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Appraisal of state-of-the-art

Applicability of reverse microdialysis in pharmacological and toxicological studies

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Abstract

A recent application of microdialysis is the introduction of a substance into the extracellular space via the microdialysis probe. The inclusion of a higher amount of a drug in the perfusate allows the drug to diffuse through the microdialysis membrane to the tissue. This technique, actually called as reverse microdialysis, not only allows the local administration of a substance but also permits the simultaneous sampling of the extracellular levels of endogenous compounds. Local effects of exogenous compounds have been studied in the central nervous system, hepatic tissue, dermis, heart and corpora luteae of experimental animals by means of reverse microdialysis. In central nervous studies, reverse microdialysis has been extensively used for the study of the effects on neurotransmission at different central nuclei of diverse pharmacological and toxicological agents, such as antidepressants, antipsychotics, antiparkinsonians, hallucinogens, drugs of abuse and experimental drugs. In the clinical setting, reverse microdialysis has been used for the study of local effects of drugs in the adipose tissue, skeletal muscle and dermis. The aim of this review is to describe the principles of the reverse microdialysis, to compare the technique with other available methods and finally to describe the applicability of reverse microdialysis in the study of drugs properties both in basic and clinical research.

Keywords: Reverse microdialysis; Delivery methods; Sampling methods; Pharmacological and toxicological agents; Basic research; Clinical applications; Neurotransmission

1. Introduction

Microdialysis sampling is an increasingly employed research method for the study of pharmacokinetic and pharmacodynamic behavior of therapeutic and toxicological agents (Höcht, Opezzo, & Taira, 2004). Traditionally, microdialysis has been used for the determination of interstitial levels of endogenous compounds and drugs. Actually, microdialysis has found important application in the field of blood pharmacokinetics, tissue distribution of drugs, pharmacokinetic–pharmacodynamic (PK–PD) modelling so much in animals as in human (Höcht et al., 2004).

A more recent application of microdialysis is the introduction of a substance into the extracellular space via the microdialysis probe (Galvan, Smith, & Wichmann, 2003). The inclusion of a higher amount of a drug in the perfusate allows the drug to diffuse through the microdialysis membrane into the tissue. This technique is actually called as reverse microdialysis (Chan & Chan, 1999) (Fig. 1).

Reverse microdialysis, based on the dialysis principle, not only allows the local administration of a substance but also permits the simultaneous sampling of the extracellular levels of endogenous compounds (Fig. 1). Therefore, reverse microdialysis serves both as an administration and sampling technique. To date, reverse microdialysis has been mainly used for the study of the effect of local drug administration in brain concentration of neurotransmitters and metabolites. However, the effect of drugs in other tissues, such as liver, dermis, corpora lutea and heart has also been studied by means of reverse microdialysis. More recently, reverse microdialysis has been introduced for the evaluation of pharmacodynamics of therapeutic agents in the clinical setting. The large body of work published (more than 300 publications) underscores the importance of the reverse microdialysis technique for the study of pharmacological and toxicological aspects of therapeutic drugs.

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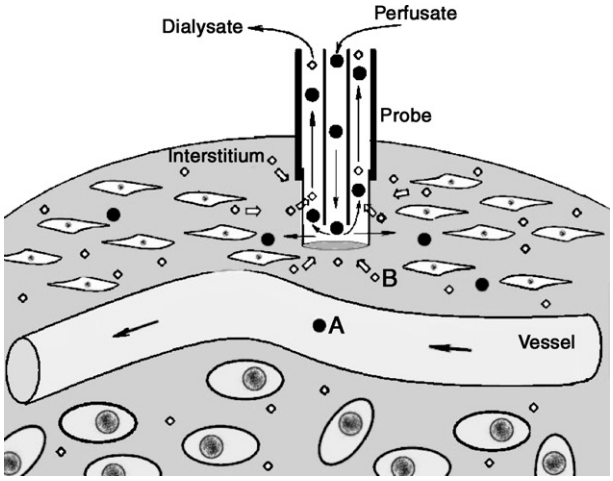

Fig. 1. Representation of the reverse microdialysis principle. A concentric microdialysis probe inserted in the extracellular space of a tissue is perfused with a solution containing the exogenous compound (black circles) to administer. The solution is pumped through the inner cannula allowing the diffusion of the exogenous compounds to the surrounding tissue. Simultaneously, endogenous compounds (white rhombus) diffuse from the extracellular space into the microdialysis probe and are collected in the dialysate for further analysis.

The aim of this review is to describe the principles of the reverse microdialysis, to compare the technique with other available methods and finally to describe the applicability of reverse microdialysis in the study of drugs properties in both basic and clinical research.

2. Principles of reverse microdialysis

In the past twenty years the microdialysis technique has become a method of choice in the study of tissue concentrations of both endogenous and exogenous substances. The microdialysis sampling technique, as we know it today emerged from the neurosciences, where it was originally used for measuring concentrations of neurotransmitters in rat brain (Ungerstedt & Pycock, 1974).

In this technique, a probe that is inserted into tissue mimics the function of a capillary blood vessel (Fig. 1). The probe has a hollow fiber that is permeable to water and small molecules, and when it is perfused with a physiologic fluid, molecules are exchanged through the dialysis membrane by diffusion in both directions in favour of a gradient of concentration. Later, dialysate samples are analyzed using highly sensitive techniques. Therefore, microdialysis samples endogenous compounds, because levels of neurotransmitters and metabolites are higher in the extracellular space than in the perfusion fluid. On the other hand, the inclusion of a higher concentration of a drug or endogenous compound in the perfusate allows the substance to diffuse through the dialysis membrane to the tissue (Höcht et al., 2004).

The microdialysis technique is not performed under equilibrium conditions because the perfusate is constantly being pumped through the probe, and therefore the concentration of the endogenous compounds in the dialysate is some fraction of that in the surrounding tissue. This fraction is called relative recovery (Plock & Kloft, 2005). Also, the concentration of the drug administered through the microdialysis probe is a fraction of the concentration of the same in the perfusate.

The basic setup for a microdialysis experiment consists of a microdialysis probe, a perfusion pump, and an analytical method with the required sensitivity to quantify small concentrations of substances in small volumes of sample (de Lange, de Boer, & Breimer, 2000).

3. Methodological considerations

3.1. Microdialysis probe

The microdialysis probe is perhaps the nucleus of the microdialysis experiment. The microdialysis probe typically consists of a tubular dialysis fiber that is connected with an inlet and an outlet tube. The inlet and outlet tubes are connected with thin and flexible tubing with a perfusion pump and with a fraction collector, respectively. It is very important that they do not interact with the perfusate or the surrounding tissue. Many laboratories have designed their own probe as its construction lasts not longer than several minutes but today there are commercial approved probes for studies in human soft tissues and brain.

In general, probes have a longitudinal, a semicircular or an I-shape design. Various designs have been described: concentric cannula probe, linear probe, shunt probe. Several modified probes designs such as: spinal loop dialysis catheter (Marsala, Malmberg, & Yaksh, 1995), flexible intravenous probe (Evrard, Cumps, & Verbeeck, 1996) shunt intraarterial microdialysis probe (Höcht, Opezzo, & Taira, 2003) has also been reported. Microdialysis probes have been described in previous reviews (Höcht et al., 2004; Plock & Kloft, 2005) and are beyond the scope of the manuscript.

3.2. Microdialysis membrane

The choice of the membrane type and size is an essential element to optimize the microdialysis probe for a particular experiment. Conventional microdialysis probes are constructed with 20 kDa molecular weight cut-off membranes enabling the measurement of small molecules such as glucose, lactate, pyruvate and glutamate. Common substances used as membrane materials are cuproamonic rayon, celluloses, polycarbonate, polyethersulfone or cuprophan (Höcht et al., 2004). Recently, a 100 kDa molecular weight cut-off microdialysis membrane has been introduced to allow detection of larger molecules such as cytokines (Hutchinson et al., 2005).

Important aspect to be considered in a reverse microdialysis experiments with regard to the dialysis membrane is the molecular weight of the compounds to be administered or sampled, the size of the microdialysis membrane and the interaction of the membrane with the perfusate or the surrounding tissue.

Despite the molecular weight cut-off of the membrane, the molar mass of the substance of interest has to be taken into consideration. As discussed previously, only substances with a