UDC 545.3:616.61-78

Studying the Dynamics of Changing the Concentration of Acetate Ions in the Blood of Patients in the Course Dialysis Method Using Capillary Electrophoresis

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(Received July 3, 2012; revised August 13, 2012)

Abstract

The content of acetate ions in the blood of patients after cardiac surgery in the course of hemodialysis using different dialyzing solutions was investigated for the first time. Using the method of capillary electrophoresis it was revealed that in the case when the bicarbonate dialysate contains acetate ions in a small amount (3 mmol/L), the concentration thereof in blood exhibits a 12-fold increase, which causes enhancing the risk of cardiovascular instability in the course of hemodialysis.

Key words: capillary electrophoresis, acetate ion, acetate-free dialysis, SLED

INTRODUCTION

The acetate buffer solution used as a component of dialysis systems, has found wide application since the middle of 1960ths due to a good solubility and stability in the concentrates of dialyzing solutions, as well as rapid transformation in an organism to form the equivalents of bases. However, a high concentration of acetate ion in the dialysate (35–40 mmol/L) and a decreased rate of the acetate metabolism in patients with renal dysfunctions result in the accumulation of the substance in an organism. According to the literature, the accumulation of acetate ions in the blood induces hypoxia with a subsequent activation of the proinflammatory cytokine cascade, cyclo- and lipooxygenase activation, the synthesis of prostaglandins and thromboxane. Clinical manifestations of acetate ion accumulation in the course of dialysis could be presented by such complications as lowering the blood pressure (BP) and heart rhythm abnormalities [1–3].

At the present time, in connection with numerous complications the acetate buffer in the dialysate uses to be replaced by a more physiologic bicarbonate buffer. However, the standard bicarbonate dialysate also contains some acetate ion (between 3 and 7 mmol/L) needed to maintain a necessary pH value of the dialyzing solution (pH ≤ 7.4) in order to prevent the precipitation of calcium carbonate.

The authors of [4–6] have demonstrated that for a number of patients, this concentration of the acetate ions in the dialysate can provoke a 4- to 10-fold increase in the content thereof in the blood with respect to the normal value. However, such studies were performed only concerning the patients with terminal chronic renal disease (tCRD). Similar data for patients with acute renal damage (ARD), including that after cardiac surgery, are not

available from the literature. Meanwhile, these categories of patients have a fundamental difference: the postoperative period of cardiac surgery in patients with ARD is often accompanied by cardiac insufficiency, severe impairing the microcirculation of blood, reducing the rate of metabolic processes, *i. e.*, the factors those promote the accumulation of acetate ions in the course of hemodialysis.

Modern dialysis technique such as SLED (Sustained Low-Efficiency Dialysis), presuppose a low-intensity therapy mode, *i. e.*, that at a lower feed rate of the dialysis liquid (6–8 L/h) and, accordingly, a less intake of acetate ions into the blood (18–54 mmol/h). Taking into account the fact that normally the rate of the metabolism of acetate ions is high enough (200–300 mmol/h), the accumulation thereof in an organism, as a matter of principle, should not occur.

However, our pilot study demonstrated that in the course of using the acetate containing bicarbonate dialysate the episodes of blood pressure lowering and heart rhythm disorders were observed significantly more often than in the case of using the acetate-free dialysate, which, to all appearance, could indirectly indicate the intradialytic cumulation of acetate ions in the blood [7]. To confirm this hypothesis it was required for the quantification of the level of acetate in the course of the SLED sessions in dynamics.

Previous studies of the dynamics of acetate ion metabolism in the blood demonstrated that the normal physiological concentration thereof is sufficiently low (0–100 μmol/L), however, in the course of dialysis, the concentration could reach 2800 μmol/L [8, 9]. In other words, intradialytic changes in the level of the acetate ion in the blood are observed in a fairly wide range of concentrations, therefore, to carry out the studies in this field it is necessary to use such methods of analysis those provide the possibility of selective acetate ion determination in bioassays at a concentration level ≤100 μmol/L.

Unfortunately, the number of published papers concerning the determination of acetate ions in biological substrates is rather scanty. The authors of [10] used an acetyl coenzyme A synthetase method referenced to a commercial development by BioAssay Systems Co., which method allows determining the acetate ion within the concentration range of 200–20 000 μmol/L in the mode of photometric detection or within the range of 130–2000 μmol/L in the mode of fluorimetric detection. Earlier research works based on the use of gas chromatography also provided the sensitivity at a level of 200 μmol/L [11, 12]. The authors of [13] used an ion-exclusion chromatography for the determination of a number of anions in saliva, however no acetate ion presence in the samples under analysis was revealed, therefore the possibility of acetate determination was confirmed by means of using the model solutions with the concentration of the analyte not lower than 220 μmol/L. The most sensitive and rather laborious method for determining the acetate ion was described in [9]; it is based on a preliminary extraction of acetate ion by means of vacuum distillation followed by gas chromatographic determination of the content at a level of 26 μmol/L.

Thus, the issue concerning the methodological support of research connected with studying the dynamics of changing the concentration of acetate ions in biological substrates in the course of different processes is of urgent importance.

Within the framework of this work, for the purpose of determining the acetate ions we developed a technique using the method of capillary electrophoresis (CE). This method belongs to the methods of the separation analysis, and therefore using thereof for the investigation of complicated systems such as blood serum seems to be quite reasonable.

The principle of the CE method consists in the fact that in the solution placed in a narrow quartz capillary ($d \leq 100$ μm), on the one hand, the movement of charged particles occurs under the influence of applied electric field, and on the other hand, a passive flow of liquid is observed. As a result, the sample is separated into individual components, since the parameters of electromigration (electrophoretic mobility) are specific for each species of charged particles. In order to detect sample components under determination in a flow, one most often uses a direct (for analytes with high molar absorption coefficients) photometry or indirect one (for non-absorbing components) [14].

EXPERIMENTAL

The determination of acetate ion in the samples of blood serum was carried out with the

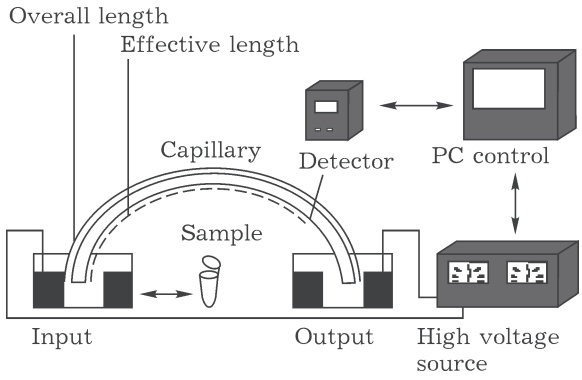

Fig. 1. Schematic diagram of capillary electrophoresis unit.

use of a "Kapel 105" CE unit equipped with a quartz capillary tube (diameter 75 μm, total length 70 cm, the length from the inlet to the detector amounting to 50 cm) with UV detector at the wavelength of 250 nm. As the separation electrolyte we used a solution containing chromate ion (UV absorbing anion), cetyltrimethylammonium bromide (cationic surfactant for reversing the electroosmotic flow) and diethanolamine to maintain the pH value to be equal to 9.0.

The sample under analysis was input into the capillary by means of applying external pressure to the reservoir, wherein the inlet end of the capillary is immersed (pneumatic input: pressure 30 mbar, duration 10 s). The operating voltage in the course of the analysis was equal to 25 kV; for decreasing the duration of the procedure the analysis was performed with applying the pressure value equal to 50 mbar. A schematic diagram of the CE unit is presented in Fig. 1.

Sample preparation for analysis

In the case of analyzing the samples of complicated composition, whereto the biological substrates of humans and animals belong, it is necessary to eliminate the influence of the protein components. The presence of these components in the sample could result in non-reproducible data of the analysis due to blocking the capillary walls, whereby the migration time changes from sample input to input to decrease the efficiency of the separation of sample components. In order to eliminate this effect, as a rule, the sample should be diluted, but similar approach, on the one hand, results in a loss of sensitivity, whereas on the other hand it does not provide a complete elimination of the matrix effect exerted on the results of the analysis.

Within the framework of the studies under performing, we have proposed the following procedure of sample preparation: the samples were thawed immediately before the analysis in a closed vessel at a room temperature, to take then a 0.25 mL aliquot, whereto was added 0.25 mL of acetonitrile in order to precipitate the proteins. The sample was then centrifuged at 900*g* during 10 min. To another test-tube was taken 100 μL of the supernatant, 100 μL of acetonitrile was added thereto with stirring to subject then to the procedure of CE analysis.

This approach provided the removal of the protein components from the sample and at the same time it allowed significantly reducing the electrical conductivity due to the dilution of the sample introduced into the capillary by an organic solvent with a low dielectric constant. This causes the most favourable conditions to provide for performing the concentration immediately within the capillary (stacking) and reducing the detection limit of the analyte [14].

Another feature of the object of the study consists in a high level of chloride ion content (96–106 mmol/L), which is 1000 times higher than the concentration of the component under determination and, as a matter of principle, could result in the superposition of the signals from analyte and macrocomponent (chloride ions). In order to overcome the mentioned effect, the analysis conditions were optimized by means of choosing the voltage and pressure in the course of separation as well as the volume of injected sample. As a result, quite satisfactory resolution was achieved for the signals of analyte and interfering component (Fig. 2).

The absence of acetate ions loss in the course of serum protein precipitation was confirmed by the results of an "introduced–found" experiment, wherein the sample trial before the precipitation stage was added with a known amount of acetate ion in the form of solution. It is demonstrated (Table 1) that is within the error of analysis the results of the "introduced–found" experiment were in a satisfactory agreement.

At this stage of the investigation the results of the "introduced–found" experiment confirm the validity of the technique devel-

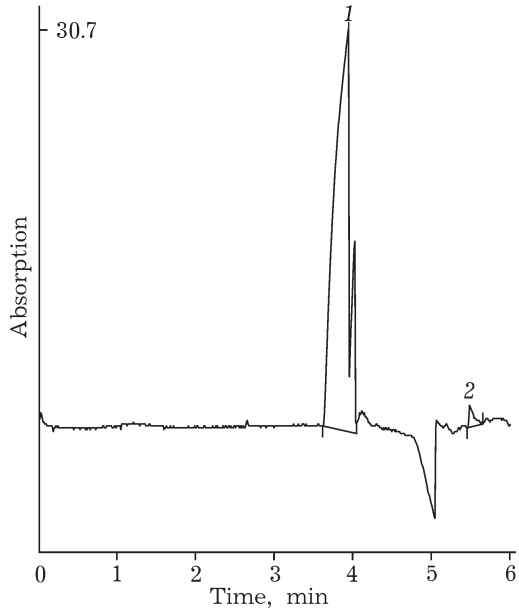

Fig. 2. Electrophoregram of blood serum samples: *1* – chloride ion, *2* – acetate ion. Assay conditions: chromate buffer with the addition of cetyltrimethylammonium hydroxide and diethanolamine; capillary: L_{eff}/L_{total} = 50/60 cm, inner diameter 75 μm; sample injection: 30 mbar · 10 s; voltage 25 kV; pressure in the course of the analysis 50 mbar; detection: λ = 254 nm, indirect.

oped, since the method of CE is based on the separation of the sample components. The detection limit of the technique was evaluated from the magnitude of acetate ion analytical signal (peak area) three times exceeding the variance of the background signal. It has been revealed that the acetate ion detection limit in the blood serum with the use of this method corresponds to 20 μmol/L.

A typical electrophoregram of blood serum is demonstrated in Fig. 2.

The random error (relative standard deviation) of acetate ion determination in the serum samples were evaluated as the result of statistical processing the sampling of 10 parallel determinations for the sample of average composition (confidence level being equal to 0.95). It is demonstrated that the error does not exceed 10 %.

TABLE 1

Results of the "introduced–found" experiment, μmol/L

Exp. No.	Introduced	Found	Found taking into account the content in the sample
1	40	102±10	62±10
2	40	100±10	60±10
3	40	110±12	70±12
4	100	170±17	70±17
5	100	164±16	64±16
6	100	172±17	72±17
7	200	260±26	60±26
8	200	265±27	65±27
9	200	258±26	58±26

Note. Acetate ion content in the averaged serum samples (65±7) mmol/L

Objects of investigation

We examined 70 patients with ARD after cardiovascular surgical procedure treated in the course of the postoperative period with the use of SLED technology. The group examined included 46 men and 34 women aged from 23 to 81 years old (the mean age of patients was equal to (58.7±11.7) years).

Depending on the type of dialysate the patients were divided into two groups. For the group 1 (n = 35), the SLED was performed using a standard bicarbonate-containing dialysate 3 mmol/L of acetate ion.

For the group 2 (n = 35), the SLED was performed using a "Krebsol" acetate-free dialysate wherein acetate was replaced by a solution of hydrochloric acid (3 mmol/L).

The control group 3a comprised 14 patients aged from 38 to 74 years old (mean age being equal to (52.6±1.3) years) with uncomplicated postoperative clinical course, who were examined at the first day after cardiovascular surgical operations. The group 3b included 15 healthy volunteers.

For the participants of the control groups who did not take any treatment, we determined a basal level of acetate ions in the blood serum.

For the groups of patients treated by means of the SLED techniques with different dialysates, the concentration of acetate ions in the blood was determined three times: before treatment, in 1 h after the beginning of the therapy procedure and at the end of the procedure.

The statistical analysis was performed using a Statistica (7.0 for Windows) software package. The results are presented as the mean and

TABLE 2

Comparison of blood acetate ion concentration in the groups under study

Groups	Number of observations	Acetate ion concentration of blood, μmol/L		
		Median	25–75 %	Rank
Healthy volunteers	15	40	30–80	20–160
Patients, uncomplicated postoperative period	14	45	30–60	20–100
Patients treated by means of SLED method	70	100*	40–330	20–540

*$p < 0.01$ as compared to control groups.

the standard deviation (M±SD), the median, the 25th and 75th percentiles. Differences in the data were considered to be statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Studied A comparative effect of SLED with different dialysates exerted on acetatemia dynamics was studied in 70 patients in the course of treatment. From Table 2 one could see that for the patients of the control group the level of acetate ion in the blood is within the range of normal physiological value.

For the studied sampling of patients with ARD, the initial pre-dialysis acetate ion concentration level exhibited a 2.5-fold increase on the average as to compare with the control groups. Thereby, in 40 cases (57 %) the concentration of acetate ion was corresponding to the normal value, whereas in the rest cases (43 %) the concentration exceeded the normal level (from 120 to 540 μmol/L). Thus, almost a half of the patients with ARD demonstrated initial decreasing the rate of acetate ion metabolism.

The results of studying the dynamics acetatemia in the course SLED are presented in Table 3.

One could see that the patients treated with the use of SLED technology with acetate-free dialysate exhibit the acetate ion level in the blood to be not changed in the course of the SLED session. On the contrary, the group treated with the use of SLED with acetate-containing bicarbonate dialysate exhibited a 1.5-fold increase in the concentration of acetate ions in the average after 1 h since starting the dialysis procedure. By the time of ending the SLED session, the acetate ion concentration exhibited a 5.6-fold increase on the average as compared with the stage before the treatment and up to a 12-fold increase as compared with the control groups.

Thus, despite the low concentration of acetate ions (3 mmol/L) in the dialyzing solution and low-intensity parameters of the dialysis therapy, increasing the acetate ion level in the blood was clinically significant. Therewith the degree of increasing the level of acetate ion and the range of varying its concentration (maximum up to 2100 μmol/L) in the case when the acetate-containing bicarbonate dialysate is used, are in a good agreement with literature data for the groups of patients with tCRD those were treated by means of hemodialysis with acetate-containing bicarbonate dialysate [5, 15, 16].

TABLE 3

Comparative dynamics of acetatemia in the course of SLED with different variants of dialysate ($n = 35$)

Values	Acetate ion content, μmol/L					
	SLED with acetate-containing bicarbonate dialysate			SLED with acetate-free bicarbonate dialysate		
	before treatment	1 h	after treatment	before treatment	1 h	after treatment
Median	100*	150*	560*	100	90	90
25–75 %	60–240	80–400	100–980	30–360	30–300	40–230
Rank	20–460	20–1300	20–2100	20–540	20–600	20–520

*$p < 0.01$.

In our study we considered the group of patients with ARD of cardiac surgery profile only, so in order to confirm these results further studies are required, including other categories of patients being in need of the dialysis therapy.

CONCLUSION

The investigation performed demonstrated the application of the CE method to be promising in order to determine the concentration acetate ions in the blood. This method demonstrated a high resolving power over a wide range of values, including the case of determining a low concentration of acetate ion. Thereby, the error of measurement was low and did not affect the clinical interpretation of the data obtained. At the same time, the CE method is characterized by availability, a relatively low cost of single determination and short analysis duration.

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