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Relationship of dialysate and serum phospholipid concentrations and ultrafiltration during continuous ambulatory peritoneal dialysis

Zależności pomiędzy stężeniami fosfolipidów w dializacie i surowicy a ultrafiltracją podczas ciągłej ambulatoryjnej dializoterapii otrzewnowej

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INTRODUCTION

Substances with surfactant activity are present on surface of mesothelial cells and can be found in peritoneal effluent as well. Among these are surface active phospholipids, produced mainly by mesothelial cells are prominent [1, 2, 3, 4]. Similar surfactant-like activity is also produced in pleura, pericardium, alveoli, and can be found on articular surfaces [3, 5]. In cells covering the peritoneal cavity, electron microscopic studies have demonstrated the existence of lammellar bodies that serve as the place of phospholipids production. These substances are then transported on the surface and cover the peritoneal membrane [3, 6, 7, 8]. It has been found that the dialysate phospholipids concentration in continuous ambulatory peritoneal dialysis (CAPD) patients ranged between 11-25 mg/l [11]. The basic constituent of phospholipids in effluent from peritoneal dialysis patients is phosphatidylcholine (81%) [9, 10]. The surface of the mesothelial membrane also contains phosphatidylserine and phosphatidyletanolamine [4]. It is accepted that phospholipids on mesothelial surface serve as a natural barrier, sealing up intracellular connections, protecting against the invasion of microorganisms and limiting protein loss. By decreasing friction, they facilitate the movement of mesothelial surfaces against each other, preventing the formation of adhesions [3, 5, 12]. The carbohydrate chains of phospholipids are orientated towards the free surface of the mesothelium and are responsible for its hydrophobic properties. Phospholipids covering the mesothelium and present in lamellear bodies are connected with hydrophilic proteins SP-A and SP-B, the same as proteins existing in pneumocytes [3, 13]. These proteins facilitate surfactant secretion and its surface adsorption. They are involved in immunological processes, mainly in phagocytosis [13]. In

lung alveoli there are also SP-B and SP-C surfactant-building proteins, but their presence in dialysate has not been confirmed [13]. The role of phospholipids in ultrafiltration processes is not clear. Beavis et al. [14] did not affirm important relations between phospholipids concentration and ultrafiltration volume. Di Paolo et al. [15] and Ziegler et al. [10] observed that over time, the amount of phospholipids in peritoneal effluent decreases. In patients with low ultrafiltration and during periods of peritonitis, phospholipid concentrations were lower [16]. Low concentrations of phospholipids have also been found in patients with a prior history of peritonitis [10]. With a view to improving the filtration features of the peritoneal membrane, exogenous phospholipid supplementation has been studied with uncertain results. Querques et al. [17] did not observed a statistically significant influence of this therapy on ultrafiltration volume after 15 days of intraperitoneal application of phosphatidylcholine in patients treated with CAPD. However, Di Paolo et al. [15] reported that addition of phosphatidylcholine in the peritoneal cavity may improve ultrafiltration in patients with low values. An increase in ultrafiltration after oral and intravenous phosphatidylcholine supplementation has also been reported [15, 16, 18]. The overall results are inconsistent. There are studies indicating that in patients on CAPD, fluids enriched with phosphatidilcholine may have better results in peritoneal ultrafiltration [19]. On the other hand, Di Paolo [15] found no effect of phosphatidylcholine on those with normal rates of ultrafiltration. The lack of improvement of ultrafiltration after oral supplementation of phosphatidylcholine has also been reported [20, 21]. In animal studies, the intraperitoneal administration of phosphatidylcholine in healthy rabbits did not improve the transport properties of the peritoneal membrane [22] although phospholipids added to peritoneal cavity decreased the frequency of postoperative adhesions [23, 24]. There has been discussion of the potential role of phospholipids adsorbed on mesothelial surface in improving semipermeability [25]. The passage of lipid soluble substances may be facilitated [5].

The purpose of the present study was to determine if correlations existed between dialysate and serum phospholipids concentration and the ultrafiltration rate in patients treated with continuous ambulatory peritoneal dialysis.

MATERIALS AND METHODS

The study was conducted on patients with chronic kidney disease, treated with peritoneal dialysis, during standard peritoneal equilibration test (PET) [26]. After 8 hours dwell time with dialysate composition containing 2.5% glucose, the peritoneal effluent was removed. In a recumbent position, 2000 ml of a new 2.5% peritoneal fluid was introduced at a rate of 200 ml per minute. During this time, the patients changed their position from side to side to mix new dialysate. After 4 hours of dwell time, the dialysate was drained. Specimens of dialysate and serum were collected and the ultrafiltration rate was established.

There were 40 patients (24 males and 16 females). The average age was 51.5 ± 15.8 years (range from 30 to 79 years). Patients were clinically stable, without peritoneal symptoms. The average time of treatment with peritoneal dialysis was 26.4 ± 20.6 months (range from 4 to 72 months). Among causes of chronic kidney failure were: glomerulonephritis (19 patients), type 2 diabetes mellitus (9 patients), interstitial nephritis (3 patients), amyloidosis (3 patients), polycystic kidney disease (2 patients), and mercury intoxication (1 patient). In three cases the cause of kidney failure could not be established.

The specimens of dialysate and serum collected in the 4 hour PET test were used to examine phospholipids concentration. Serum samples taken from patients' ulnar artery were centrifuged at 2000 x

g for 10 minutes and stored at – 70 °C. Dialysate effluent was maintained at the same temperature prior to analysis.

Estimation of phospholipids concentration was performed in serum and in dialysate using enzymatic method with standard diagnostic kit (Wako Chemicals GmbH) in automatic biochemical analyser (Hitachi 902; Roche Diagnostics Poland). The principle of this method is the hydrolysis of phospholipids by phospholipase D, and then estimation of choline released in Trinder's reaction [27]. Choline is oxydated to betaine with use of choline oxidase, with hydrogen peroxide formation. The reaction of hydrogen peroxide and aminoantipiryne and phenol causes a colored reaction, which is measured spectrophotometrically with the length of waves $\lambda = 505$ nm.

Before examinations serum was defrosted, and standard procedure performed to estimate phospholipids concentration. Dialysate effluent, after thawing, was liophylised and 7.5 x concentrated before phospholipid examination. Then it was treated in a similar fashion as the serum was. Results of examination for dialysate were divided by a 7.5 concentration factor. Concentrations of phospholipids in serum were given in g/l, and in dialysate in mg/l.

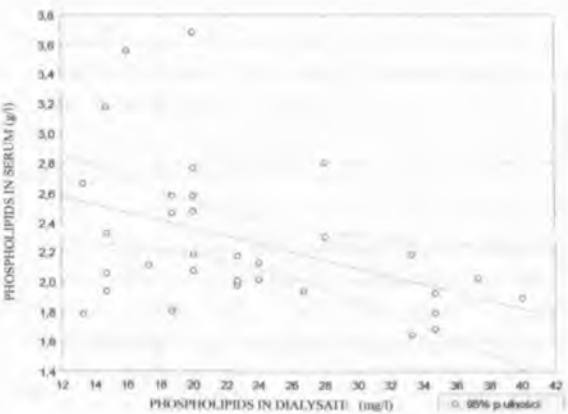
RESULTS

The concentration of phospholipids in dialysate averaged 23.43 ± 7.74 mg/l (range 13.3 to 40 mg/l). Corresponding serum concentration of phospholipids averaged 2.27 ± 0.48 g/l (range 1.65 to 3.69 g/l). There was statistically significant negative correlation between phospholipids concentration in serum and in dialysate ($r = - 0.4146$; $p = 0.0183$) (Fig. 1).

The ultrafiltration volume was 2146 ± 541 ml (range 67 to 2900 ml). The correlation between the amount of ultrafiltration and phospholipid concentration in serum was near statistical significance ($r = - 0.3246$; $p = 0.0571$). This correlation evaluated in tau-Kendall test in the same group of patients had statistically negative importance ($Z = - 2.0096$; $p = 0.0445$). There were no significant correlations between the concentration of phospholipids in dialysate and ultrafiltration volume ($r = 0.0840$; $p = 0.631$).

Fig. 1. Relations between phospholipids concentration in serum and indialysate in 4 hour of peritoneal equilibration test in patients treated with CAPD

(y=2,9029 – 0,0270 x; r= - 0,4146; p = 0,0183



DISCUSSION

Others have shown that dialysate effluent contains phospholipid substances which serve as a surfactant [2, 3, 5]. They are produced by mesothelial cells and play important physiological role, protecting mesothelial surface, decreasing friction and acting as barier against protein loss and bacterial invasion [1, 2, 3, 4, 25]. Phospholipids with surface activity are also present in other physiological fluids including synovial fluid [3, 5]. The main constituent of phospholipid is phosphatidylcholine. This exists in different biological fluids in different proportions. For example, in dialysate about 81% of the phospholipid is phosphatidylcholine [9, 10]. Phospholipid substances present on mesothelial cells surface and in peritoneal fluid exist in the form of lamellar bodies. They are produced by mesothelial cells and may be found in other biological liquids [3, 6, 7, 8]. Phospholipid concentrations in dialysate fluid have been reported to range from 11 to 25 mg/l [11]. During peritoneal dialysis therapy phospholipids are washed out from the mesothelial surface into the dialysate effluent. The concentration of phospholipids in dialysate fluid decreases over time, [10, 15]. Lower concentrations of phospholipids in dialysate have been observed during and after peritonitis, as well as in patients with low ultrafiltration [10, 16]. Conversely, CAPD patients characterized by high mesothelial transport, have been found to have significantly higher phospholipids concentration in dialysate in comparison to low transporters [28]. Others have observed significantly higher choline concentrations in serum of CAPD patients in contrast to healthy people, but not when choline bound with phospholipids was estimated. In the same time there was marked increase of concentration of choline and phosphatidylcholine in dialysate and decrease of free choline concentration in serum during every dialysis session, which may be caused by acute choline loss to dialysate during the first 4 hours of dialysis. After 6 hours, gradual increasing of choline concentration in serum took place, however phosphatidylcholine levels didn't change in the same time. It has been suggested that phospholipids included in the dialysate could influence ultrafiltration, but there is no general agreement. Di Paolo et al. [15] considered that the reduction of ultrafiltration could be caused by decreasing of surfactant concentration in the dialysate. The same observations were made by Ziegler et al. [10]. However Beavis et al. [14] did not show such a correlation. There is no information regarding the possible influence of phospholipids in serum on ultrafiltration.

We attempted to estimate if there is a correlation between phospholipid (surface active substances) concentration in dialysate and in serum and the level of ultrafiltration in patients treated with CAPD. Our results showed that the concentration of phospholipids in dialysate in 4 hours of a standard peritoneal equilibration test in CAPD patients was between 13.3 and 40 mg/l (average value = 23.43 ± 7.74 mg/l). Phospholipids concentration in serum in the same group of patients in the same time was from 1.65 to 3.69 g/l (average value 2.27 ± 0.48 g/l). We did not observe a statistically significant correlation between phospholipids concentration in dialysate and the volume of ultrafiltration in CAPD patients. However, we noted the existence of a significant negative correlation between ultrafiltration rate and serum concentration of phospholipids in this group of patients. The likelihood that intraperitoneal supplementation of phosphatidylcholine and its intravenous or oral supplementation could increase ultrafiltration in CAPD patients has been discussed but with little agreement [15, 16, 17, 18, 19, 20, 21, 22]. Based on our studies we conclude, that phospholipids present in serum could influence ultrafiltration rate in patients treated with CAPD, but these issues need further studies.

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ABSTRACT

Objective: Substances with surfactant activity are present on the surface of mesothelium and in peritoneal effluent, protecting the mesothelium and improving tissue lubrication. Among these substances, phospholipids (PL) appear to play crucial roles. In patients treated with continuous ambulatory peritoneal dialysis (CAPD) PL concentration in dialysate decreases over time. PL may increase mesothelial semipermeability although the significance of PL in improving ultrafiltration is not clear.

The aim of our study was to determine if there was a correlation between serum and peritoneal PL concentrations and their ultrafiltration rates in CAPD patients as determined with peritoneal equilibration tests (PET).

Material and methods: The study was conducted in 40 CAPD patients (24 M, 16 F) aged 51.5 ± 15.8 years. The mean CAPD duration in this group was 26.4 ± 20.6 months. Dialysate effluent and serum for examination of PL concentration were taken after 4 hour dwell time. Patients were in a stable clinical state, without peritonitis. Concentration of total PL was examined with colorimetric method. For dialysate PL examinations dialysate was lyophilized and 7.5-fold condensed.

Results: Phospholipids concentration in dialysate was 23.43 ± 7.74 mg/l and in serum 2.27 ± 0.48 g/l. There was a significant negative correlation between PL concentrations in serum and in dialysate ($r = -0.4146$; $p = 0.0183$). There was no significant correlation between ultrafiltration and dialysate PL concentration ($r = -0.0739$; $p = 0.631$). We found a significant negative correlation between ultrafiltration and PL concentration in serum in CAPD patients in 4 hours of PET ($Z = -2.0096$; $p = 0.0445$).

CONCLUSION: We conclude that serum concentration of phospholipids may influence ultrafiltration rate in CAPD patients.

Key words: Peritoneal dialysis, phospholipids, ultrafiltration

STRESZCZENIE

Cel: Substancje o aktywności powierzchownej są obecne na powierzchni mezotelium i w płynie otrzewnowym, chroniąc otrzewną i poprawiając jej właściwości "smarowania". Wśród nich fosfolipidy (PL) wydają się odgrywać kluczową rolę. U chorych leczonych ciągłą ambulatoryjną dializą otrzewnową (CADO) stężenie PL w dializacie zmniejsza się z czasem trwania dializ. PL mogą poprawiać półprzepuszczalność otrzewnej, jednak ich znaczenie w zwiększaniu ultrafiltracji nie jest jasne.

Celem naszego badania była ocena czy istnieją zależności pomiędzy surowiczymi i otrzewnowymi stężeniami fosfolipidów oraz ultrafiltracją u pacjentów leczonych CADO, podczas testu równoważenia otrzewnowego (PET).

MATERIAŁ I METODY: Badanie przeprowadzono w grupie 40 chorych leczonych CADO (24 M, 16 K) w wieku 51.5 ± 15.8 lat. Średni czas leczenia CADO wynosił 26.4 ± 20.6 miesięcy. Dializat i surowicę do oznaczenia stężenia PL pobierano w 4 godzinie testu PET. Pacjenci byli w stabilnym stanie klinicznym, bez cech zapalenia otrzewnej. Stężenie całkowitych PL oznaczano kolorymetrycznie. Dializat przed oznaczeniami liofilizowano i zagęszczano 7,5-krotnie.

WYNIKI: Stężenie fosfolipidów w dializacie wynosiło 23.43 ± 7.74 mg/l a w surowicy 2.27 ± 0.48 g/l. Stwierdzono istotną ujemną zależność pomiędzy stężeniem PL w surowicy i dializacie ($r = - 0.4146$; $p = 0.0183$). Nie znaleziono istotnych zależności między ultrafiltracją i stężeniem PL w dializacie ($r = - 0.0739$; $p = 0.631$). Stwierdzono istotną ujemną zależność pomiędzy ultrafiltrają i stężeniem PL w surowicy u chorych leczonych CADO, w 4 godzinie testu PET ($Z = - 2.0096$; $p = 0.0445$).

WNIOSKI: Wnioskujemy, że stężenie fosfolipidów w surowicy może wpływać na ultrafiltrację u chorych leczonych CADO.

Słowa kluczowe: Dializa otrzewnowa, fosfolipidy, ultrafiltracja

DECLARATION OF INTEREST:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.