Arch Pharm Res Vol 30, No 12, 1635-1645, 2007

Archives of Pharmacal Research
http://apr.psk.or.kr

Intracerebral Microdialysis Technique and its Application on Brain Pharmacokinetic-Pharmacodynamic Study

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(Received June 24, 2007)

Intracerebral microdialysis (IC-MD) has been developed as a well-validated and powerful technique for decades. As a practical sampling tool, it can gain the continuous dialysates of endogenous and exogenous substances in extracellular fluid (ECF) of awake freely moving animals. Also, variform IC-MD probes (IC-MDPs) have grown more exquisite. The implantation of the IC-MDP in certain tissue of brain allows monitor drug distribution and measure drug and corresponding neurotransmitters levels in brain ECF after administration for brain pharmacokinetic-pharmacodynamic (B-PK-PD) study. So it is suitable for IC-MD to B-PK-PD study (IC-MD/B-PK-PD). The performance of IC-MD/B-PK-PD can not only elevate the degree of precision and accuracy of experimental data, minimize the individual difference by reduced number of animals, but also give important information for the prediction and optimization of drug effective dose in preclinical study. In this review, we have discussed various IC-MD/B-PK-PD studies of analgesic, antiepileptic and antidepressant drug. The role of IC-MD/B-PK-PD in confirming and assessing the drug effect before clinic trials is highlighted.

Key words: Intracerebral microdialysis, Pharmacokinetic-pharmacodynamic, Extracellular fluid, Analgesic drug, Antiepileptic drug, Antidepressant drug

INTRODUCTION

Intracerebral microdialysis (IC-MD) is an established technique for sampling dialysates from extracellular fluid (ECF) of certain tissue of brain where IC-MD probe (IC-MDP) implanted in awake freely moving animals to monitor the dynamic variance of endogenous and exogenous substances.

As we known, IC-MD has been developed and has gained a number of advances in brain science research for decades. It was originally developed as early as 1972 by Delgado *et al.* who used a dialytrode with a push-pull perfusion device for performance of intracerebral perfusion in monkey's brain (Delgado *et al.*, 1972). Since then, IC-MD has opened up a gate to neurochemistry studies for neuroscientists. In 1974, Ungerstedt and Pycock firstly manufactured a linear IC-MDP implanted transcranially to monitor dopamine (DA) level in brain for neurological pharmacology study (Ungerstedt and Pycock, 1974). It is signified that the pharmacology studies of drugs in central nervous system (CNS) are potential. In the 1980's, IC-MD was used extensively in neuroethology studies connecting the time-course of behaviors with neurochemistry changes (Westerink, 1995; Peerdeman *et al.*, 2000; Bourne, 2003). In 1985, Vezzani A. *et al.* demonstrated a method for capturing electroencephalography (EEG) with the IC-MDP, permitting measurement at the same brain location for neuroethology study (Vezzani *et al.*, 1985). Certainly, the evolution of IC-MD and its applications in neuroscience study have paved the way for their utilizations in following drug studies.

In the 1990's, since IC-MD was introduced into the pharmaceutical studies, it had been applied to pharmacokinetic (PK) and pharmacodynamic (PD) studies widely. According to a great many studies, it has been proved that IC-MD can offer a means of continuous drug sampling and have ability to monitor the drug distribution and metabolism in animals for brain PK study (Tsai, 2003). Special emphasis is made to describe that using multiple MD in blood and brain of conscious animals, drug concentrations in blood and brain ECF can be determined

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simultaneously (de Lang *et al.*, 1997). Compared with concentrations of two compartments, PK parameters and blood-brain barrier (BBB) penetration can be elucidated. On the other hand, with the IC-MD and analysis techniques, both drug and neurotransmitters levels in brain ECF can be determined from dialysates for PD study (Boschi and Scherrmann, 2000; Plock and Kloft, 2005; Li *et al.*, 2006). That allows study drug effect responded to the changes of neurotransmitters levels and the concentration-effect profile of drug can be illustrated. However, either PK or PD studies only presents drug time-course profile or concentration-effect profile, respectively. Therefore, it is necessary for scientists to use IC-MD for B-PK-PD study (IC-MD/ B-PK-PD) to investigate the drug effect along with the concentration changes in time course.

Since the conception of "effect compartment" was presumed, B-PK-PD model had attracted scientists' interests and evolved a series of researches, aiming at the prediction of the time course of drug effects *in vivo*. From the current studies, a novel mechanisms-based B-PK-PD model concept (Breimer and Danhof, 1997) has been well accepted. B-PK-PD study is used to identify the properties of drug in brain, allow the measurement of the time course of drug effects under normal and pathophysiological conditions, and provide scientific evidences for optimal dose regiment. IC-MD/B-PK-PD model applications in pharmaceutical study have undergone significant changes over more than twenty years. IC-MD/B-PK-PD model is a mathematical and incorporated model to connect the concentration-time profile of drug with drug effect together, providing an assessment of conclusions in outcomes interest of such as the drug effect concentration to achieve a certain efficacy profile. The applications have shifted from descriptive models to probabilistic models, which is an integration of numerous data with complex functional relations from multiple compounds and scaling multiple endpoints.

Furthermore, with the rapid growth of analysis techniques, such as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), ultra-violet spectroscopy (UV) and mass spectrometry (MS), on-line IC-MD couple to analytical system (Khan and Shuaib, 2001; Dash and Elmquist, 2003) are much better to facilitate IC-MD/PK-PD study efficiently.

The review will introduce the principle and characteristics of IC-MD, the advantages and limitations of methods for in vivo recovery determination, with special attention paid to IC-MDP, and focus on the applications of IC-MD/ B-PK-PD study.

INTRACEREBRAL MICRODIALYSIS

Principle of IC-MD

IC-MD is a novel and popular technique for studying a wide range of substances, acrossing a semi-permeable IC-MDP membrane, and getting dialysates continuously from the ECF of the conscious animal's brain to determinate the concentrations of drugs and their metabolites, and to monitor the variational levels of neurotransmitters relatively (Khan and Shuaib, 2001). The dialysate can be gained on real time through IC-MDP, which is inserted into certain tissue of brain.

The principle of IC-MD can be elucidated in terms of Fick's first law of diffusion (Plock and Kloft, 2005), in other words, that is the passive transport of endogenous and exogenous substances across the membrane due to the concentration gradient between the ECF and the perfusion fluid within the IC-MDP lumen (See Fig. 1).

On the basis of dialysis principle of a semipermeable membrane, IC-MDP plays action as an artifical blood vessel, which was the first described by Ungerstedt (1991). The dialysis membrane in IC-MDP acts like a filter in charge of the passport of running various substances in the ECF.

Characteristics of IC-MDP

IC-MD system consists of IC-MDP, microinjection pump, sample-collected microvial, and stereotaxis instrument. Specially, IC-MDP is the core assembly unit in the IC-MD system. The original appearance of IC-MDP was traced back to 1972. The first IC-MDP called dialytrode used for

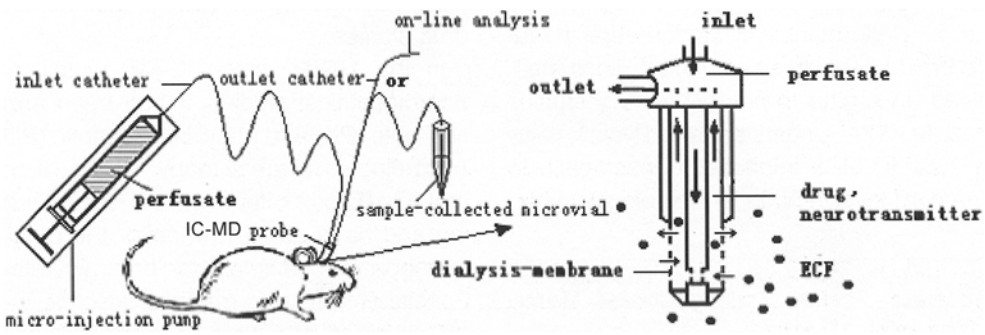

Fig. 1. The principle of IC-MD

long-term intracerebral perfusion in caudate nucleus and amygdala of the awake monkeys. In 1974, the linear IC-MDP was described. It is composed of a single length of dialysis tubing, which stretched from one side of the head to the other. It can be used for investigation of chemical activity in brain tissue by contacting with the membrane but was not useful for differentiating the activity of individual brain regions (Plock and Kloft, 2005). In the earlier 1980's, I-shaped and U-shaped IC-MDP came up subsequently (Davies *et al.*, 2000; Horn and Engelmann, 2001; Orlowska-Majdak, 2004). I-shaped IC-MDP consists of an inlet cannula and an outlet cannula side by side, and can be used for implantation into certain tissue of brain. While, the U-shaped IC-MDP consists of two cannulas serving as inlet and outlet with a dialysis tube at the ends of them. The cannulas are aligned side by side so that the dialysis tubing forms a loop appearance. The U-shaped IC-MDP is easy for researchers to be insert into specific brain tissues, such as prefrontal cortex, hypothalamus, hippocampus, cerebellum, and occipital cortex. But it still caused more tissue damage than desirable (Swanson *et al.*, 2006). In 1983, IC-MDP was constructed progressively to be more sophisticated and variegated. The concentric IC-MDP consists of a thin dialysis tube with an inner diameter and a semipermeable membrane at the tip of the probe. It has great advantages of less tissue damage and great accuracy concerning placement of target tissue in brain.

In conclusion, a variety of IC-MDPs in brain can be divided into horizontal probes and vertical probes, and each type of IC-MDPs has own features (See Table I). On the basis of different features of IC-MDPs, optimal IC-MDP can be choose to use in experiments in order to elevate the accuracy of experimental data. Moreover, IC-MDP is selected in consideration of the specific molecular weight and material of each type probe, as well the surgical accessibility and the anatomical feature of certain tissue of brain, in accordance to different experimental goals.

However, there are special IC-MDPs manufactured by investigators in laboratory themselves (Horn and Engelmann, 2001). Landgraf R. *et al.* made a special triple-MDP, with a infusing stainless steel cannula glued to the two dialysis cannula of U-shaped IC-MDP (Landgraf *et al.*, 1992). Obrenovitch *et al.* constructed IC-MDP with miniature electrode inserted in it, allowing the measurement of electroencephalogram (EEG) along with the dialysis samples (Obrenovitch *et al.*, 1993).

Apart from the IC-MDP applications to a single tissue in brain, the multiple-MDPs can be conducted in the brain of a single animal simultaneously. In 1986, Hernandez *et al.* first constructed a miniature and concentric IC-MDP, which was constructed from 36 gauge stainless steel tubing inside of 26 gauge tubing, with a cellulose hollow fiber tip 0.2 mm in diameter and 2 mm long, in order to investigate multiple IC-MD experiment simultaneously in rats (Hernandez *et al.*, 1986). In 1989, Hernandez L. *et al.* implanted three removable IC-MDPs into prefrontal cortex, nucleus accumbens and striatum in the same rat successfully to measure the change levels of DA induced by haloperidol (Hernandez and Hoebel, 1989). Recently, the implantation of multiple probes into striatum and nucleus accumbens simultaneously to monitor DA levels (D'Souza and Duvauchelle, 2006; Netigh *et al.*, 2004). Specially, the dual-MDPs system is a common tool to pharmacology study (Benturquia *et al.*, 2004). It consists of two concentric IC-MDPs, which were glued together by a piece of 26 gauge stainless steel tube, with an interprobe distance of 0.5 mm. Our lab (Li *et al.*, 2007) used dual-MDPs to get dialysates from olfactory bulb and cerebellar nucleus successfully, in order to study the delivery of ^{125}I-cobrotoxin after intranasal administration to brain in freely moving rats.

Advantages and limitations of IC-MD

IC-MD has a number of advantages for sampling dialysates (Peerdeman *et al.*, 2000; Boschi and Scherrmann, 2000; Plock and Kloft, 2005; de Lang *et al.*, 1997; Li *et al.*, 2006; Horn and Engelmann, 2001) (See Table II).

However, there are still some problems need to be solved in the applications of IC-MD. The main limitations of this technique are showed as following: (1) Invasive nature of implantation of the IC-MDP may cause the reaction in brain, and gliosis influence on BBB function. (2)

Table I. Comparison of selected parameters of IC-MDPs

Parameter	Probe type			
	Horizontal probe	Vertical probes		
	Transcerebral probe	I-shaped probe	U-shaped probe	Concentric prove
Destruction of tissue	High	High	High	Low
Recovery	High	High	High	Low
Preferred brain areas	Transcerebral, but can not fix position to individual brain regions	Structures of low volum	Structrures of low volum, structures near the base of the brain	Structures of high volum,pronounced three dimensional structrures

Table II. The characteristics and advantages of IC-MD

Characteristics	Advantages
In vivo	The experimental sampling can be gained from the conscious animal, which provides the steady physiological state
Real time	Long-term and continue sampling can be gained with no net change in fluid volum, called "high temporal resolution"
High efficiency	The dialysate can be sampled automatically and the number of experimental animals can be reduced
On-line	The technique can be amenable to analysis directly coupled with analysis technique on line
Filtering	The dialysis principle can provide protein-free samples, which can avoid the clean-up procedures and eliminating the potential enzymatic degradations
Multiple probes implantation	Multiple site samplings can be performed in different tissues of brain simultaneously, allowing the reduction of the number of animal used for preclinical study
Multiple drug administration routes	The technique can allow drug administration via different routes
PK study	The ability to monitor the unbound drug concentration in both brain and blood simultaneously in a single animal, apart from MD in blood or other tissue
PD study	The levels of exogenous and endogenous substances can be monitored induced by drug administration for PD study, as an active signs during the drug delivery process

Calibration is necessary for assessment of recovery, as recovery does not reach 100%. (3) The requirement of sensitive analytical methods to detect small concentrations with small volumes because of diluting effect of dialysis. (4) Size limitation for molecules to be determined. (5) The highly lipophilic drugs stick to tubing and probe components, and the functional molecular weight will cut off, complicating the relationship between dialysate and extracellular concentrations, so IC-MD sampling may not be suitable for all potential analytes. (6) Determination of mean concentrations over a time interval. (7) The chronic IC-MD with permanently implanted probes may be limited by severe technical problems and tissue changes.

Recovery and calibration methods

IC-MDP acts as a generic collector that provides samples that reflect the ECF concentrations of compounds. However, the concentration in the dialysate never (except in the no-net-flux situation) exactly represents the concentration in the periprobe fluid, as the IC-MD is not typically carried out at equilibrium conditions. Therefore, the analyte concentration in the dialysate will be only a fraction of the actual concentration in the surrounding ECF. The ratio between the analyte concentration in the dialysate and that in the ECF is called relative recovery.

In order to determine the true concentrations in vivo, the IC-MDP has to be calibrated before reaching conclusions about concentrations in the ECF. There are several approaches have been proposed for quantifying microdialysis. The advantages and limitations of calibration methods for determination of relative recovery in vivo are showed in Table III (Jacobson *et al.*, 1985; Lonnroth *et al.*, 1987; Stahl *et al.*, 2002; Larsson, 1991).

IC-MD/B-PK-PD STUDY

Description of IC-MD/B-PK-PD model

IC-MD provides a good opportunity to estimate B-PK-PD parameters simultaneously in vivo in a single animal. IC-MD/B-PK-PD is frequently used to determine the concentrations of drug and monitor the change levels of drug-induced neurotransmitters as PD markers in brain ECF of drug active sites where MDP is implanted, whilst getting blood samples to determine the drug concentration by blood MD (Zhou and Gallo, 2005). As a result, compared with concentration-time profile of drug between in the blood and brain ECF, PK parameters can be obtained. Meanwhile, PD parameters can be showed in the form of concentration-effect profile on account of the measurements of drug concentrations and corresponding neurotransmitters. Therefore, the drug concentration-time-effect relationship will be elucidated in B-PK-PD model. It is clearly exhibited an improvement of both the accuracy and quality of data by the minification of inter-animal variation using a single animal.

For the appraisal of drug action, IC-MD/B-PK-PD model can provide information in drug development (Breimer and Danhof, 1997). Firstly, drug effect on the basis of the mechanisms of drug effect can be confirmed, and a strategy in assessment of novel drug candidates for certain drug effect in CNS is offered. Triple-MDP applied for fast B-PK-PD evaluation of dopaminetic activity of two novel neurosearch drug candidates (NS-A, NS-B) was described by Weikop *et al.*. In contrast to PK-PD characteristics

of cocaine and methylphenidate, the result was showed that the rate of NS-A drug transport into the BBB was slow and the mechanism of multi-drug resistance was complex, while the NS-B drug exemplified a fast kinetics of drug transport into brain and a delayed effect phenomenon on extracelluar DA. The two candidates drugs presented different PK profiles and similar PD profiles to the methylphenidate (Weikop *et al.*, 2004). Secondly, hysteresis phenomenon that effect versus concentration profile exhibited a dramatic hysteresis can be explained. Chenel *et al.* investigated the distribution of norfloxacin in CNS and the phenomenon of delayed EEG effect in rats after a convulsant dose of norfloxacin (Chenel *et al.*, 2004). As a result of PK-PD parameters in both plasma and brain, the reason for delayed norfloxacin EEG effect is not due to the slow drug distribution to brain, but also the restriction of drug distribution at the effect site. Moreover, the relationship among the dose, concentration and drug effect can be described. It is benefit for prediction and optimization of effective dose regiment of drug in preclinical study. For instance, phenserine is a new potent and highly selective acetylcholinesterase (AChE) inhibitor for the treatment of Alzheimer's disease (AD). It was certified that phenserine had long duration of action coupled with its short PK half-life, produced a long-lasting stimulation of brain cholinergic function at well tolerated doses, and selectively inhibited AChE, while potential butyrylcholinesterase (BChE) side effects was minimized (Greig *et al.*, 2000).

Advantages of IC-MD/B-PK-PD model

As far, there have been several sampling techniques for preclinical B-PK-PD study, such as position emission to-

Table III. The advantages and limitations of calibration methods

Methods	Advantages	Limitations
Flow rate method	The calibration is accomplished by varying the perfusate flow fate	A long sampling time for small flow rates; poor temporal resolution
No-net-flux method	No assumptions on periprobe behavior of the drug have to be made in steady-state measurements; independence from mass transfer in the tissue	Time consuming; being inadequate for monitoring concentration in ECF and probe recovery as a function of time
Dynamic no-net-flux method	The measurements can be allowed under transient conditions	A large number of study individuals are required
Retrodialysis method	The method combines the use of the analyte itself for relative recovery determination (retrodialysis by drug) with the use of an internal standard (retrodialysis by calibrator) to monitor probe recovery; being applicable for clinical studies	This method requires that the internal standard should match the characteristics of the analyte

Table IV. Comparison of IC-MD with other techniques for B-PK-PD study

	IC-MD	PET	MRS
Analytes	Any	Positron emitting isotope (^{18}F, ^{11}C, ^{13}N, ^{15}O)	NMR-active nuclei (^{19}F, ^{13}C, ^{15}N, ^{1}H, ^{31}P)
Invasiveness	Mini-invasive	Non-invasive	Non-invasive
In vivo	Yes	Yes	Yes
Monitored compartment	Extracellular fluid of tissue concentration (Unbound)	Total tissue concentration (Bound and unbound)	Total tissue concentration (Unbound)
Multiple sites	Yes	Yes	Yes
Technical complexity	Low	High	High
Continuous monitoring	Yes	Yes (Duration restricted by physical half-life of employed radioisotope)	Yes
Time resolution	High	High	Poor
Spatial resolution	High (Focal sampling)	Moderate (1-5 mm)	Poor (>10 cm)
Sensitivity	High (10^{-9}-10^{-3} mol/L)	High (10^{-12} mol/L)	Poor (10^{-5}-10^{-3} mol/L)
Selectivity	High	High	High
On-line	Yes	Yes	Yes
Cost	Low	High	High

mography (PET) and MR spectroscopy (MRS). Comparison of IC-MD and other techniques, it is suggested that the IC-MD has advantages over other techniques, such as excellent temporal and spatial resolution, high selectivity in determination the analyte of interest without interference from metabolites, and serial continuous samplings in a single animal in steady state (de Lang *et al.*, 1997; Brunner and Langer, 2006). Thus, it is concluded that the IC-MD is a more practical and low-cost technique for B-PK-PD (See Table IV).

In summary, the most advantage of IC-MD/B-PK-PD model is reduction of the quantity of animals. That not only minimizes the individual difference between animals, but also elevates degree of precision and accuracy of experimental data.

APPLICATIONS OF IC-MD/B-PK-PD STUDY

Analgesic drug

The efficiency of IC-MD allows conduct experiments in a large quantity of samples for B-PK-PD study related to the analgesic drug in the CNS. An example of investigating B-PK-PD study of cocaine was given (Hedava and Pan, 1997). The model in this study permitted measure the concentrations of cocaine and its metabolites in both plasma and brain ECF of awake freely moving rats and monitor the neurochemical response involved the changes in DA level. It showed that the *AUC* (area under the plasma concentration time curve) ratios in brain ECF and plasma of cocaine, norcocaine and benzoylecgonine were 1.2, 0.33, 0.13, respectively. The rapid penetrations into brain of cocaine and its metabolites were monitored. Meanwhile, a linear concentration-effect profile in the nucleus accumbens was measured. It was showed that the DA reuptake process was inhibited completely when the concentrate of cocaine approached at a very high state. Therefore, this study provided a powerful evidence for IC-MD/B-PK-PD study.

As for morphine, a published report researched it in a great extent. The object of experiment was to interpret the phenomenon of the delay in antinociceptive effect of morphine in rats. Combined with the unbound concentration of morphine in blood and brain ECF, the ratios of unbound *AUC* between brain and venous blood were 0.22 and 0.28, for the 10 and 40 mg/kg dose, respectively. The difference indicated an active efflux of morphine across the BBB. The half-life of morphine in brain was 32 min longer than in blood. This demonstrated that a rate-limited elimination of morphine in brain by redistribution of the drug from brain tissue rather than being controlled by the drug PK in blood. The concentration-effect relationship exhibited a hysterisis with an effect delay half-life of 5 min at CNS level can be speculated that either rate-limited mechanisms at receptor level or the further distribution in the brain. This article confirmed that determination of the unbound concentrations of morphine in blood and brain by in vivo MD had a capability to establish B-PK-PD model to elucidate the origin of the effect delay (Bouw *et al.*, 2000). Another reported article at present applied the IC-MD-HPLC coupled to an electrochemical detector to study the B-PK/PD correlations of morphine in the rat, whilst the EEG was recorded continuously during the experiment (Groenendaal *et al.*, 2005). The time-course of the drug change in amplitude of the delta-frequency band (0.5-4.5Hz) of the EEG as PD endpoint after drug administration can be investigated. Combination of both the concentration-time and the effect-time relationship revealed a complex concentration-effect relationship, and used for quantitative analysis o-phthalaldehyde (OP_3) receptor mediated responses in vivo.

For another analgesic drug, a case of a delayed drug effect analysis about Morphine-6-glucuronide (M6G) where IC-MD/B-PK-PD model was set up was reported by Bouw *et al.* (2001). The rate and extent of BBB equilibration of M6G were determined, and the contribution of BBB transport to the antinociceptive effect delay of M6G by IC-MD was quantified. With the implantations of MDPs in striatum and arterial blood sampling, the determinate result of the unbound concentration ratio of M6G in brain ECF to blood was 0.22, as a BBB equilibration, indicating the presence of active efflux mechanism in the BBB transport of M6G, and the transport of M6G was at a slower rate. An effect delay half-life of 53 min with respect to brain ECF concentrations exhibited a clear hysteresis of concentration-antinociceptive effect relationship. This delay was likely due to a combination of drug distribution in brain tissue and PD study in relation to the limited rate mechanisms at the receptor level.

Antiepileptic drug

A series of IC-MD/B-PK-PD studies for antiepileptic drug carried out in animals. As we known, nitric oxide (NO) has been shown to be involved in seizure activity process, and make effect in CNS for epilepsy treatment by modulation of nitric oxide synthase (NOS). The scientists assessed the effect of 7-nitroindazole (7-NI) to understand the relationship between NOS activity in the CNS and the concentrations in serum and brain tissue (Bush and Pollack, 2001). NO was converted predominately to NO_3^- and NO_2^- (NOx^-), due to the extremely short half-life in vivo of NO. Therefore, NOS activity was assessed by quantitating NOx^- in the dialysate with a modification (Feelisch, 1993), by the means of implanting probe in the hippocampus of brain. The ratio of 7-NI concentrations in hippocampal ECF to the unbound concentrations in serum was 0.92 ± 0.05. It was indicated that the 7-NI distributed

rapidly passively across the BBB. Meanwhile, PD parameters presented that NOx^- concentration decreased during administration. The relationship between NOx^- concentration in hippocampus and 7-NI concentration was clearly sigmoidal with a maximum in NOx^- concentration of 54%, and the 7-NI has ability of inhibiting NOS activity with an apparent IC_{50} (inhibitory concentration, 50%) of 17 ug/mL. The IC_{50} data was helpful to the determination of 7-NI dosing regiments to maintain an optimal degree of NOS inhibition.

Several papers have confirmed that the sodium valproate (VPA) has obvious PK interactions with some antiepileptic drugs, increases the drug concentration in plasma, and enhances antiseizure activity (Cuadrado *et al.*, 2003; Cunningham and Jones, 2000). The researchers investigated lamotrigine (LTG), VPA and the two drugs combined concentrations and their effects on the basal and changed levels of extracellular glutamate (GLU), aspartate (ASP), taurine (TAU), 5-hydroxytryptamine (5-HT) and DA resulted from drug release (Ahmad *et al.*, 2005). With IC-MDP inserted into the hippocampus of freely moving rats, it was found that LTG (10 mg/kg) given alone did not alter basal levels of GTU, ASP or TAU significantly, neither did VPA (300 mg/kg). But when given together, the levels of extracellular GLU and ASP were descended, while the level of TAV was rise significantly. In additional, 5-OH was dramatically prolonged increased and DA got to an increase degree when given LTG alone, which cannot evoke by two drugs together. Moreover, these extracellular neurotransmitters did contributions to the efficacy of combined drugs in the treatment of a range of seizure types. Under the B-PK-PD study, probable PK and PD interaction of the two drugs were certified. With a generally similar model, Clinckers *et al.* (2005a) detected that the variation of DA and 5-HT levels, as PD markers, were useful for the selection of anticonvulsant threshold concentrations of oxcarbazepine and 10,11-dihydro-10-hydroxycarbamazepine.

An integrated IC-MD/B-PK-PD study coupled with EEG was studied (Cleton *et al.*, 2000). With regard extracellular GABA concentration as PD marker, the influence of amygdala kindling on PD of tiagabine was determined. Tiagabine produced an increase in the amplitude of the 11.5-30 Hz frequency band of EEG. The relationship between drug and EEG, and the relationship between drug and GABA concentration were non-linear. The shift in concentration-EEG effect relationship to lower concentration was due to the increase of baseline GABA level.

In additional, IC-MD/B-PK-PD model used to explain drug effect on the basis of mechanisms of pharmacology. Clinckers *et al.* used IC-MD to study the influence of multidrug transporters on the BBB passage of oxcarbazepine, concomitant used hippocampal monoamines as PD markers for the anticonvulsant activity (Clinckers *et al.*, 2005b). The experimental data exhibited that oxcarbazepine was a substrate for multidrug transporters at the BBB, and exerted effect associated with the increase of 5-HT and DA levels in ECF.

Antidepressant drug

Since IC-MD allows the research for active drug concentrations and corresponding neurotransmitters at the effect site in brain, a consequent study to B-PK-PD model is employed to antidepressant drug study. For example, IC-MD can be used to get dialysates of 5-HT, 5-HIAA, noradrenaline (NA) by implanting IC-MDP in the frontal neocortex. With this technique, the PK and PD features of venlafaxine (VEN) were studied by Wikell *et al.* (2001a, 2001b, 2000). The VEN concentration in brain parenchyma was three times higher than that in the serum. The levels of 5-OH and NA were increased, while 5-HIAA in brain dialysate was decreased along with subcutaneous injection administration of VEN. With generally similar pattern of experiment in rats, the PK and PD features of citalopram have been described in past studies (Apelqvist *et al.*, 2000; Berqqvist *et al.*, 1997). By the virtue of widespread applications of IC-MD, it was permitted that a much better understanding of PK and PD characteristics of antidepressant drugs can be achieved. According to the previous studies, the CNS drug such as tranylcypromine (Ferrer and Artiqas, 1994), imipramine (Ichikawa *et al.*, 1998), YM992 (Mano *et al.*, 2002) and TC-1734 (Gatto *et al.*, 2004) have been characterized with PK and PD parameters and demonstrated their effects of drug actions.

Recently, B-PK-PD model in ovariectomized (OVX) rats was conductedto describe PK and PD properties of desvenlafaxine succinate (DVS), which was serotonin (5-HT) and norepinephrine (NE) reuptake inhibitor (SNRI) (Alfinito *et al.*, 2006). The *Cmax* (max concentration) of desvenlafaxine in plasma, brain (total brain minus hypothalamus) and hypothalamus were 0.007, 0.0108 and 0.0095 mol/L, respectively. The terminal half-lives of desvenlafaxine in plasma, brain and hypothalamus were 3.0, 2.1 and 2.5 h, respectively. The concentrations of 5-HT and NE in dialysates were increased by drug administration. These data revealed that DVS had a rapid brain penetration in an excellent extent and selectively increased 5-HT and NE levels in the hypothalamus for mitigation of depressive disorder symptoms. Similarly, duloxetine which was a balanced reuptake inhibitor of 5-OH and NE was confirmed a good candidate of antidepressant drug (Wong, 1998; Bymaster *et al.*, 2005). Bundgaard *et al.* assessed the applicability of IC-MD in conjunction with automated serial blood sampling for simultaneous PK and PD characterizations of the escitalopram (Bundgaard *et al.*, 2006). The temporal B-PK-PD correlations indicated that the

catheterization in blood and the production of 5-HT in hippocampus did not influence the penetration rate and exposure of escitalopram in brain ECF, and the escitalopram had a high volume of distribution in brain. Combined with PK parameters and the drug effect, adaptive regulations of the serotonergic system following acute selected 5-HT reuptake inhibitor treatment can be investigated.

CONCLUSIONS

IC-MD is a powerful and versatile tool, which is applied in free moving conscious animals, for examination of in vivo drug disposition in brain ECF and acquirement of information referring to pertinent drug time-concentration profiles. The applicability of IC-MD/B-PK-PD is certificated for characterization of multiple relationships between drug concentration and neurotransmitters, and inferred the concentration-time-effect profile, also can minimize inter-animal variation using a reduced number of animals. The integrated model of IC-MD in conjunction with blood sampling is suitable for measurement time-concentration of drug to exhibit the clearance and half-life in brain and plasma (Bickel, 2005). The parallel decline of unbound drug concentration in brain and plasma elucidates exchange and equilibration between these two compartments. Besides, the IC-MD is amenable to facilitate the simultaneous multiple MDP implantation in a single animal to examine the pharmacological agents and effects on the change levels of endogenous substances induced by drug. The local levels of endogenous substances in certain tissue of brain, such as striatum, hippocampus, nucleus accumbens, can be showed in the drug concentration-effect profile, as critical PD markers.

Recently, a classic model called the mechanism-base B-PK-PD model is used to provide necessary information for prediction and optimization of dose regiment of CNS drug in preclinical study (de Lang *et al.*, 2005; Clinckers *et al.*, 2005). Furthermore, the relevant data of B-PK-PD parameters can be as references to evaluate the effect of drug candidates in preclinical (Danhof *et al.*, 2005). In another case, IC-MD coupled to quantitative EEG recording was confirmed to explain for the effect hysteresis phenomenon, demonstrating that the most of delayed drug EEG effect is not only caused by the drug distribution within the CNS, but also the PD parameters of blood, might be interpreted the restriction of the effect site during drug distribution, because that the BBB transport and brain distribution often do not occur instantaneously. Moreover, drug concentration-effect with the time-course profiles is used to analyze dialysates following different dose. With the repeated experiment, the threshold dose for drug effect can be presumed. Hence, the IC-MD/B-PK-PD model could be useful to design optimal dose regimens for preclinical study.

However, there are several factors affecting effectiveness of IC-MD with PK-PD study. Scientists indicated that perfusate temperature may be important in pathological conditions when periprobe tissue lost its capability to compensate temperature effects. Recovery at body temperature of 37°C is considered to be benefit for keeping animals in a good condition. Researchers used a hydraulic heating pad and pump to maintain a uniform body and perfusion fluid temperature at 37°C in the experiment procedure (Khan and Shuaib, 2001). Low recovery is often considered to be a drawback of the MD technique. When flow rates are at 0.1-0.5 μL/min, the recoveries can be reached close to 90%. The relative recovery of sampled compounds decreases when the flow rates (between 0.5 and 2 μL/min)increases, whereas the absolute recovery is constant. The perfusion fluid not only takes neurotransmitters away from the ECF, but the small molecules and ions are also removed from the brain simultaneously. Therefore, the perfusion fluids should be isotonic to ECF and contain essential ions composition to keep homeostatic balance of extracellular environment. The changed ionic concentration may cause a change release of many neurotransmitters. An artificial cerebrospinal fluid (aCSF) is the medium commonly used as the perfusion fluid in IC-MD. The abilities of substances to come across the dialysis membrane usually depend on the characteristics of membranes. Some peptides may bind to various polymers, leading to a low recovery, due to the interaction between dialyzed compounds and membrane material. Relative recovery is also closely relative to the dialysis membrane size. It is important to choose optimal membranes to elevate ralative recovery in vitro. Due to the invasiveness of implanted probes in the brain, an abnormal release of neurotransmitter and the integrity of the BBB might be disturbed. The extracellular levels of neurotransmitters will decrease gradually 3-4 days because of a glial barrier around the cannula tract. So it is important to ensure that the experimental animal is sufficiently recovered from the surgery. Generally, a recovery period for surgery of about 24 h is considered satisfactory, and IC-MD experiments are limited in time. As to the diluting effect of dialysis, it is necessary to require more sensitive analytical methods to detect small concentrations, especially connection of between IC-MD and analytical system on line.

In conclusion, IC-MD is of great value in direct evaluation of endogenous substances in multiple sites and continuous sampling in the same individual and high sensitivity in small volumes with probes. IC-MD/B-PK-PD model has been accepted and put into practice commonly in pharmaceutical study, and has contributed greatly to the understanding the relationship between drug effect and concentration changes during administration (Heinzen

and Pollack, 2004; Yassen *et al.*, 2005; Jonker *et al.*, 2005). It also can be expected to afford more samplings from more tissues in brain, exploring the mechanisms of drug concerned with neurotransmitters in more detail, and giving useful information to preclinical study. Furthermore, the synergy between IC-MD/B-PK-PD and current analysis techniques is more value to given a new perspective of drug discovery development in a high efficiency manner.

ACKNOWLEDGEMENTS

We are very grateful to Prof. Youichi ABC (Department of Pharmacology, Kagawa Medical University, Ikenobe, Kagawa, Japan) for the guidance of microdialysis technique, and also to his laboratory where the corresponding author had ever studied in. Financial support for this work is provided by the Project No. 30371781 of the National Natural Science Foundation of China.

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