprofen in plasma and the total concentrations in CSF were determined by the above mentioned RP-HPLC method [13]. Before injection into the chromatograph, the drug was extracted from acidified biological samples with diethylether in the presence of indomethacin (internal standard). The limit of quantification was 10 μg/l in total plasma, ultrafiltrate and CSF.

2.3.4. Pharmacokinetic parameters

The plasma (total and free) and CSF concentrations versus time profiles of ketoprofen were defined and the following pharmacokinetic parameters were determined:

- the observed maximal concentration (C_{max}) and the time to C_{max} (t_{max});
- the area under the concentration–time curve (AUC), which was calculated for total plasma (AUC_T), free plasma (AUC_F) and CSF (AUC_{CSF}) between 0 and 24 h by the trapezoidal rule (Siphar® program, Simed, Créteil, France);
- the AUC_{CSF}/AUC_T (R_T) and AUC_{CSF}/AUC_F (R_F) ratios were used to quantify overall drug transit to CSF;
- the unbound fraction (f_u) in percentage was given by the formula:

$$f_u = \left(\frac{AUC_F}{AUC_T}\right)100$$

3. Results

The total plasma, free plasma and CSF pharmacokinetic profiles of ketoprofen for both formulations are shown in Figs. 1–3, respectively. The mean (S.D.) concentrations are listed in Table 1. The pharmacokinetic parameters are reported in Table 2.

3.1. Plasma pharmacokinetics

Ketoprofen was rapidly absorbed after ip administration. The time to C_{max} of total ketoprofen

in plasma was observed at 30 min for NC suspension and CMC solution (Fig. 1). The free concentrations of ketoprofen were also maximum at 30 min after dosing (Fig. 2).

After administration of ketoprofen-loaded NCs, there was a second, but lower peak of total ketoprofen concentrations at 10 h. However, the corresponding AUC_T (215 mg/l × h) was comparable with that obtained with the CMC solution of ketoprofen (227 mg/l × h). The unbound fraction (f_u) was higher after administration of NC suspensions than after that of CMC solutions (2.5 and 1.8%, respectively). Similarly, the former resulted in a higher AUC_F than the latter (5.41 and 4.03 mg/l × h, respectively).

3.2. CSF pharmacokinetics

Whatever the formulation applied, ketoprofen entered rapidly the CSF, the maximum concentration being observed at the earliest sampling time at 30 min. Furthermore, neither the CSF concentrations, nor the AUC_{CSF} showed substantial differences between the two formulations used. The AUC_{CSF}/AUC plasma ratio was shown to allow the overall drug transit into CSF to be quantified [15]. Regarding the total plasma concentrations of ketoprofen, this ratio (R_T) was 0.006 and 0.007 for the CMC solution and the NC suspension, respectively. Considering the free plasma concentrations, this ratio (R_F) was lower after administration of ketoprofen-loaded NCs (0.28) than after that of CMC solution (0.38).

4. Discussion

The present study aimed to determine the pharmacokinetics of racemic ketoprofen in plasma and CSF after an administration of a polymeric carrier (NC suspension) in comparison with a reference formulation (CMC solution).

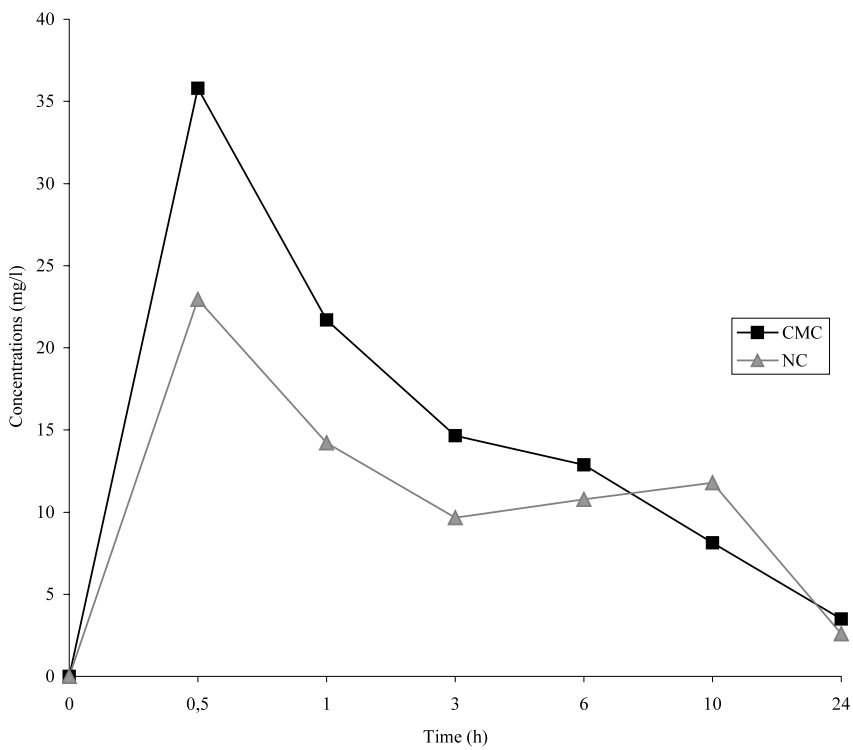

Fig. 1. Total concentration vs. time curves of ketoprofen in plasma after administration of CMC solution and NC suspension.

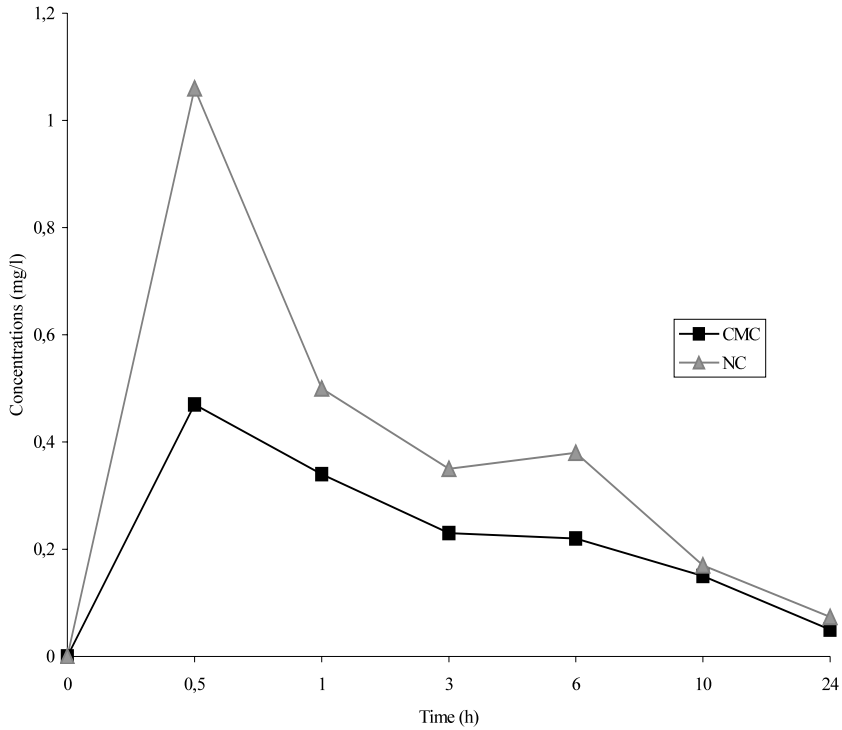

Fig. 2. Free concentration vs. time curves of ketoprofen in plasma after administration of CMC solution and NC suspension.

NCs are drug carriers of lactic acid polymers. They are composed of an oily core surrounded by a polymeric envelope. The inner of NCs being oily, the diffusion of the drug through the polymeric wall into the aqueous medium is governed by its partition coefficient [11,16].

The administration of NCs generated an AUC_T value very similar to that observed after injection of the CMC solution (215 and 227 ml/l × h, respectively). However, these formulations generated different pharmacokinetic profiles of ketoprofen in plasma (Fig. 1). Ketoprofen administered in CMC solution was rapidly absorbed and the C_{max} was followed by a phase of elimination whereas two peaks of concentrations were noted after administration of NC suspensions. The second and lower peak might be due to a progressive release of the drug from NCs during the elimination phase.

Ketoprofen was extensively bound to plasma proteins, the unbound fraction being no more than 2.5%. Similar findings have already been reported in humans with an average unbound fraction of about 1% [18]. Of note, the extent of ketoprofen binding to plasma proteins appeared to be somewhat lower after administration of NCs. Since organic acids may displace ketoprofen from its protein binding sites, it could be hypothesized that benzyl benzoic acid, the oily phase of NCs, might act as a displacer [19,20]. Moreover, benzoic acid is a precursor of hippuric acid, which was shown to produce a two-fold increase in the free concentrations of both ketoprofen enantiomers [21].

If only the free drug in the plasma is able to cross the blood–brain barrier [14,15,17], an increase in the free plasma concentrations would be expected to result in an increase of its concentrations in CSF. The central nervous compartments are surrounded by a diffusional barrier, which is mainly constituted of lipid bilayers. Thus the blood–CSF barrier has been compared with a second cell membrane [15,22]. Accordingly, the main process by which a drug passes into and out

of the CSF is passive diffusion [14,15,17]. Ketoprofen entered rapidly the CSF after administration of each formulation. However, the extent of ketoprofen binding to plasma proteins did not appear to influence the overall drug transit into CSF as estimated by AUC_{CSF}/AUC_{plasma} ratio (R_T or R_F). Similar findings have been reported for oxicam NSAIDs [23] and other classes of drugs [24], suggesting that the fraction of drug that may permeate into the central nervous system

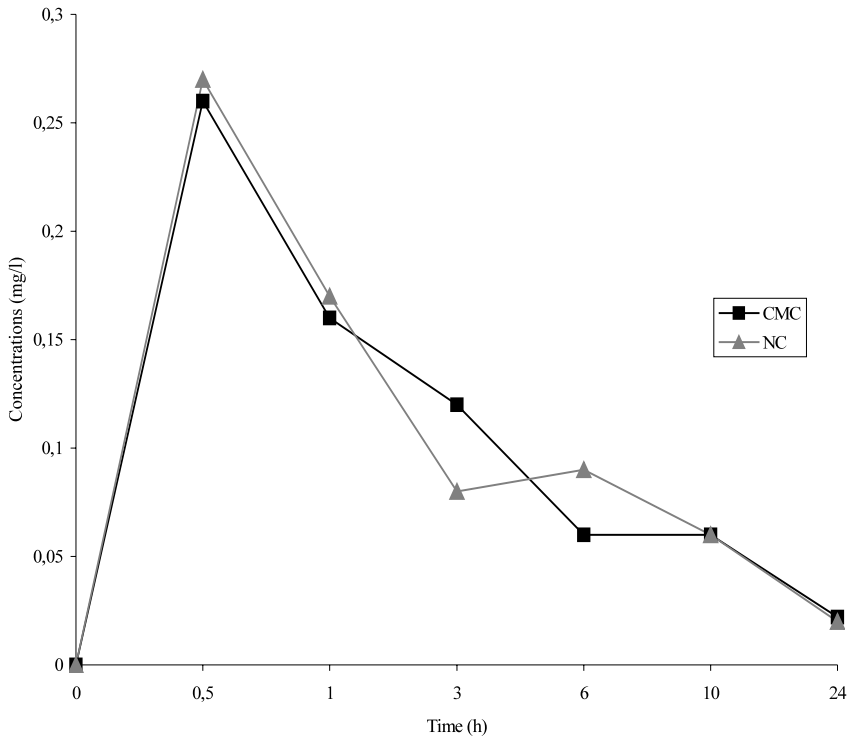

Fig. 3. Concentration vs. time curves of ketoprofen in CSF after administration of CMC solution and NC suspension.

Table 1
Mean (S.D.) concentrations (mg/l) of total (C_T) and free (C_F) ketoprofen in plasma and in CSF (C_{CSF}) at the different sampling times

Formulations	Time (h)					
	0.5	1	3	6	10	24
Ketoprofen CMC						
C_T	35.80 (6.34)	21.69 (6.10)	14.65 (0.87)	12.87 (1.38)	8.14 (1.45)	3.48 (0.46)
C_F	0.47 (0.11)	0.34 (0.11)	0.23 (0.07)	0.22 (0.03)	0.15 (0.04)	0.050 (0.020)
C_{CSF}	0.26 (0.08)	0.16 (0.09)	0.12 (0.09)	0.06 (0.01)	0.06 (0.03)	0.022 (0.006)
Ketoprofen NC						
C_T	22.94 (5.94)	14.21 (2.62)	9.67 (4.10)	10.77 (3.58)	11.78 (3.49)	2.60 (0.89)
C_F	1.06 (0.29)	0.50 (0.13)	0.35 (0.07)	0.38 (0.22)	0.17 (0.05)	0.074 (0.031)
C_{CSF}	0.27 (0.04)	0.17 (0.07)	0.08 (0.01)	0.09 (0.03)	0.06 (0.01)	0.02 (0.007)

CMC, carboxymethylcellulose solution; NC, nanocapsule suspension.

Table 2
Mean (S.D.) pharmacokinetic parameters of ketoprofen in plasma (total concentrations), ultrafiltrate (free plasma concentrations) and CSF after ip administration of two formulations

Formulations	T_{max} (h)	C_{max} (mg/l)	AUC (mg/l × h)	f_u (%)
Ketoprofen CMC				1.8
Plasma	0.5	35.8 (6.34)	227.21	
Ultrafiltrate	0.5	0.47 (0.11)	4.03	
CSF	0.5	0.26 (0.08)	1.53	
Ketoprofen NC				2.5
Plasma	0.5	22.94 (5.94)	215.32	
Ultrafiltrate	0.5	1.06 (0.29)	5.41	
CSF	0.5	0.27 (0.04)	1.53	

CMC, carboxymethylcellulose solution; NC, nanocapsule suspension.

through the blood–brain barrier is not only the unbound fraction but also the fraction dissociated from the proteins in the capillaries [22]. In other words, both the plasma unbound and initially bound fractions of ketoprofen might be available for transfer into CSF. CMC solution and NCs resulted in very similar AUC_T and AUC_{CSF}. These findings are consistent with the hypothesis that drug lipophilicity seems to be the main parameter that governs the blood-to-CSF exchanges [8,17,22].

Acknowledgements

The authors are greatly indebted to E. Deridet and B. Martinez for their skilled technical assistance.

References

[1] N. Ammoury, H. Fessi, J.P. Devissaguet, M. Allix, R.G. Plotkine, M. Boulu, Effect on cerebral blood flow of orally administered indomethacin-loaded poly(*iso*-butylcyanoacrylate) and poly(D,L-lactide) nanocapsules, J. Pharm. Pharmacol. 42 (1990) 558–561.

[2] A.M. Evans, Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs, Eur. J. Clin. Pharmacol. 42 (1992) 237–256.

[3] S.S. Guterres, H. Fessi, G. Barratt, F. Puisieux, J.P. Devissaguet, Poly(D,L-lactide) nanocapsules containing non-steroidal anti-inflammatory drugs: gastrointestinal tolerance following intravenous and oral administration, Pharm. Res. 12 (1995) 1545–1547.

[4] M. Bonati, J. Kanto, G. Tognoni, Clinical pharmacokinetics of cerebrospinal fluid, Clin. Pharmacokinet. 7 (1982) 312–335.

[5] B. Bannwarth, F. Demotes-Mainard, T. Schaeverbeke, L. Labat, J. Dehais, Central analgesic effects of aspirin-like drugs, Fundam. Clin. Pharmacol. 9 (1995) 1–7.

[6] D. Bustamante, C. Paeile, J.C. Willer, D. Le Bars, Effects of intravenous nonsteroidal antiinflammatory drugs on a C-fiber reflex elicited by a wide range of stimulus intensities in the rat, J. Pharmacol. Exp. Ther. 276 (1996) 1232–1243.

[7] A. Morgan, D. Clark, CNS adverse effects of nonsteroidal anti-inflammatory drugs. Therapeutic implications, CNS Drugs 9 (1998) 281–290.

[8] M. Matoga, F. Pehourcq, F. Lagrange, G. Tramu, B. Bannwarth, Influence of molecular lipophilicity on the diffusion of arylpropionate non-steroidal anti-inflammatory drugs into the cerebrospinal fluid, Arzneimittelforsch 49 (1999) 477–482.

[9] D. Quintanar-Guerrero, E. Allemann, E. Doelker, H. Fessi, Preparation and characterization of nanocapsules from preformed polymers by a new process based on emulsification–diffusion technique, Pharm. Res. 15 (1998) 1056–1062.

[10] H. Fessi, F. Puisieux, J.P. Devissaguet, N. Ammoury, S. Benita, Nanocapsule formation by interfacial deposition following solvent displacement, Int. J. Pharm. 55 (1989) R1–R4.

[11] N.S. Magalhaes, H. Fessi, F. Puisieux, S. Benita, M. Seiller, An in vitro release kinetic examination and comparative evaluation between submicron emulsion and polylactic acid nanocapsules of clofibride, J. Microencapsul. 12 (1995) 195–205.

[12] P. Calvo, J.L. Vila-Jato, M.J. Alonso, Comparative in vitro evaluation of several colloidal systems, nanoparticles, nanocapsules, and nanoemulsions, as ocular drug carriers, J. Pharm. Sci. 85 (1996) 530–536.

[13] F. Péhourcq, F. Lagrange, L. Labat, B. Bannwarth, Simultaneous measurement of flurbiprofen, ibuprofen and ketoprofen enantiomer concentrations in plasma using

L-leucinamide as the chiral coupling component, J. Liq. Chromatogr. 18 (1995) 3969–3979.

[14] T. Nohjoh, H. Suzuki, Y. Sawada, Y. Sugiyama, T. Iga, M. Hanano, Transport of cefodizime, a novel third generation cephalosporin antibiotic, in isolated rat choroid plexus, J Pharmacol. Exp. Ther. 250 (1989) 324–328.

[15] R. Nau, G. Zysk, A. Thiel, H.W. Prange, Pharmacokinetic quantification of the exchange of drugs between blood and cerebrospinal fluid in man, Eur. J. Clin. Pharmacol. 45 (1993) 469–475.

[16] L. Marchal-Heussler, P. Thouvenot, M. Hoffman, P. Maincent, Comparison of the biodistribution in mice of 111indium oxine encapsulated into poly(lactic-co-glycolic)-D,L-85/15 and poly(epsilon caprolactone) nanocapsules, J. Pharm. Sci. 88 (1999) 450–453.

[17] B. Bannwarth, P. Netter, J. Pourel, R.J. Royer, A. Gaucher, Clinical pharmacokinetics of nonsteroidal anti-inflammatory drugs in the cerebrospinal fluid, Biomed. Pharmacother. 43 (1989) 121–126.

[18] L. Deschamps-Labat, F. Péhourcq, M. Jagou, B. Bannwarth, Relationship between lipophilicity and binding to human serum albumin of arylpropionic acid non-steroidal anti-inflammatory drugs, J. Pharm. Biomed. Anal. 16 (1997) 223–229.

[19] T. Sakai, T. Maruyama, H. Imamura, H. Shimada, M. Otagiri, Mechanism of stereoselective serum binding of ketoprofen after hemodialysis, J. Pharmacol. Exp. Ther. 278 (1996) 786–792.

[20] L.N. Sansom, A.M. Evans, What is the true clinical significance of plasma protein binding displacement interactions, Drug Saf. 12 (1995) 227–233.

[21] F. Lagrange, F. Péhourcq, M. Matoga, B. Bannwarth, Binding of ketoprofen enantiomers in various human albumin preparations, J. Pharm. Biomed. Anal. 23 (2000) 793–802.

[22] W.M. Pardridge, Transport of small molecules through the blood–brain barrier: biology and methodology, Adv. Drug. Deliv. Rev. 15 (1995) 5–36.

[23] P. Jolliet, N. Simon, F. Brée, S. Urien, A. Pagliara, P.A. Carrupt, B. Testa, J.P. Tillement, Blood to brain transfer of various oxicams: effects of plasma binding to their brain delivery, Pharm. Res. 14 (1997) 650–656.

[24] H. Tanaka, K. Mizojiri, Drug protein binding and blood–brain barrier permeability, J. Pharmacol. Exp. Ther. 288 (1999) 912–918.