MINIREVIEW: UTILIZATION OF IN VIVO ULTRAFILTRATION IN BIOMEDICAL RESEARCH AND CLINICAL APPLICATIONS

ABSTRACT

Ultrafiltration (UF) is a filtrate selection method with a wide range of biomedical and clinical applications, including detoxification of blood in hemodialysis and peritoneal dialysis. New is, however, the use of UF as a convenient in vivo sampling method that, for example, has been used in diabetics. Ultrafiltration avoids complicated and time-consuming recovery calculations that are necessary when using in vivo microdialysis, as recoveries of low molecular weight molecules are near 100%. The subcutaneously or intravenously placed UF probes have been studied for off-line sample analysis and for continuous on-line monitoring, in a wide variety of species, including dogs, rats, pigs and humans.

This review discusses the potential of in vivo UF as a continuous tissue sampling technique in clinical research areas, and in several major biomedical applications including glucose and lactate monitoring and drug kinetic studies.
INTRODUCTION AND SCOPE

Ultrafiltration (UF) is used for the separation or purification of chemicals. UF has a wide variety of applications, from the mere separation of chemicals in the laboratory setting to the detoxification of blood in hemodialysis and peritoneal dialysis in case of kidney failure (Hynes-Gay and Rankin, 2000; Ronco and Clark, 2001). New is, however, the use of in vivo UF as a convenient multiple sampling method.

In 1987 the first study was published on in vivo UF as a sampling technique alternative to microdialysis (Janle-Swain et al., 1987). Already in that article, its potential as a clinical device and a research tool was recognized. It was suggested that UF could be used in hospitalized patients in whom frequent biochemical parameters have to be monitored, without the necessity of repeated blood sampling (Janle-Swain et al., 1987). In the first pioneering studies, in vivo UF was used for sampling in awake, unrestrained animals such as dogs, but its use has been broadened since to rats, mice, rabbits, sheep, pigs, horses and humans. Thus far, most in vivo UF experiments are performed subcutaneously, but intravenous measurements and the ultrafiltration of saliva and bone have also been investigated. About 30 scientific papers on the use of in vivo UF as a sampling technique have appeared.

Recently another mini review on membrane sampling (microdialysis (MD) and ultrafiltration) from biological tissues appeared (Garrison et al., 2002), but that review mainly focussed on microdialysis in pharmacokinetics and pharmacodynamics. The present overview focuses upon the biomedical applications of in vivo UF and gives suggestions for clinical applications of UF, for example to monitor metabolism. First, we explain the principles of UF and outline the similarities and differences between MD and UF, discussing the advantages and disadvantages of UF as compared to MD. We will discuss the biomedical applications of in vivo UF on the basis of several UF studies, including subcutaneous and intravenous UF for monitoring glucose and lactate. Finally, combinations with analytical devices for off-line sampling and on-line analysis are proposed.

PRINCIPLES OF ULTRAFILTRATION

In vivo UF collects a filtrate of body fluids by applying negative pressure as a driving force. The molecular cut-off value of the semipermeable filtration membrane determines the maximum size of particles that can pass through the membrane. Also the configuration and charge of a molecule affects the membrane passing: for example, some small highly charged molecules such as gentamicin do not cross a polyacrylonitrile membrane. There are several materials that can be used as capillary semipermeable membranes, for example regenerated cellulose, polycarbonate-ether, and polyacrylonitrile (Hsiao et al., 1990). Mostly membranes with a molecular cut-off value between 20 kD and 50 kD are used. By thus excluding larger particles like large proteins and cellular elements, the ultrafiltrated sample can be directly analyzed (Deterding et al., 1992; Paez and Hernandez, 1997).

The rate of fluid collection is determined by the amount of negative pressure applied, the membrane surface area and the hydraulic resistance, which is composed of the UF membrane type, the protein layers on the membrane surface, the surrounding tissue and the capillary walls near the UF probe. Membranes with high molecular weight cut-offs have
lower resistance and thus allow higher flow rates. The flow-rate in subcutaneous UF probes ranges between 0.05 to 10 µl/min (0.07 to 14 ml/day), the outer surface area of current probes lies between 2 to 15 mm².

Frequent subcutaneous sampling of ultrafiltrate depends on the rapid replacement of interstitial fluid by the blood vessels. In tissue with limited blood flow rate and low replenishment of interstitial fluid only low sampling rates are possible. There are species differences in the applicability of UF, for example, the rat subcutaneous space allows better UF-sampling than human subcutaneous space (unpublished results). The only tissue unsuited for UF is the (rat) brain; the extracellular space is too small and the influx of blood plasma filtrate too slow for sampling (even at very low flow rates of 50 nl/min (unpublished results)). Most UF studies have been performed in subcutaneous tissue (Janle-Swain et al., 1987; Ash et al., 1992; Linhares and Kissinger, 1992; Ash et al., 1993; Linhares and Kissinger, 1993a; Linhares and Kissinger, 1993b; Moscone et al., 1996; Schneiderheinze and Hogan BL, 1996; Kaptein et al., 1997; Kaptein et al., 1998; Tiessen et al., 1999), but UF sampling has also been performed in bone and muscle (Spehar AM et al., 1998; Janle et al., 2001), saliva (Linhares and Kissinger, 1992) and blood (Kaptein et al., 1997; Tiessen et al., 2001; Leegsma-Vogt et al., 2001; Savenije B et al., 2002).

Two types of UF probes have been developed (see figure 6-1), of which one is commercially available (figure 6-1a) (Bioanalytical Systems, UF probe). The two types of UF probes have distinct characteristics and sample different environments when placed subcutaneously: the amount of negative pressure applied (low versus high) determines if subcutaneous tissue or plasma water is collected.

The commercial probe consists of one or more loops of hollow, semi-permeable polycrylonitrile (PAN) fibers (300 micron diameter, 30 kD cutoff, effective surface area about 15 mm²) that are joined to a single non-permeable conducting tube (Janle-Swain et al., 1987) (figure 6-1a). The UF probe comes with or without a porous cuff for tissue ingrowth for longer duration placements up to six months. The filtrate passes through a “Y” connector with one limb leading to a sampling port, and to a negative pressure source that drives the UF process (Janle-Swain et al., 1987). By using a relatively high negative pressure (fast UF), fluid and dissolved metabolites are directly pulled from the capillaries to the ultrafiltrate membrane and thus, the ultrafiltrate closely resembles the plasma water concentration of blood.

These commercially available UF probes have been used for off-line analysis of discrete UF samples from a variety of tissues. In the subcutaneous space the typical filtration rate obtained by the three-fiber probe under high negative pressure is between 50 and 100 microliters per hour (UF-3-12, 3 fiber, 12 cm probe). Two drawbacks of the relatively large size of the UF probe are that the probe has to be implanted through a 14 gauge cannula with (local) anesthesia, which can cause some tissue damage during implantation, and, that it is not suited for intravenous UF, possibly except in the larger blood vessels.

Moscone et al (Moscone et al., 1996) have developed a small UF probe (single hollow fiber with a diameter of 300 µm, and a length of 4 cm, surface area 2.5 mm², with a stainless steel spring inside to prevent collapsing of the fiber) that can only be used for continuous sampling at very low flow rates (slow UF, 50-150 nl/min) (figure 6-1b). The negative pressure (about 625 x10^4 mPa or 47 mm Hg) is created by a disposable syringe: the use of the syringe pump and a restriction creates a pulse-free flow (Moscone et al., 1996). Because of its small size, the probe causes minimal tissue damage and can be introduced to
the subcutaneous space via adapted needles without local anesthesia. With slow UF, the chemical balance in the extracellular fluid is minimally disturbed and easily replenished, so what is actually measured are the ECF concentrations, reflecting the metabolic changes created by the cells in the intercellular space.

Figure 6-1: Graphic representations of a subcutaneous (figure 1a) and an intravenous (figure 1b) ultrafiltration method. The probe of the fast UF method (figure 1a) is large, and consists of three UF fibres that are placed subcutaneously. The porous cuff allows for tissue ingrowth for longer duration placements up to six months. The filtrate passes through a “Y” connector with one limb leading to a sampling port, and to a negative pressure source that drives the UF process. The slow UF method uses a single hollow fiber probe that is small enough to be used intravenously (figure 1b). The negative pressure is created by a disposable syringe, which together with a restriction creates a pulse-free flow. This probe has also been used subcutaneously (data figure 1a from Ash et al, 1993).

Although very thin tubing is used with both UF probe types, there is a lag time between the time the sample leaves the tissue and sample collection, caused by the dead volume of the tubing and the flowrate. Especially in studies in which the timing of measurement between two compartments has to be precise, for example, simultaneous pharmacokinetic studies in the blood and the subcutaneous space, such lag time may hinder the simultaneous measurement. However, by calculating the lag time, by dividing the dead volume of the tubing by the flow rate, the time the sample left the tissue can be determined.
MD and UF have several characteristics in common, but there are important differences (figure 6-2, table 6-1). Both the MD technique and the UF technique separate chemicals by moving them across a semipermeable membrane. In MD, the separation is exclusively due to a concentration gradient and diffusion of the analytes, whereas in UF, analytes pass through the membrane by convection together with the body fluids in which they are dissolved (Janle and Kissinger, 1996). Both techniques measure the free concentration of the analyte.

For both MD and UF, off-line and on-line analysis techniques are available (see “Combination with analytical devices”), and the fluid collection rate is similar, between 0.05 and 10 µl/min. Whereas in the UF sample the analyte concentration directly reflects the tissue concentration, and the recovery is thus 100% (under 3000 M.W., over this size the capillary walls will detain some of the solutes), the tissue concentration of the analyte from a MD sample needs to be calculated taking into account the diffusive characteristics of the membranes, the composition of the perfusion fluid, the flow rate of fluid through the membranes and the recovery of the analytes (Osborne et al., 1991; Ungerstedt, 1991; Linhares and Kissinger, 1993b). As so many variables influence the recovery in MD, calibration is complicated in vivo, but necessary to estimate the concentration in the perfused tissue. Most of the calculation-methods are time consuming, can be applied only at steady state situations, and there is risk of creating artifacts while performing the calculations. Different methods of calculation have been developed, each with its advantages and disadvantages. It is beyond the scope of this overview to discuss these calculation methods. For a detailed description on several calculation methods for MD recovery, see Stenken (1999).

The flow rate used in MD can be higher than the flow rate used in UF, but the higher the flow rate, the lower the recovered concentration of the analyte. Although there is a continuous transfer of fluid and small molecules between the blood and the extracellular...
space (Linhares and Kissinger, 1993b), as a result of the sampling process in MD, analytes can be depleted from the tissue around the probe when the analytes diffuse into the dialysis fluid (Kaptein, 1998). MD may cause artifacts in the tissue due to disturbance of the ECF, or drainage of the analyte or other compounds present in the extracellular fluid (Rosdahl et al., 1993). UF has no adverse effects on the sampling tissue (Linhares and Kissinger, 1993b).

Table 6-1: a comparison between microdialysis and ultrafiltration.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Microdialysis</th>
<th>Ultrafiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Driving force</td>
<td>Pump</td>
<td>Negative pressure</td>
</tr>
<tr>
<td>Method of analyte retrieval</td>
<td>Diffusion</td>
<td>Filtration</td>
</tr>
<tr>
<td>Retrieved fluid</td>
<td>Protein free, ready for analysis</td>
<td>Protein free, ready for analysis</td>
</tr>
<tr>
<td>Sampling in tissue</td>
<td>Sampling in different tissues, including brain</td>
<td>Sampling in different tissues except brain</td>
</tr>
<tr>
<td>Sample collection rate</td>
<td>0.1-10 µl/min</td>
<td>0.05-10 µl/min</td>
</tr>
<tr>
<td>Highest sampling frequency</td>
<td>Every minute</td>
<td>Continuous</td>
</tr>
<tr>
<td>Flow rate control</td>
<td>Precise</td>
<td>Method-dependent</td>
</tr>
<tr>
<td>Calculation after retrieval</td>
<td>Calculation of recovery, tissue concentration</td>
<td>No calculation necessary for small molecules</td>
</tr>
<tr>
<td>Recovery</td>
<td>5 - &gt;95 %</td>
<td>&gt;95 % for small molecules</td>
</tr>
<tr>
<td>Sampling</td>
<td>Off-line, on-line</td>
<td>Off-line, on-line</td>
</tr>
<tr>
<td>Drainage</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Blood or fluid loss</td>
<td>No loss of blood or other fluids</td>
<td>No loss of blood or other fluids</td>
</tr>
<tr>
<td>Total measuring time</td>
<td>At least 21 days</td>
<td>At least 21 days</td>
</tr>
<tr>
<td>Drug delivery</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Limits in molecular weight</td>
<td>MW&lt;3000</td>
<td>Fast UF: MW&lt;3000, slow UF no limitations</td>
</tr>
<tr>
<td>Species</td>
<td>Various animals, man</td>
<td>Various animals, man</td>
</tr>
<tr>
<td>Measurement of chemicals</td>
<td>Free concentration of analyte</td>
<td>Free concentration of analyte</td>
</tr>
<tr>
<td>Measurement in vitro, in vivo</td>
<td>Both</td>
<td>Both</td>
</tr>
</tbody>
</table>

A major advantage of MD is the possibility of local drug delivery via the inflowing perfusate, which is conveniently used in many neurotransmitter-studies. A near-100% relative recovery can be created with MD when perfusing at very low flow rates (Kaptein et al., 1998), however, often large losses of perfusion fluid through the probe are seen when using ultra-slow MD. To prevent this loss of perfusion fluid, a high osmotic compound that cannot pass the dialysis membrane, for example dextran, can be added to the perfusate.
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(Rosdahl et al., 1997). Such addition can interfere with online detection methods such as biosensors; moreover, dextran may absorb aromatic compounds (e.g. indoles, catechols, steroid hormones).

In summary, UF gives recoveries of 100% for low molecular weight molecules because the recovery is essentially independent of the flow rate and there is no dilution factor (Linhares and Kissinger, 1992; Linhares and Kissinger, 1993a; Janle and Kissinger, 1996). UF thus avoids the complicated and time-consuming recovery calculations of MD. UF does however not allow the local application of drugs. But, in contrast to MD, UF is more practical at very low flow rates. UF is not suitable for brain sampling, but is very useful in on-line and off-line analysis at shorter time-intervals than MD.

BIOMEDICAL APPLICATIONS OF UF

The applicability of in vivo UF has been demonstrated in several biomedical and clinical applications, including ion dynamics, mineral and drug kinetics, protein studies and glucose and lactate monitoring. As most studies have focused on subcutaneous and intravenous glucose and lactate monitoring, these applications will be discussed separately (next section: Glucose and Lactate).

Ion dynamics

The applicability of UF for monitoring ion dynamics was studied by Linhares and Kissinger (1993b), who investigated insulin-dependent potassium uptake. Both increased or decreased levels of potassium can have fatal effects, creating cardiac arrest and muscle paralysis respectively. High levels of insulin cause the transfer of potassium from the intercellular space to the intracellular space of the cell, decreasing the intercellular levels (Linhares and Kissinger, 1993b). The objective was to monitor this phenomenon to substantiate the conclusion that the active sampling process of UF does not cause cell rupture and indeed monitors the intercellular fluid. The interstitial potassium level decreased after an acute dose of insulin; intracellular levels of potassium were not observed, thus proving the use of UF to measure the flux of ions in and out of cells (Linhares and Kissinger, 1993b).

Spehar et al. (1998) studied ion dynamics using UF in the subcutaneous and intramuscular space in horses. UF sodium, potassium and calcium levels from the intramuscular and subcutaneous space were compared to plasma concentrations, and the temporal change in the calcium concentration was determined in response to an intravenous infusion with calcium. They found that the sodium and potassium concentration equilibrate rapidly between the vascular and interstitial space, and that the interstitial fluid concentrations of these ions reflect the plasma concentrations. Calcium concentrations, however, differed according to site, indicating that factors other than diffusion play a role in determining calcium concentrations in subcutaneous fluids (Spehar AM et al., 1998).
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Mineral metabolism

Two articles have appeared on the measurement of minerals in bone, muscle and subcutaneous tissue using UF in sheep (Janle and Sojka, 2000; Janle et al., 2001). Calcium, magnesium and phosphorus are important for the strength and shape of the bone, as for other functions in the body. The study of mineral metabolism can help to understand bone physiology and pathology (Janle et al., 2001). By comparing the calcium, magnesium and phosphorus concentration in different tissues using fast UF and in blood it appeared that the calcium and phosphorus concentration in bone, muscle and subcutaneous tissue is lower than the plasma phosphorus concentration, whereas the magnesium concentration is lowest in blood (Janle and Sojka, 2000; Janle et al., 2001). These studies show that UF has potential in the development of anti-osteoporosis drugs, in nutritional studies and in the study of bone physiology and pathology (Janle et al., 2001).

Drug kinetics

UF has been used in drug kinetic studies of acetaminophen and theophylline. Linhares and Kissinger (1993a) compared subcutaneous UF with subcutaneous MD, by placing both a UF probe and a MD probe in awake rats. Also, multiple UF probes were placed for comparison of drug concentrations for the validation of the UF technique (Linhares and Kissinger, 1993a). MD was performed with a flow-rate of 3 µl/min, UF with a flow-rate of 2.4 µl/min. Samples were collected every 5-30 minutes and analyzed immediately. Although the concentration of theophylline found with MD was lower than that found with UF, the disposition of the drug was similar (figure 6-3). The acetaminophen disposition measured with two UF probes placed in the same rat was also similar. The disposition of subcutaneous acetaminophen, as measured with UF, compared very well to MD experiments monitoring acetaminophen in the jugular vein (Scott et al., 1991; Linhares and Kissinger, 1992). The disposition curves, taken from the same subject at separate days, were practically identical. UF is thus an effective tool for the monitoring of drug disposition and pharmacokinetics in awake rats.

Figure 6-3: Simultaneous monitoring of the theophylline disposition in a single animal with a capillary ultrafiltration probe (triangles) and a microdialysis probe (circles). Although the concentration in the dialysate is lower than that in the ultrafiltrate, both methods follow the disposition of theophylline similarly (Data from Linhares and Kissinger, 1993).
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Proteins

MD probes with membranes with a high molecular weight cut-off are unsuitable for conventional and low-rate MD, as the perfusion-fluid will be lost during passage of the probe. However, the measurement of pharmacokinetics and metabolism of proteins and nucleic acids can be of interest both systemically and in local tissues (Hall, 1995). For example, proteins are often released as chemical messengers, for instance, cytokines (molecular weights: 20-90 kD) during inflammation and infection (Schneiderheinze and Hogan BL, 1996). UF allows the measurement of proteins using membranes with a high molecular cut-off weight. Schneiderheinze and Hogan (Schneiderheinze and Hogan BL, 1996) have studied the subcutaneous protein sampling in vitro and in vivo using small UF probes (length 10-15 mm). For model proteins with molecular weights up to 68 kD the UF recovery was higher than 90%. In vivo UF sampling in conjunction with slab gel electrophoresis and silver staining detected three recovered proteins (molecular weight 9100-26800 D) present in the extracellular space of rats (Schneiderheinze and Hogan BL, 1996).

GLUCOSE AND LACTATE

Subcutaneous glucose and lactate

Frequent monitoring of glucose in diabetics is very important to prevent diabetic complications. Many studies have aimed to develop methods to conveniently measure glucose, as fingerprick blood glucose measurements are painful and tedious. UF has shown to be a useful technique for the monitoring of glucose dynamics in animal models of diabetes (Janle et al., 1992a; Janle et al., 1992b; Janle et al., 1995). In fact, the first article about subcutaneous UF (Janle-Swain et al., 1987) was concerned with glucose monitoring. In their set-up, a capillary filtrate collector consisting of three UF fibers was implanted subcutaneously to monitor glucose levels in freely moving, diabetic and non-diabetic dogs. A UF sample was collected every 15-30 min for periods of 3-5 hours at near maximal negative pressure and flow rate. It was shown that the UF concentration at high flow rate reflects the blood plasma glucose values well. Implanted UF devices functioned up to 60 days at a flow rate between 20-120 µl/h, and, after 3 months of implantation, only minimum surrounding fibrous connective tissue response was seen. Sc UF probes could be maintained for at least 6 months (Janle-Swain et al., 1987).

Ash et al (1992) used fast UF for the subcutaneous monitoring of glucose levels in diabetic patients. Seven diabetics were equipped with a capillary filtrate collector (CFC), tubing and a vacutainer tube for 1 month. The CFC’s had an average flow rate of 40-50 µl/h (Ash et al., 1992). None of the patients exhibited exit site infection. Although there were some problems with bacterial contamination in the filtrate resulting in erroneous glucose measurements, the study gave promising results: the UF glucose concentrations correlated well with the concurrent blood glucose levels (Ash et al., 1992). One year later this study was expanded with 10 more diabetic patients, the results were similarly promising (Ash et al., 1993).

Slow UF has also been applied for subcutaneous tissue glucose measurements. Kaptein et al (Kaptein et al., 1997) measured glucose every minute for several hours in rats and compared the subcutaneous levels to the arterial blood glucose concentration. At such high
time resolution, it appeared that the subcutaneous glucose concentration did not rapidly follow changes in blood. The curves would not simply be explained by a time-delay; the relation between these compartments was that of interrelated pools, the subcutaneous pool being derived from the blood (Kaptein et al., 1997). This is different from the results found by using fast UF (Janle-Swain et al., 1987), but, as stated earlier, fast UF measures chemicals in the blood plasma water, whereas slow UF measures compounds in the interstitial space.

Tiessen et al (Tiessen et al., 1999) studied whether subcutaneous glucose and lactate levels, measured with slow UF, were solely dependent on intravascular concentrations, or also influenced by local factors such as cellular uptake and tissue metabolism. The UF glucose and lactate concentrations were measured simultaneously in six volunteers who were subjected to an oral glucose tolerance test. The emerging curves of the analytes were different for different subjects, emphasizing that the subcutaneous glucose is not directly and exclusively linked to the blood compartment (Tiessen et al., 1999).

**Intravenous glucose and lactate**

Efforts have been undertaken to apply slow UF for measurements of plasma concentrations in blood itself. A problem with placing UF membranes within the blood stream is the risk that the probe membrane may become coated with blood clots soon after being inserted in the blood stream (Stjernstrom et al., 1993). However, this does not necessarily interfere with convection of the metabolites (Stjernstrom et al., 1993). The membrane used in MD and UF is often made of polyacrylonitrile, which is also used in haemodialysis and haemofiltration and tested well for biocompatibility (Baeyer von H et al., 1988; Kaptein et al., 1997). Contact with blood plasma causes a thin protein and fibrin layer on the outer and inner surface of the membrane (Baeyer von H et al., 1988; Morti and Zydney, 1998) even in the presence of heparin (Baeyer von H et al., 1988). Once fibrin is formed, blood-surface interaction is terminated because a new natural wall is formed and heparin action is now sufficient to prevent coagulation (Baeyer von H et al., 1988). The contact of polycrylonitrile with blood has little effect on the filtration of small molecules such as urea and vitamin B12, but it causes a significant reduction in the filtration of large molecular weight molecules > 10 kDa (Morti and Zydney, 1998).

MD has been performed in blood in several studies in the blood vessel itself in rats (Telting-Diaz et al., 1992), in healthy male volunteers (Paez and Hernandez, 1997), and in intensive care patients (Stjernstrom et al., 1993), or via an extracorporeal MD sampling circuit in anaesthetized dogs (Rabenstein et al., 1996; Freaney et al., 1997). Intravenous MD could be realized for up to 24 hours (Stjernstrom et al., 1993) and is easy and safe (Paez and Hernandez, 1997). Measured in the blood itself, the corrected dialysate glucose was not different from plasma glucose (Telting-Diaz et al., 1992; Stjernstrom et al., 1993; Paez and Hernandez, 1997).

UF in blood has only been performed with the small slow UF probes in rats (Kaptein et al., 1997; Leegsma-Vogt et al., 2001), pigs (Tiessen et al., 2001), and broiler chickens (Savenije B et al., 2002). Kaptein et al. (Kaptein et al., 1997) showed that the glucose concentration in an ultrafiltrate of the jugular vein placed near the heart mimicked simultaneously sampled arterial blood, although very high concentrations just after a glucose injection appeared flattened, likely to be due to dead spaces in the connections.
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The first application of UF on myocardial pathophysiology encompassed the venous glucose and lactate concentration around myocardial ischemia in pigs (Tiessen et al., 2001). As the early detection of myocardial ischemia is of major importance, monitoring changes in glucose and lactate levels in the cardial venous efflux can become clinically useful. Lactate is produced when poorly perfused tissue shifts from aerobic to anaerobic metabolism. Thus, the monitoring of lactate may offer an opportunity to detect tissue oxygen deficits in time for preventive intervention. An UF probe was integrated in a cardiac catheter and placed in the coronary sinus. The myocardial infarction was created by the inflation of a balloon catheter in the left anterior descending coronary artery. During the procedure, blood samples were taken to compare with UF measurements. The UF glucose and lactate measurements followed the blood measurements perfectly. The lactate concentration rose sharply after the deflation of the balloon catheter, which suggests the existence of a tight link in time between cardiac pathological events and metabolic shifts in the venous efflux, interesting for their potential diagnostic value (figure 6-4). This study (Tiessen et al., 2001) demonstrated that an UF catheter could sample blood ultrafiltrate continuously for hours in the coronary sinus, monitoring venous efflux on a minute-to-minute basis.

The intravenous monitoring of glucose and lactate concentrations can also be useful during brain damage, as the uptake of glucose is impaired during head trauma and lactate points to oxygen deficiency. Using a rat model, Leegsma-Vogt et al (2001) studied the arteriovenous glucose and lactate levels before, during and after experimental brain trauma at a minute-to-minute basis with continuous UF. Rats were monitored for several hours before and after the induction of brain damage induced by the intracranial inflation of an arterial embolectomy balloon, while other rats served as controls. The rats received two UF probes, one in the aorta and another in the jugular vein, for the arterio-venous measurement of brain metabolism. Under control conditions, there was a net glucose influx and lactate efflux. After the inflation of the balloon, the glucose uptake and the lactate efflux decreased.

Savenije et al (Savenije B et al., 2002) have studied in vivo for the first time the combination of intravenous ultrafiltration with collection tubing, allowing the ultrafiltrate to be collected for 24 hours. This was studied by placing a UF probe in the wing-vein of
broiler chickens, with the collection device placed in a plastic bag and sutured to the wing. The chickens were group-housed; the UF-collection device allowed stress-free sampling in their naturalistic environment. The collection tubing was analyzed afterwards with a time-resolution of less than 5 minutes.

UF is thus very well suited for intravenous measurements. Research has shown that the materials used are safe, although intravenous UF may need some more study to prove the safety in humans. Intravenous UF can not only be used for continuous blood monitoring, but may serve as a diagnostic tool in e.g. heart failure and brain damage as well.

COMBINATION WITH ANALYTICAL DEVICES

In both MD and fast UF, the samples can be collected with a conventional collection device (fraction collectors, often adapted to the sample size of less than 5 µL) that can be processed automatically. Such off-line sampling allows a wide variety of analytic techniques, including the most often applied HPLC. However, with HPLC time resolutions of less than 5 minutes are not easily achieved. Slow UF samples cannot be collected in separate samples, mostly because of their small size (often< 0.5 µL), but can be collected in a sample storage tube for subsequent analysis with a time resolution of less than 5 minutes and minimal longitudinal diffusion (Moscone et al., 1996) or can be directly coupled to small size biosensors or flow injection analytic systems with a time resolution of one minute (Kaptein et al., 1997; Tiessen et al., 2001; Leegsma-Vogt et al., 2001).

There is a general tendency to use smaller samples for analysis. UF can contribute to this cause, as it does not dilute the sample. There are several detection techniques available that can manage small samples, like microbore or capillary HPLC, mass spectroscopy, (bio-) sensors and capillary electrophoresis.

Most slow UF studies mentioned in this overview have used biosensors for the detection of glucose and lactate. These biosensors can detect a sample as small as 20 nl/min, and use respectively glucose oxidase or lactate oxidase combined with horseradish peroxidase to selectively detect glucose or lactate in the sample. However, a disadvantage of the selectivity of biosensors is that only one analyte can be detected per biosensor. Other detection systems, such as capillary electrophoresis or mass spectroscopy, can measure several analytes in one sample.

CONCLUDING REMARKS

MD has become very popular due to the ability to sample for an extended period of time in the laboratory and clinic with either on-line analysis or automated sample collection systems (Linhares and Kissinger, 1992). UF probes can function similarly (Linhares and Kissinger, 1992).

The specific advantages of UF, compared to MD, allow samples to be taken more frequently because a smaller volume can be collected, and UF is potentially better usable for quantitative in vivo monitoring because no dilution factor has to be considered. UF has been applied in human subjects and probes can remain in situ for up to 1 month. UF probes can easily be combined with (bio)sensors or other methods of micro-analysis (e.g. capillary electrophoresis, microbore-HPLC). Depending upon the operating rate of UF (slow or fast)
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the probes can sample interstitial concentration or plasma water concentration in
neighboring capillaries. The small sample volume of UF probes (if removed frequently)
cannot easily be analyzed by conventional methods, however, larger samples collected
every 10-20 minutes can be easily handled and analyzed by conventional methods. Slow
UF can be performed in virtually every tissue including blood.

UF can be a good alternative for MD. In most cases, there will be no difference in tissue or
plasma water concentrations determined by UF and MD. However, when brain substances
are measured, or drugs have to be delivered during the experiment, MD is the best choice,
but when high recoveries or frequent samples are needed, UF will be the preferred sampling
method.

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