Use of microdialysis in translational pharmacological research

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Introduction

Translational research in life sciences refers to a process where scientific discoveries (e.g. drugs, medical devices) are translated into practical applications (therapies). The discoveries at “the bench”, including the experimental research on animal models of human diseases should then be translated to a clinical level, i.e. to the patient's “bedside”. One of the major reasons of low success rate in introducing new medicines and therapies in CNS pharma industry is a poor correlation between the preclinical readouts and the outcome in clinical trials (often in phase IIb and III). In fact, fewer than 1 in 10 medicines that are tested in human clinical trials will succeed.

One strategy believed to increase the translational potential of preclinical models to clinical practice is the use of common techniques for screening the defined biomarkers of the disease states and therapeutic efficacy of tested drugs. Non-invasive neuroimaging techniques including functional and pharmacological MRI (fMRI, phMRI), MRI spectroscopy and PET are the strongest candidates in this effort, mostly due to their broad establishment in clinical practice. However, the imaging techniques possess a very limited capability to monitor and quantify the surrogate markers of the disease states. In addition, imaging techniques suffer of high running costs and costs for the equipment, limitations of testing only one animal in time, a need of anaesthesia, poor spatial and temporal resolution. These facts make the imaging techniques difficult to use for rapid and quantitative screening of candidate drugs in the late discovery phase.

Microdialysis, voltammetric and biosensor techniques undoubtedly became the most widespread in vivo monitoring technologies in experimental neuropharmacology and neuroscience, for review, see [1,2,3]. However, the invasive character of these methods confines their clinical applicability mostly to neuromonitoring in intensive care, open and reconstructive surgery or to the use of microdialysis in organs such as skin or skeletal muscles. A major advantage of skin and muscle microdialysis is that monitoring can be carried-out on healthy volunteers, which makes it possible to use microdialysis as a truly translational technique for evaluation of drug effects from animal to human - for vascular microdosing and phase I trials.
Methods

The animal microdialysis experiments were carried out on anaesthetized mice, rats or guinea pigs. Briefly, the animals were anaesthetized with isoflurane using a Univentor 400 anesthesia unit (AgnThos, Lidingö, Sweden). The body temperature of the animal was controlled by a thermometer and a heating pad maintained at 37°C. One to two skin microdialysis probes were inserted into the dermis of the abdominal or upper neck skin of each animal. The distance between the probes was 5 mm. One probe (OP-100-4, membrane length: 4 mm, diameter: 0.26 mm, Eicom Corporation, Japan) was used for sampling histamine, amino acids, lactate, glucose, glycerol and other small molecules. The second probe (3000 kDA, 5 mm length, Microbiotech, Stockholm, Sweden) was used for sampling large molecules including neuropeptides and cytokines. In a similar way, the experiments were performed in the gastrocnemius muscles of anaesthetized rats and mice. Human microdialysis experiments were carried out on healthy volunteers using CMA 66 microdialysis catheters (CMA Microdialysis, Stockholm, Sweden) or custom-made Microbiotech linear catheters. Concentrations of histamine and amino acids were determined by HPLC methods [1,2,3], lactate, pyruvate, glycerol and glucose were determined by enzymatic assays and using CMA 600 Microdialysis Analyser, the levels of neuropeptides and cytokines were determined by commercially available ELISA kits.

Results and Discussion

Clinical microdialysis is a proven technique for monitoring local chemistry of an organ including brain at conditions associated with traumatic injury, hypoxia, ischemia and related pathological states. Less common is the use of microdialysis in clinical pharmacological studies, which are mostly limited to pharmacokinetic studies in human skin. In some countries including Sweden, it is ethically precluded to perform therapeutic interventions on unconscious patients in neurointensive care. In spite of this, we could participate with analytical services, in a first ever clinical phase II study using brain microdialysis on TBI patients ongoing pharmacological treatment with a substance aiming to reduce the brain damage and improve the functional outcome. On the other hand, a low translational validity of various animal models of stroke and TBI is still a major challenge, a typical example is the case of the neuroprotective drug NXY-059 [4].

The purpose of the current effort is to use skin and/or muscle microdialysis, which is equally applicable both on healthy animals and humans for monitoring effects of locally administered test compounds on the biomarker molecules, which might be associated with common PNS and CNS pathways. Using the ultrasensitive HPLC methods based on intramolecular excimer-forming fluorescence derivatization [for review, see 5], we have determined some typical representatives of such molecules including neurotransmitters 5-HT [6], noradrenaline [7,8], whereas other sensitive techniques were used for determination of acetylcholine [unpublished data], histamine [9], markers of energy and metabolic state [10] and of changed blood flow [11] following local infusion or topical application of test compounds. Similarly, we and others have
used skin microdialysis for sampling and determination of inflammation and pain responding molecules including neuropeptides, cytokines and PGE$_2$ \cite{12}.

It is concluded that skin microdialysis offers an effective means in translational pharmacological research to evaluate the effects of the test compounds on peripheral neurotransmission and vascular blood flow in relation to local inflammatory and nociceptive stimuli.

References