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Measurement and pharmacokinetic analysis of buspirone by means of brain microdialysis coupled to high-performance liquid chromatography with electrochemical detection

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Abstract

The feasibility of an electrochemical detection system with on-line microdialysis coupled with sensitive microbore high-performance liquid chromatography for the measurement and brain pharmacokinetic analysis of buspirone was investigated. A microdialysis probe was inserted into the right striatum of male Sprague–Dawley rats, which had been administered buspirone (10 mg/kg, i.v.). Dialysates were automatically injected through an on-line injector into a cyano microbore column coupled to an electrochemical detector. Samples were eluted with a mobile phase containing 0.1 *M* monosodium dihydrogenphosphate–acetonitrile–diethylamine (85:15:0.1, v/v/v, pH 3.0, adjusted with orthophosphoric acid) at a flow-rate of 0.06 ml/min. A biphasic phenomenon with a rapid distribution phase followed by a slower elimination phase was observed from the brain buspirone concentration–time curve. The results indicate that the brain pharmacokinetics of buspirone appear to conform to a two-compartment model.

Keywords: Microdialysis; Sampling methods; Buspirone

1. Introduction

Buspirone, 8-[4-[(2-pyrimidinyl)piperazinyl]-butyl]-8-azaspiro[4,5]decane-7,9-dione, is a novel and effective anxiolytic agent [1–3]. It exhibits an anxiolytic effect similar to that of diazepam, without sedative, muscle relaxing or anticonvulsant properties [4–6]. The drug concentration–time course in the target brain areas is of particular importance when assessing the effect of psychotropic agents. In general, the concentrations of drugs in the central nervous system are accessed by analyzing brain tissue of animals killed at specific time points after the administration of the drugs [7].

Methods for the determination of buspirone, either by selective-ion monitoring gas chromatography–mass spectrometry [8], or by HPLC with ultraviolet detection [9,10], and electrochemical detection (ED) [11,13] have been reported. The gas chromatographic–mass spectrometric determination is not only cumbersome and time-consuming but also requires technical skill [8]. In addition, conventional HPLC with ultraviolet detection or ED is not sensitive enough for the determination of buspirone in small sample volumes. Recently, microbore columns instead of conventional columns coupled with ED have advantages of very high sensitivity and a relatively small sample introduction.

Recent advances in brain microdialysis techniques have enabled the direct measurement of various neurotransmitters in the brain but pharmacokinetic investigations of psychotropic drugs by this method

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are limited [14,15]. Since drug monitoring is crucial for the rational therapeutic use of drugs, the feasibility of employing the brain microdialysis method for pharmacokinetic studies is of particular importance.

Of interest in elucidating the central disposition of buspirone in the brain, a precise and sensitive method using an on-line microdialysis system coupled with microbore HPLC–ED was developed to measure buspirone in brain dialysates. In addition, the pharmacokinetics of buspirone in rat brains was also investigated.

2. Materials and methods

2.1. Materials and reagents

Buspirone was purchased from Research Biochemical International (RBI, Natick, MA, USA). Acetonitrile and orthophosphoric acid were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Chromatography

The HPLC–ED system consisted of a chromatographic pump (BAS, PM-80, Bioanalytical System, West Lafayette, IN, USA) at a flow-rate of 0.06 ml/min for buspirone analysis using a cyano microbore column (BAS, Sepstik CN-5μ, 150×1 mm I.D., particle size 5 μm) in series after an on-line injector. The mobile phase consisted of 0.1 *M* monosodium dihydrogen orthophosphate–acetonitrile–diethylamine (85:15:0.1, v/v/v, pH 3.0 adjusted with orthophosphoric acid). The mixture was filtered with a 0.22-μm millipore membrane and degassed by helium. The injection volume was configured with a 10-μl sample loop on an on-line injector (CMA-160, CMA/Microdialysis, Stockholm, Sweden). Buspirone was measured using an electrochemical detector (BAS 4C). The potential for the glassy carbon working electrode was set at +1.10 V with respect to a Ag/AgCl reference electrode. The output from the electrochemical detector was recorded using Waters Millennium 2020 software.

2.3. Microdialysis

Adult, male Sprague–Dawley rats (250–320 g) were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The rat was cannulated with a PE-50 tube at the right femoral vein for drug administration. After the femoral vein cannulation, the rat was placed in a Kopt stereotaxic frame and its body temperature was maintained at 37°C with a heating pad. A microdialysis probe (CMA-12; CMA/microdialysis) with a tip length of 4 mm and an outer diameter of 0.5 mm was implanted into the right striatum with its tip located at AP 0.4 mm, ML −3.0 mm, DV −7.0 mm, from the bregma and dura surface, respectively [16]. The probe was perfused with Ringer solution (147 m*M* Na^+, 4.0 m*M* K^+, 2.2 m*M* Ca^{++}) at a flow-rate of 1 μl/min, by a microinjection pump (CMA-100). The outflow from the dialysis probe was connected to an on-line injector (CMA-160) and HPLC–ED [17,18].

2.4. Recovery

The recovery of the dialysis probe for buspirone was the ratio of its concentration in the dialysate, i.e. the outlet from the probe (C_{out}) to its concentration of buspirone in the medium surrounding the probe (C_{in}).

$$\text{The recovery}_{\text{invitro}} = C_{out}/C_{in}$$

2.5. Pharmacokinetic analysis

Calibration curves were constructed based on the analysis by HPLC–ED of various concentrations of buspirone (0.01–1 μg/ml) and were used to determine the concentrations of buspirone in rat brain dialysates. Following a 2-h period for stabilization, dialysates were automatically injected every 8 min for 144 min after drug administration (10 mg/kg, i.v.). The volume of i.v. buspirone (10 mg/ml) solution administered was 1 ml/kg. After the administration of the drug, the catheter was then immediately flushed with 0.5 ml normal saline.

All brain dialysate concentration–time data were processed by the computer program "PCNONLIN" (SCI Software, Lexington, KY, USA), with recip-

rocal concentration weights ($1/C$) for the calculation of pharmacokinetic parameters.

The data were compared with pharmacokinetic models (one-vs two-compartment) according to the criteria of Akaike's information criterion (AIC) [19] and Schwartz criterion (SC) [20], with minimum AIC and SC values being regarded as the best representation of the concentration–time course data. The following equation applies to a two-compartment pharmacokinetic model:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

In Eq. (1), A and B are the concentration (C) intercepts for the fast and the slow disposition phases, respectively. α and β are disposition rate constants for the fast and the slow disposition phases, respectively. The distribution half-life ($t_{1/2,\alpha}$) and elimination phase half-life ($t_{1/2,\beta}$) of buspirone in brain dialysate were defined as $0.693/\alpha$ and $0.693/\beta$, respectively.

The noncompartmental method for calculating disposition parameters of trazodone in the brain is based on the theory of statistical moments [21]. The area under the concentration–time curve (AUC) of a plot from time zero to infinity is often referred to as the area under the moment curve (AUMC) [21]. The ratio of AUMC to AUC for buspirone in the brain is a measure of its mean residence time (MRT) [22].

3. Results

Under the conditions described above, the retention time of buspirone was found to be 6.2 min (Fig. 1). Fig. 1A shows a standard sample of buspirone (0.5 μg/ml). Fig. 1B shows a chromatogram of a blank brain dialysate. No discernible peaks were observed within the time frame in which buspirone was detected. Fig. 1C shows a chromatogram of a dialysate sample containing buspirone (0.64 μg/ml) obtained from brain microdialysis 24 min after buspirone (10 mg/kg, i.v.) administration. The in vitro recovery of buspirone of the microdialysis probe based on a 1 μg/ml standard, was 40%.

The reproducibility of the method was also determined by examining both intra- and inter-assay

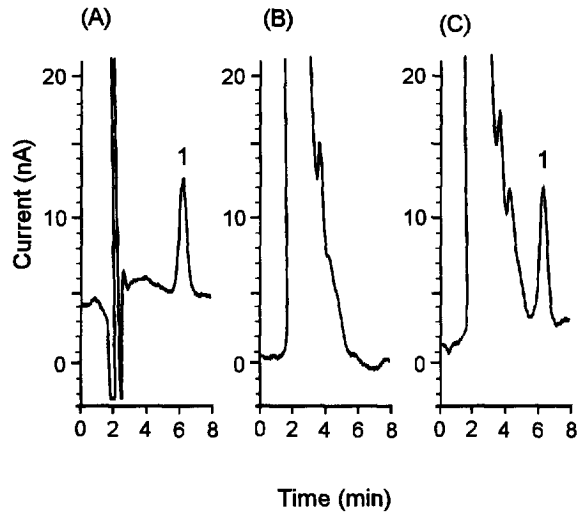

Fig. 1. Typical chromatograms of (A) a standard sample containing 0.5 μg/ml buspirone, (B) a blank brain dialysate and (C) a dialysate sample containing buspirone (0.64 μg/ml) collected from a rat after buspirone (10 mg/kg, i.v.) administration. 1: buspirone.

variabilities. The intra-assay ($n=6$) for the determination of buspirone at concentrations of 0.05, 0.5, and 1 μg/ml were acceptable with R.S.D. values less than 8% (Table 1). The inter-assay R.S.D.s ($n=6$) for buspirone at the same concentrations were less than 10% (Table 1).

Table 1
Intra- and inter-assay precision and accuracy in buspirone determination ($n=6$)

	Nominal concentration (μg/ml)		
	0.05	0.5	1
Intra-assay			
Mean	0.046	0.47	1.03
S.D.	0.003	0.03	0.06
% R.S.D.	6.5	6.4	5.8
Accuracy (%)	−8.0	−6.0	3.0
Inter-assay			
Mean	0.045	0.54	1.04
S.D.	0.004	0.04	0.06
% R.S.D.	8.9	7.4	5.8
Accuracy (%)	−10	8.0	4.0

Precision (% R.S.D.) = [standard deviation (S.D.)/mean concentration] × 100.
Accuracy (%) = [(Mean conc. − Actual conc.)/Actual conc.] × 100.

Table 2
Brain pharmacokinetic parameters of buspirone (10 mg/kg, i.v.) in rats

Parameters	Estimate
AUC (μg min/ml)	50.06±2.13
$t_{1/2,\alpha}$ (min)	8.74±0.60
$t_{1/2,\beta}$ (min)	47.14±8.59
AUMC (μg min^2/ml)	1679±240
MRT (min)	33.22±4.17

Data are expressed as mean±SEM ($n=6$).

The limit of quantitation is defined as the lowest concentration on the standard curves which can be measured with acceptable accuracy and precision. The limit of quantitation was 0.01 μg/ml for buspirone. However, the detection limits for buspirone, at a signal-to-noise ratio of 3, was 1 ng/ml.

A two-compartment open model with individual animal data after buspirone i.v. administration was proposed by the computer program "PCNONLIN". Analysis of data yields Eq. (2).

$$C = 2.35e^{-0.081t} + 0.41e^{-0.018t} \quad (2)$$

The brain pharmacokinetic parameters, as calculated by the PCNONLIN program and derived from these data, are shown in Table 2.

4. Discussion

Compared to conventional HPLC systems [12], microbore columns decrease band broadening of analytes so that sharper peaks are obtained. Furthermore, the low flow-rates in a microbore HPLC–ED system provide a smoother baseline to achieve lower detection limits. Also, the time of contact of the analytes with the working electrode is prolonged and results in higher coulometric yields [23,24]. Hence, microbore HPLC–ED systems can enhance detection sensitivity and achieve optimum detection limits. Furthermore, the microbore HPLC–ED system requires only small quantities of samples which is compatible for microdialysis sampling methods. In addition, the on-line analysis improves analytical reproducibility and obviates the need for preservatives in the samples and other tedious manual procedures.

In the present study, a microbore HPLC–ED system was applied to the determination of buspirone in rat striatal dialysates from an on-line microdialysis system in rats receiving an i.v. administration of buspirone. The limit of quantification and the detection limit of buspirone were 10 ng/ml and 1 ng/ml, respectively. A biphasic phenomenon with a first-order elimination rate constant for buspirone was observed from the brain dialysate concentration–time curve. The results indicate that the brain pharmacokinetics of buspirone appear to conform to a two-compartment model.

Noncompartmental methods for calculating disposition parameters of buspirone in brain dialysate are based on the theory of statistical moments [21]. After administration of buspirone (10 mg/kg, i.v.), MRT and $t_{1/2,\beta}$ were 47.14 and 33.22 min, respectively. MRT is a function of both distribution and elimination. Elimination half-life ($t_{1/2,\beta}$) is the time required to eliminate 50% of the dose, whereas MRT_{iv} is the time required to eliminate 63.2% of the dose [21]. Our results suggest that buspirone in rat brain exhibits fast and slow disposition phases.

In conclusion, the present results suggest that the brain microdialysis method may be applicable to further pharmacokinetic studies of psychotropic or neurotropic agents in the brain.

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