Biocybernetics and Biomedical Engineering
2008, Volume 28, Number 2, pp. 21–33

Modern Application of Membrane Technique in Therapeutic and Diagnostic Medical Systems

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This study presents actually available membrane systems devoted to therapeutic and diagnostic applications. In particular LDL apheresis systems including new Two Stage Membrane System with Recirculation (TSMS) and the microdialysis technique are discussed. Application of the membrane systems to therapeutic purposes with utilization of methods improving the selectivity of LDL cholesterol removal cause decrease of albumin losses. Application of quasi-continuous monitoring using microdialysis technique during intensive treatment provided in some cases a completely new quality data, which may be helpful in the profound understanding of the pathophysiology of the specified diseases.

K e y w o r d s: membrane techniques, LDL apheresis, microdialysis technique, monitoring system, interstitial fluid, diabetes

1. Introduction

At the present time the membrane techniques are used in two purposes: therapeutic and diagnostic (measurements). The membrane technique used in the therapeutic purposes make possible removal of metabolism products (small and middle particles) and exogenous and endogenous toxins. The membrane techniques used in diagnostic – measurement purposes make possible continuous monitoring of biochemistry in extracellular space in living tissue. In all membrane processes two physical phenomenons are utilized: diffusion and convection. This study presents actually available membrane systems devoted to therapeutic and diagnostic applications. In particular the LDL apheresis systems including new Two Stage Membrane System with Recirculation and the microdialysis technique are discussed.

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2. Membranes Application to the Therapeutic Purposes

The main classification of membrane application for the therapeutic purposes is presented in Fig. 1.

Presently the most commonly used membrane technique is dialysis employed for removal of nitrogenous end-products of catabolism from and correcting the salt, water, and acid-base derangements in blood of patients (with acute and chronic kidney failure). Dialysis is used for removal of exogenous toxins too (drugs and another chemical substances poisoning) from patients' blood.

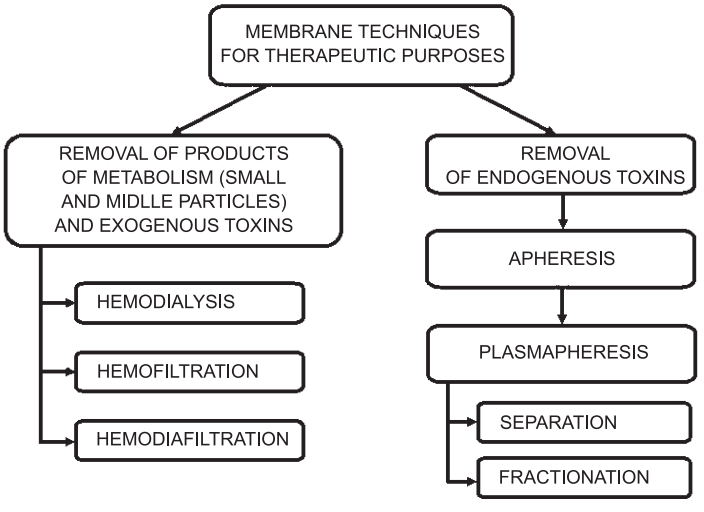

Fig. 1. Scheme of membrane application for the therapeutic purposes

Apheresis is a process in which blood components (like plasma, plasma components, leucocytes, erythrocytes, platelets) are mechanically separated and removed, while rest of the blood is returned to the patient together with required substitution fluid. The basic apheresis processes are plasmapheresis and cytapheresis. Basic processes of the membrane plasmapheresis are the plasma separation and fractionation procedures [1].

Main disadvantage of the membrane separation procedure is necessity of usage of the substitution fluids. Substitution of the pathological plasma with the plasma received from the donor is recently applied rarely because of infection hazard caused by infectious jaundice B, C or HIV viruses. Plasma fractionation procedure could be performed without application of the substitution fluid. The plasma fractionation procedure, in standard way, is realized using cascade filtration (Fig. 2). In principle patient blood is given to a separator and then, after separation of the morphological elements from the plasma, the morphological elements are returned to the patient

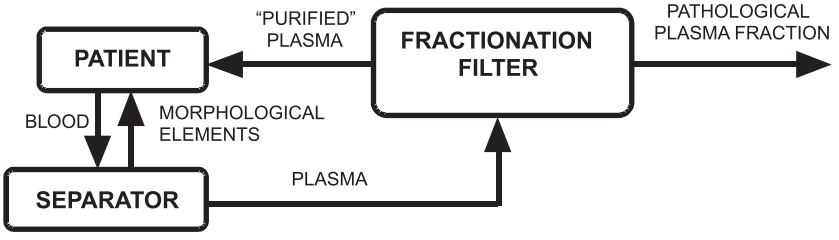

Fig. 2. Scheme of cascade filtration

whereas the plasma is fed to the fractionation membrane. Separation process of the pathological part of the plasma takes place on the fractionation membrane and then the "purified" plasma is returned to the patient blood circulation.

2.1. LDL Cholesterol Apheresis Using Membrane Technique

Low – density – lipoprotein cholesterol (LDL cholesterol) is a risk factor for atherosclerosis. LDL cholesterol accumulates in the fatty streaks of the vessel walls and causes: inflammation, endothelial dysfunction and eventually luminal narrowing. Molecular weight (MW) and relative density of LDL, HDL and VLDL cholesterols are presented in Table 1 below.

Table 1. Molecular weight and density of chosen plasma lipids

	MW	Relative density [g/l]
LDL cholesterol	2.300.000 – 2.700.000	1006 – 1210
HDL cholesterol	125.000 – 320.000	1063 – 1210
VLDL cholesterol	3.000.000 – 128.000.000	950 – 1006

In patients with heterozygous familial hypercholesterolemia LDL cholesterol can be lowered by diet and drugs. Patients with homozygous or severe heterozygous familial hypercholesterolemia need further drastic lowering of LDL cholesterol by extracorporeal removal of LDL cholesterol. For this purpose LDL cholesterol fractionation procedure is used [2]. Improvement of this procedure should result in high removal of the pathological plasma components and, equally important, lowest unwanted protein losses caused by limited permeability of the membrane and adsorption to the membrane structure. Currently available plasma fractionation membranes allow for high level of removal of the plasma pathological substances. The main problem occurring during removal of LDL cholesterol from the patient blood during the membrane filtration, carried out in ambient temperature, directly worsening effectiveness (selectivity) of the plasma fractionation procedure, are unwanted proteins and HDL cholesterol losses. In order to improve selectivity of

the procedure several modifications of the traditional cascade filtration system have been developed, like: thermofiltration, HELP system, pulsations [3–7]. System with thermofiltration is characterized by the introduction in the second circuit of the plasma fractionation system of the plasma warming element, which heat up it within the range 37ºC to 42ºC. Advantages of this system are easy handling and effective separation of LDL and HDL cholesterol, prevention of cryogel formation on the second filter. Disadvantages of this system are necessity of introduction of the heating system and risk of plasma overheating. HELP System is a system where LDL cholesterol removal is enhanced by addition of heparin/acetate buffer to the plasma, which causes precipitation of LDL cholesterol, VLDL cholesterol and fibrinogen with the heparin (Fig. 3). These protein precipitates can then be removed from the plasma by the membrane filtration. The excess heparin is subsequently removed from the solution by adsorption and excess fluid is removed using bicarbonate dialysis.

Advantage of this system is effective separation of LDL and HDL cholesterols.

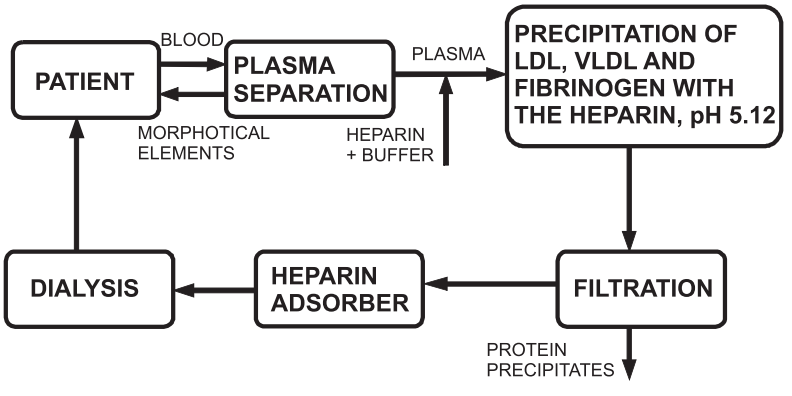

Fig. 3. Scheme of HELP System

Another advantage of this system is a possibility of use for the patients who simultaneously need dialysis procedure. Disadvantages of this system are its complicated construction and high cost.

Mechanical pulsation inside capillaries at the filter input and/or output can efficiently remove or prevent adsorption of the protein on the membrane surface [8].

Very high peaks of pressure occurring during this procedure may pass to the first membrane circuit and cause in some cases high risk of damages of the morphological elements.

All the mentioned above solutions generally cause some improvement in the selectivity of the plasma fractionation procedure, but they are not optimal and the problem especially with maintenance of the slight protein losses still exist.

In order to achieve smaller losses of protein an idea of a new membrane system was elaborated – Two Stage Membrane System with Recirculation [9].

2.1.1. Two Stage Membrane System with Recirculation

Two Stage Membrane System with Recirculation consists of two circuits (see Fig. 4). The first circuit contains: a plasma container, a peristaltic pump, inner part of the first filter capillaries, inner part of the second filter capillaries. The second circuit contains: outer part of the first filter capillaries, outer part of the second filter capillaries and a peristaltic pump. In principle the blood flows through the capillaries of the first and the second filters. The pump in the second circuit generates a high flow rate. This high flow rate circulation induces a pressure gradient along the capillaries of the first and second filters (see Fig. 5). These pressure conditions induce filtration

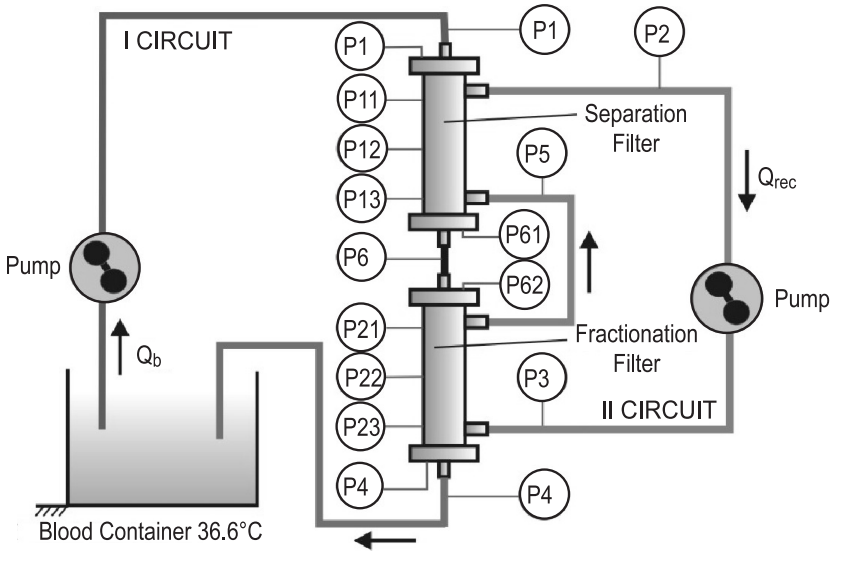

Fig. 4. Schema of Two Stage Membrane System with Recirculation

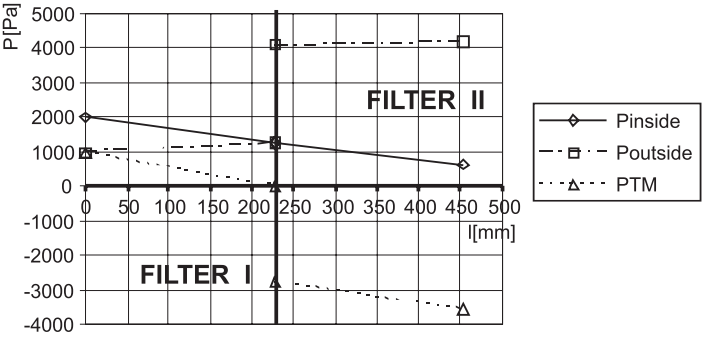

Fig. 5. Pressure distribution along the capillaries of the first and second filters (point "0" is situated exactly between the first and second filter)

along the capillaries of the first plasma separation filter and backfiltration along the capillaries of the second plasma fractionation filter. In this case, in contrast to the conventional cascade filtration, the second filter is used in the reverse operation. In such a configuration characteristics of the first and second filters decide about efficiency of the plasma separation procedure on the first stage and plasma fractionation procedure on the second stage of the system. The ideal situation is when pressure conditions induce only filtration along the capillaries of the first plasma separation filter and only backfiltration along the capillaries of the second plasma fractionation filter (point "0", in which transmembrane pressure PTM is equal to zero is situated exactly between the first and the second filters). In the tested system the capillaries are continuously flushed by plasma in the second circuit. Such process should have positive effect on effectiveness of the fractionation procedure removing weakly adsorbed protein from the membrane structure what should reduce protein losses.

The proper selection of the membrane system operating conditions is crucial in terms of the system efficiency. Preliminary *in vitro* studies indicate that the position of the "0" point is most sensitive to viscosity changes**.** Influence of the flow rates in the first and second circuits on the point "0" position has been found to be much lower than influence of viscosity.

3. Membranes Application in the Diagnostic Purposes

Application of membranes for diagnostic – measurement purposes make possible monitoring of biochemistry in extracellular space of different kinds of living tissues. The main membrane technique used for diagnostic purpose is microdialysis. This technique is used for the study of pharmacokinetic and pharmacodynamic properties of drugs in the clinical setting. It is used to measure antibiotic penetration for example of the infected tissue of lungs [10] or the interstitial space fluid of subcutaneous adipose tissue of septic patients [11]. This technique can be used as a tool for measurement of drug penetration into the interstitium of solid tumours for estimate clinical response to chemotherapy. Microdialysis may prove to be a good method for selecting compounds with the best penetration and may help to identify patients who can not be treated by chemotherapy because of poor drug penetration [12–13]. Microdialysis technique is used in neurointensive care too. It is used for monitoring secondary ischaemia, which is a common complication after brain trauma. An increase of concentration of glycerol (which is integral component of a cell membrane) in interstitial fluid indicates damage of neuronal membranes. A monitoring of lactate and pyruvate in interstitial fluid is useful too – an increase in the lactate to pyruvate ratio may be an early indicator of brain ischaemia [14]. Microdialysis technique has been shown to be useful in plastic surgery for indicating imminent ischemia in flaps used in reconstructions [15]. Microdialysis has also been used to monitor concentrations of cardiac troponin T and aspartate transaminase for up to 100 hours after

patients have undergone heart surgery [16]. Some portable microdialysis systems have been developed to allow for continuous measurement of subcutaneous glucose concentrations for up to several days. The first prototype of a system for continuous glucose measurements, so-called Ulm Sugar Watch, was designed and developed in Ulm, Germany. Currently, a few similar systems with application of the microdialysis are being developed and tested. The most interesting of them are Accu Chek (Roche Diagnostics), GlucoDay (Menarini) and GlucOnline (Roche/Disetronic) [17–20]. Microdialysis technique is a very useful tool to studying local physiology of adipose tissue, muscle and skin [21–25].

There are different types of probes commercially available. They are constructed in different ways, but all types of the microdialysis probes are designed to mimic a "blood capillary" and general principle of operation is the same. When a physiological salt solution is slowly pumped through the microdialysis probe, which is made of a semipermeable membrane, the solution equilibrate with the surrounding extracellular tissue fluid. After a while, it contains a representative proportion of the tissue fluid's molecules. For instance CMA 60 (Microdialysis, Sweden) is constructed as concentric tubes where the inner tube is made of a solid material and outer of a semipermeable membrane (Fig. 6). The perfusion fluid enters through the space between the inner tube and the outer dialysis membrane and flows to the membrane end. There is a space where the diffusion of molecules between the extracellular fluid and the perfusion fluid takes place. Then the fluid leaves this space through the inner tube and moves toward the proximal end of the probe. The effluent is collected in microvials and analyzed on the biochemical respect.

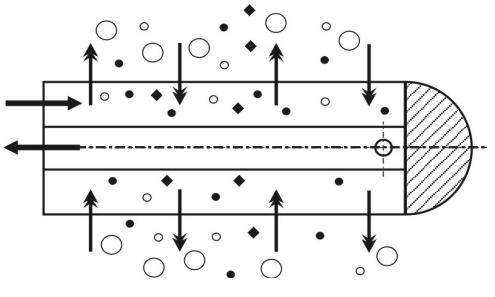

Fig. 6. Microdialysis probe

The dialyzing properties of a microdialysis probe can be defined as the relative recovery of a particular substance. The relative recovery [%] is defined as ratio of the concentration of the substance in the microdialysis probe effluent to the concentration of the medium and depends on: the "cut off" of the dialysis membrane, the length of the membrane, the membrane material, and flow rate of the perfusion fluid.

3.1. Monitoring of the Courses of Biochemical Parameters Characterizing Patient's Metabolic State

3.1.1. Pathogenesis of the Diabetes Ketoacidosis

Three of the most serious acute complications of diabetes are diabetic ketoacidosis (DKA), hyperosmolar hyperglicemic state (HHS), and lactic acidosis. During DKA and HHS the reduction of the net effective concentration of circulating insulin connected with a concomitantelevation of counterregulatory stress hormones as glucagon, catecholamines, cortisol, and growth hormone takes place. In both disorders hyperglicemia causes an osmotic diuresis due to gilcosuria and it is the reason of water and electrolytes loss, hypovolemia, dehydration, and decreased glomerular filtration rate, which further increase the severity of hyperglycemia. During lactic acidosis anaerobic glycolysis results in the production of lactate and hydrogen ions. Blood glucose levels may be low, normal, or high in diabetic subjects and lactic acidosis may also accompany ketoacidosis [26]. Current standard treatment of DKA and HHS concentrates on appropriate patient hydration, complement of lost electrolytes, and insulin treatment aimed at achievement of normoglycaemia. Treatment of lactic acidosis concentrates on stopping of accumulation of lactate acid anions in body fluids.

The main objective of the project is to characterize patients' metabolic state in DKA, HHS and lactic acidosis during application of the standard treatment by measurements of the biochemical parameters like glucose, lactate, pyruvate, and glycerol in the interstitial fluid.

3.1.2. Clinical Study

Patients with DKA, HHS, and lactic acidosis were included in to the clinical study. The patients with DKA were characterized by pH below 7.3 (norm: 7.35–7.45), bicarbonate ($HCO_3^{(-)}$) below 15 mmol/l (norm: 21–27 mmol/l), glycemia above 16.8 mmol/l (norm: 3.0–5.6 mmol/l), and presence of acetone in the urine. Requirement for including to the program a patient with HHS was glycemia above 33.6 mmol/l and effective osmolarity (counted according to the formula 2x(Na+K)+glycemia/18) above 320 mOsm/l [27]. The patients with lactic acidosis were characterised by hyperglycemia and lactate above 7 mmol/l. Preliminary investigations were carried out on 16 patients, hospitalized in the Clinic of Gastroenterology and Metabolism Diseases Medical University in Warsaw, at the age from 23 to 76. Eight patients were with type 1 diabetes, seven with type 2 diabetes, and 1 with type 3 diabetes. There were thirteen patients with DKA, two with HHS and one with lactic acidosis. The study group was very diversified on the clinic and biochemical respect. In the moment of admission individual patients pH was from 6.69 to 7.31, glycemia from 16.1 to 91.5 mmol/l, sodium from 118 to 166 mmol/l, and potassium 2.6 to 8.4 mmol/l. A CMA 60 microdialysis catheter (cut-off approximately 20 000 D)

made of polyamide, 30 mm length and 0.6 mm outer diameter (CMA Microdialysis Sweden – see Fig. 7a) was inserted into patients' abdominal adipose tissue. Perfusion fluid (T1 – CMA Microdialysis) was pumped by a CMA 107 pump (see Fig. 7a) to the CMA 60 microdialysis catheter with the flow rate equal to 0.3 μl/min. Measurements using the microdialysis technique were conducted in quasi – continuous way. Each sample of the perfusion fluid from the microdialysis catheter was collected to a microvial (CMA Microdialysis – see Fig. 7a) by 20 minutes. Biochemical parameters characterising patients' metabolic state like glucose, lactate, glycerol and pyruvate in the interstitial fluid were assessed using a microsample analyzer CMA 600 (Microdialysis, Sweden, see Fig. 7b). Glucose (every two hours), lactate and glycerol (every eight hours) from the blood were assessed as reference. Monitoring period using the microdialysis technique was 48h for each patient.

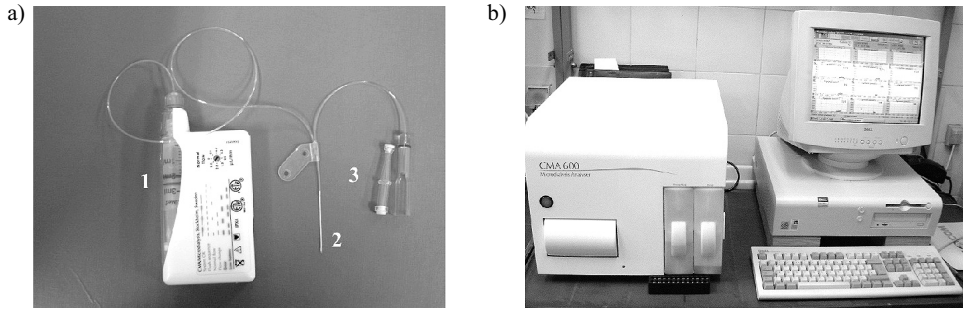

Fig. 7. Equipment used to the microdialysis technique: a) portable CMA 107 Pump (1) with CMA 60 probe connected to syringe (2) and microsamples microvial (3) b) CMA 600 Microsamples Analyzer

Characteristic exemplary time courses of the biochemical compounds (glucose, lactate, glycerol and pyruvate) concentration during first 48 h hours of the standard treatment for two DKA patients, one HHS patient (patient no XV) and one lactic acidosis patient (patient no XIV) are presented in Figures 8–11. From all the DKA patients two representative patients were chosen (patient no III and no VII) with differ character of time courses of biochemical components monitored in blood and interstitial fluid compartments.

Obtained results indicate that after initial hydration of the patient glycemia, glycerol and lactate levels correlated in very different way with the reference measurements from blood. For the patient III with DKA (Fig. 8) time course of the interstitial fluid glucose is similar to time course of the blood glucose. For the patient VII with DKA (Fig. 9) the blood glucose was about 2 times higher than in the interstitial fluid during all monitoring time. For those patients time courses for the interstitial fluid lactate reflected time courses of the blood lactate. For the patient III the glycerol in the interstitial compartment starting from 22nd hour of monitoring is dispersed

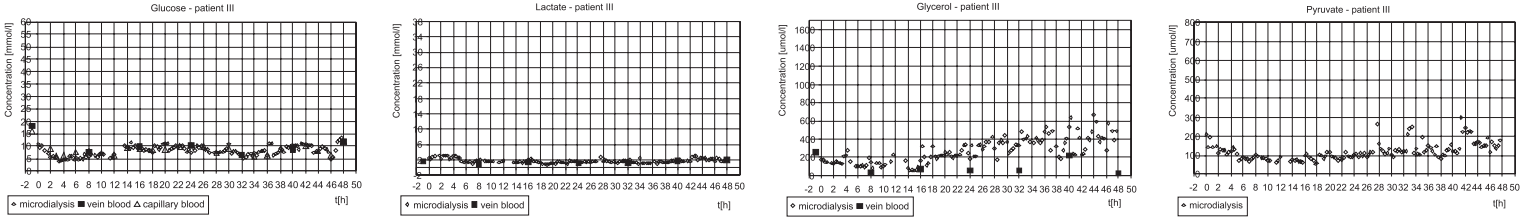

Fig. 8. Time courses of the glucose, lactate, glycerol and pyruvate concentration during the 48h observation for the patient with DKA

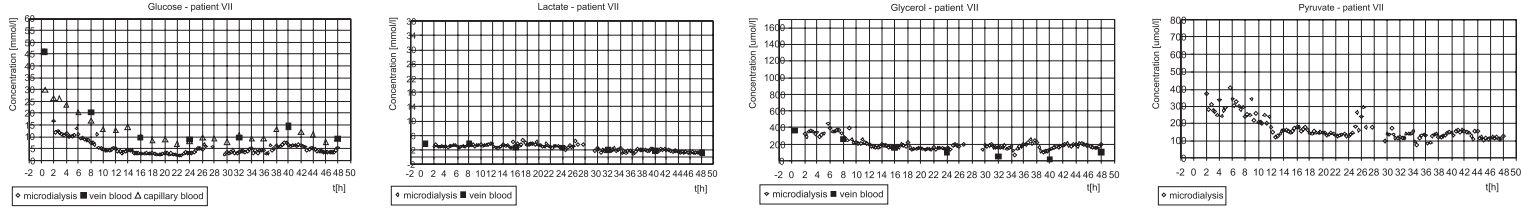

Fig. 9. Time courses of the glucose, lactate, glycerol and pyruvate concentration during the 48h observation for the patient with DKA

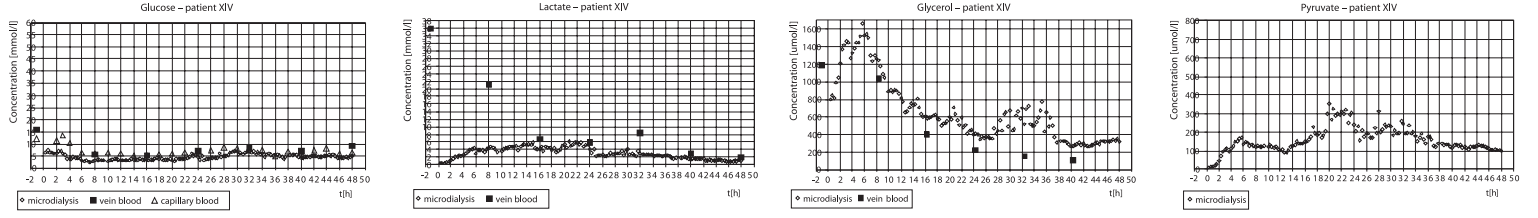

Fig. 10. Time courses of the glucose, lactate, glycerol and pyruvate concentration during the 48h observation for the patient with lactic acidosis

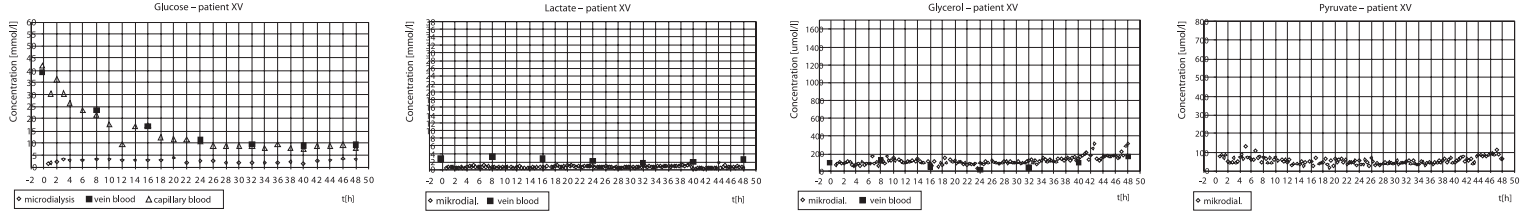

Fig. 11. Time courses of the glucose, lactate, glycerol and pyruvate concentration during the 48h observation for the patient with HHS

and significantly higher (from 2.4 to 5.6 times) than the in the blood compartment. Dispersed glycerol had in general six patients from the tested group. For the patient VII the glycerol starting from 24th hour of monitoring was 1.5–5 times higher in the interstitial fluid compartment than in the blood. Main difference between the patient with lactic acidosis and the patients with DKA was in the course of the lactate (see Fig. 10). For this patient in the 8th hour of monitoring the lactate level in the blood compartment is 5 times higher than in the interstitial fluid. Starting from 16th hour this level measured in the blood was from 1.4 to 2.9 times higher than in the interstitial fluid. For the patient with HHS glucose level (Fig. 11) in the blood compartment was significantly above glucose in the interstitial fluid (from 7.2 times in first hours of monitoring to 2.8 times during last hours). It may be caused by the poor catheter operation.

3.1.3. Summary

It was found that application of a quasi-continuous monitoring using the microdialysis technique during intensive treatment of the patients with HHS, DKA, and lactic acidosis provided in some cases completely new quality data, which may be helpful in the profound understanding of the pathophysiology of the specified diseases and improve method of patients' treatment. Due to too small quantity of the patients with HHS and lactic acidosis and high diversity of the results obtained so far, higher number of patients is required to draw meaningful conclusions.

4. Conclusion

In conclusion it can be stated that application of the membrane techniques have significant role in therapeutic and diagnostic medical systems. The membrane techniques used for the therapeutic purposes make possible removal of metabolism products (small and middle particles) and exogenous and endogenous toxins directly from patients' blood circuits. The membrane techniques used in diagnostic – measurement purposes make possible realization of continuous monitoring of biochemistry in extracellular space in living tissue. Basic problem occurring during medical therapeutic application of the membrane technique for LDL cholesterol removal are unwanted proteins and HDL cholesterol losses. In order to achieve smaller losses of proteins and HDL cholesterol a new idea of the membrane system was elaborated – Two Stage Membrane System with Recirculation. For this membrane system the proper selection of the operating conditions is crucial in terms of the system efficiency. Microdialysis as an example of a diagnostic medical system, gives a possibility to observe biochemical changes in the tissue in quasi – continuous way sometimes just before any chemical events are reflected in the changes of the systemic blood levels. For this reason the area of the medical applications of the microdialysis rapidly grows.

Since 1974 approx. 2100 scientific papers connected with clinical investigations on the technique have been published. However, till now this technique is considered as an experimental tool and further studies on applying this technique in different pathophysiological and physiological states are necessary.

Acknowledgment

Clinical studies presented in this paper were financed by research grants no 3 P05C 067 24 and N518 021 31/1238 the Polish Ministry of Science and Higher Education (formerly the State Committee for Scientific Research).

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