

EVALUATION AND MODELING OF PHARMACOKINETICS OF COPPER ION DURING HEMODIALYSIS

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Abstract

The present study investigated the kinetics of copper levels during renal dialysis. Plasma and dialysis fluid level of copper were evaluated using an Atomic Spectrophotometric Absorption method. The evaluation included the determination of mean blood level of a control group and a chronic renal failure patients group. It was found that plasma levels of copper were significantly lower in patients than in control group. The concentration of copper in dialysis fluid was ten times lower than the mean concentration in blood.

Measurement of time course of copper during dialysis allowed an estimation of transfer and pharmacokinetic model of copper in patients during dialysis. The model was conceived as a tricompartmental model: tissue compartment, blood and dialysis fluid compartment.

Following the fact that the transfer across dialysis membrane is slow, blood appeared to act as a reservoir, the difference between before and after dialysis blood levels being moderate.

Time course of copper concentration in dialysis fluid was approximately linearly dependent on the square root of time. A linear dependence of logarithm of concentration on time could be considered as an alternative model. In fact, it was difficult to make a correct hierarchy between the two models, since the number of points and time intervals were not large enough. But both models suggest the behaviour of blood as a reservoir, not too much affected by the dialysis and consequently the tricompartement model degeneration as a pseudo-monocompartmental model.

Rezumat

Studiul de față are drept scop determinarea cineticii eliminării cuprului la pacienții cu insuficiență renală în timpul dializei. Nivelul plasmatic al cuprului și cel din lichidul de dializă au fost determinate folosind o metodă spectrofotometrică de absorbție atomică. Evaluarea include determinarea nivelului cuprului în sânge la un grup martor, cu funcție renală normală și determinarea concentrației cuprului la un lot de pacienți cu boală cronică de rinichi, supuși dializei, la care s-a determinat atât concentrația cuprului în sânge, cât și în lichidul dializat. Deși concentrația medie plasmatică a cuprului a scăzut în urma

procesului de dializă, media concentrațiilor plasmatice la sfârșitul ședinței de dializă nu diferă semnificativ față de media concentrațiilor plasmatice de la începutul ședinței de dializă. Măsurarea concentrației cuprului în timpul dializei a permis o estimare a transferului și modelului farmacocinetic al cuprului la pacienții cu boală cronică de rinichi. Modelul a fost conceput ca un model tricompartimental: compartimentul tisular, sânge și lichidul dializat. Urmare a faptului că transferul în întreaga membrană de dializă este lent, sânge apare ca un rezervor, diferența între concentrația sanguină a cuprului înainte și după dializă fiind moderată. Scăderea concentrației de cupru în lichidul dializat a fost liniar dependentă de rădăcina pătrată a timpului. O dependență liniară a logaritmului concentrației funcție de timp ar putea fi considerată ca un model alternativ. Este dificilă ierarhizarea corectă a celor două modele, deoarece numărul de puncte și intervale de timp nu au fost suficient de mari. Dar ambele modele sugerează comportamentul sângelui ca un rezervor, fără a fi prea mult afectat de dializă și, în consecință degenerarea modelului tricompartimental la un model pseudo-monocompartimental.

Keywords: Copper, pharmacokinetic model, hemodialysis, Atomic Absorption Spectrometry, chronic renal disease

Introduction

Hemodialysis removes toxins from blood by allowing equilibration of their plasma and dialysate concentrations across a semi-permeable membrane.

In order to avoid significant modifications of essential ions such as potassium, sodium, bicarbonate, and calcium in blood, dialysis fluid contains these ions at concentrations comparable with their physiological level.

The dialysate concentration of other substances such as trace elements is not routinely manipulated. Substances that have lower concentrations in dialysate than in blood tend to be removed by dialysis. Although this is appropriate in the case of uremic toxins, it may lead to depletion of biologically essential substances.

Hemodialysis patients are exposed to very high volumes (>300 liters/week) of dialysate. Therefore, even low levels of toxic substances in source water could lead to concentration gradients between blood and dialysate, which in turn could lead to blood "contamination" and some substances present in dialysate but not in blood will tend to accumulate in the patient, and the lack of renal clearance in hemodialysis patients might theoretically lead to toxicity. Thus, hemodialysis patients are at theoretical risk for both deficiency and accumulation of trace elements, depending on removal by dialysis and the composition of the source water used for hemodialysis [1-6].

Many chemical elements existing in nature are also found in the human body, most of them playing an important role. Homeostatic mechanisms keep their concentration in the human body within a normal range. Their concentration in body fluids can be analytically evaluated. The biological samples used to determine these concentrations are: blood, urine, cerebrospinal fluid, hair, nails, tears and sweat. [2,7,8] The normal serum level is 110 $\mu\text{g/dL}$ [9].

Chronic hemodialysis patients generally have a copper deficiency, a condition that occurs in patients with nephritic syndrome, chronic glomerulonephritis and normal renal function. Copper deficiency in hemodialysis appears especially if it is associated with zinc overloading [3].

Materials and Methods

Clinical protocol

The study was a randomized, cohort analysis for two groups of patients: one group with normal renal function – the control group – in which were determined the copper blood levels and one group of patients with chronic renal disease in which were determined the copper blood levels, as well as the concentrations in dialysis fluid.

The inclusion criteria for the control group were: normal renal function and less than 65 years. The inclusion criteria for the second group were: chronic dialysis in a medical center, 13.5 hours of dialysis per week, age less than 65 years.

The control group included 50 subjects (26% females and 74% males), age 39.82 ± 0.65 years. They were considered healthy based on their creatinine normal values (0.93 ± 0.22 mg/dL, range 0.56-1.5).

The patient group included 34 patients (35.3 % females and 64.7 % males), age 53.62 ± 11.34 years.

The vascular approach was made by arterial-venous fistula or long - life catheter. The dialysis fluid flow was 500 mL/min.

In the course of the study, the samples were taken as follows:

- control group – 1 mL of blood in a tube with heparin for determining copper, and blood for determination of creatinine level; a normal blood level of creatinine is a marker of a normal renal function;
- patients group - 1 mL of blood in a tube with heparin before the dialysis started; 1 mL of blood at the end of dialysis; 10 mL of dialysis fluid at time intervals fixed by the protocol study. The blood creatinine levels of this group were significantly high, as a marker of a kidney disease.

The study was conducted according to the principles of Declaration of Helsinki (1964) and its amendments (Tokyo 1975, Venice 1983, Hong

Kong 1989) and Good Clinical Practice (GCP) rules. The study was approved by the Ethics Committee of UMF "Carol Davila" Bucharest and patient consent was obtained for the collection and analysis of biological samples.

Bioanalytical method

For the evaluation of the studied elements, it was used a spectrometry installation of atomic absorption spectrometry with Atomization in Graphite Furnace. – GF-AAS. [9], AAS – Varian installation: SpectrAA 880 – atomic absorption spectrometer; atomizer with Graphite Furnace GTA 100; auto sampler PSD 25; water cooler CFT 33 – Neslab; nitrogen generator, analytical nitrogen – 99.999% purity; analytical argon – 99.999% purity (Linde).

Blood samples (1 mL) were collected in anticoagulant tubes at connecting and restitution dialysis fluid.

The dialysis fluid samples (10 mL) were taken during the dialysis at 30 min, 1, 2, 3, 4 hours from dialysis started.

Blood processing method consisted in mixing 200 μ L blood with 800 μ L antifoam B and 1 mL 1.6N HNO₃. After 20 minutes, the samples were centrifugated for 10 minutes at 2000 rpm. 500 μ L supernatant were used for the injection in the system [10].

Dialysis fluid processing method consisted in addition of 1mL nitric acid 65%. After 20 minutes, the sample was centrifugated for 10 minutes at 2500 r.p.m. 500 μ L supernatant were used for the injection in the system. [10]

Dialysis fluid processing method consisted in addition of 1 mL nitric acid 65%, After 20 minutes, the sample was centrifugated for 10 minutes at 2500 r.p.m. The supernatant represented the matrix for the injection in the system.

Determination of copper levels included the following steps: automatic dilutions; 20 μ L injection.

Working parameters. Split width – 0.5 nm; background correction – none; drying – 120°C; calcination – 800°C; atomization – 2300°C; cleaning - 2500°C; calibrating in concentration; 3 points calibration curve – 0, 50, and 100 μ g/dL; recalibration rate -15. Automatically quality control: dispersion (<10%); correlation coefficient (>0.998); detection limit of method (DLM <1.0 μ g/L); detection limit of instrument (< 0.04 μ g/L) [10].

Estimation of concentration included measurement of peak height; smoothing – 9 points; replicated twice for standard and sample; wavelength – 327.4 nm.

A rational calibration curve [10] was stabilized to fit to model:

$$A/C = a + bA + cA^2$$

A - absorbance of the sample;

C - concentration of the sample;

a, b, c – calibration curve coefficient.

Correlation coefficient needed in automatically quality control was lower than 0.998.

We used Student test for the statistical comparisons, considering the data were normally distributed.

Results and Discussions

The mean copper levels were found to be 99.3 µg/dL in the control group and 61.13 µg/dL in the patients group. This decreased concentration in patients was also reported by other authors [10].

Distribution of individual data are presented in figure 1.

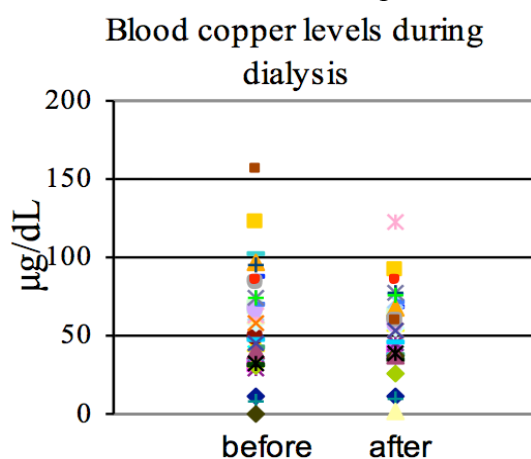


Figure 1
Blood copper levels

It can be seen that some points look to be outliers, the greatest part of data being grouped homogenously in a short interval.

Comparison of blood copper levels before and after dialysis indicates a decrease of concentration following the transfer across membrane to dialysis fluid, from 61.13 µg/dL to 55.32 µg/dL. To check if the values are normally distributed, the means were compared using Student Test. The result was that the means do not differ significantly. Elimination of data which deviated more than two standard deviations from the mean as outliers led to means much closer and again, the difference appears as non significant. There were eliminated in fact patients with very low levels

before and high levels after dialysis since is not reliable to think to a great increase of blood copper levels during dialysis. On the other hand data are well correlated lineary and the slope of the estimated line is 0.91 indicating a moderate reduction of levels during dialysis (Figure 2). This is a normal result since a part of copper migrated in fluid of dialysis. The apparent contradiction between the non-significant difference and the linear decrease can be solved if we consider that the concentration in dialysis fluid is much lower than in blood and the replacement of transferred copper by ions coming from tissues compartment is rapid enough.

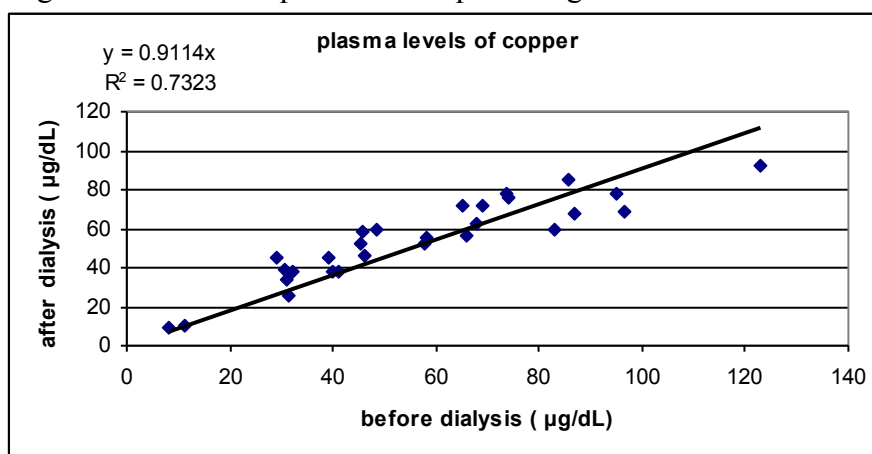


Figure 2

Linear correlation between blood levels of copper before and after dialysis.

Blood to dialysis fluid transfer. Values for copper concentrations in dialysis fluid are presented in Figure 6, and the mean copper concentrations as function of time are presented in Figure 7. Once again some data appear as outliers or correspond to a special physiopathologic class.

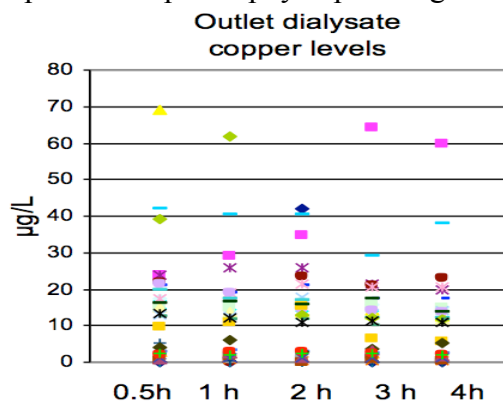
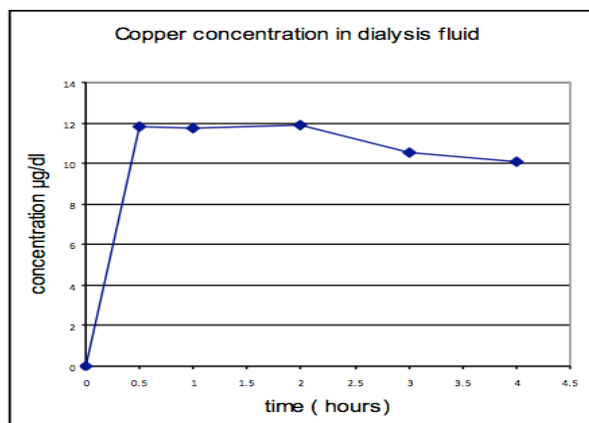


Figure 3

Copper levels in dialysis fluid

**Figure 4**

Time course of copper concentration

After elimination of patients having at least one value far more than three standard deviations from the mean, as outliers, the evolution of concentrations appeared smoother and it was possible to fit the data with solutions of different theoretical models.

Transformation of concentrations in eliminated quantities used the formula:

$$Q(t_k) = \sum_{i=0}^k DFF \frac{C(i) + C(i+1)}{2} (t_{i+1} - t_i)$$

where:

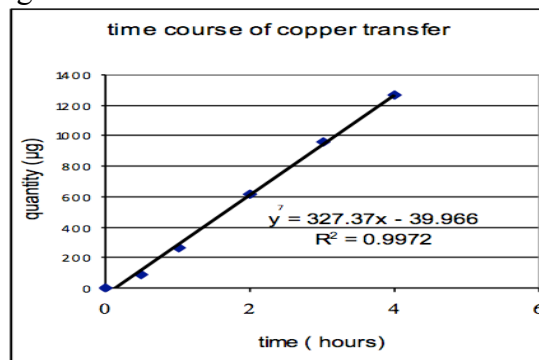
Q – eliminated quantity (µg);

DFF – dialysis fluid flow (0.5 L/min);

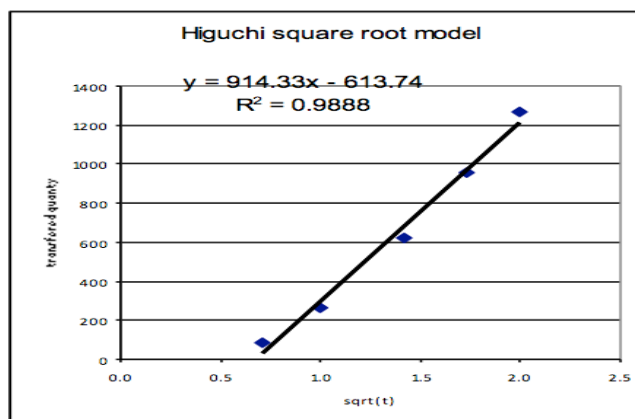
C(i) – element concentration in the dialysate fluid

t_i – time of determination (min).

Representation of eliminated quantity as function of time is presented in the figure 6.

**Figure 6**

Time course of eliminated copper quantity

**Figure 7**

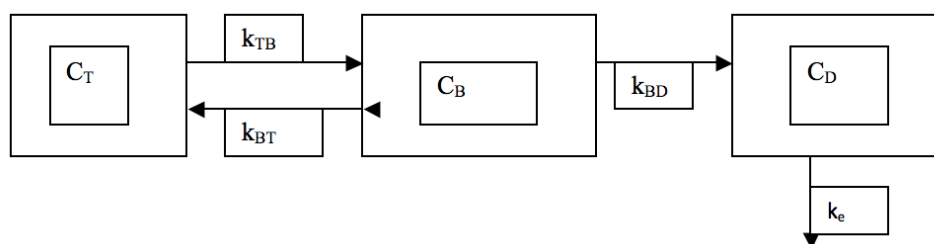
Dependence of eliminated quantity of copper on square root of time

As can be seen from the figure, the transfer is linear depending on time. Since, as it was underlined before, the concentration in blood is much higher than in plasma and is possible to consider the blood as a reservoir, we checked the application of square root law describing the release of active substances from "reservoir" pharmaceutical formulations.

Compartmental modelling

One- and two-compartmental models of creatinine are well known [11,12,14-16]. These models make it possible to analyze the course of treatment and to predict the effect of dialysis procedures. Mathematical modeling helps physicians to match dialysis therapy to the individual needs of the patient.

It is rational to think that pharmacokinetics of Cu^{2+} follow a three-compartmental model: tissue compartment, blood compartment and dialysis fluid compartment (Figure 8).

**Figure 8**

Three-compartmental model for dialysis procedure

with the associated equations:

$$\begin{aligned}\frac{dC_T}{dt} &= -k_{TB}C_T + k_{BT}C_B \\ \frac{dC_B}{dt} &= k_{TB}C_T - k_{BT}C_B - k_{BD}C_B \\ \frac{dC_D}{dt} &= k_{BD}C_B - k_eC_D\end{aligned}$$

The solution of this system for C_D is not very difficult to obtain, but it is really impossible to estimate all coefficients of transfer starting only from data of the dialysis fluid. In these conditions we simplified the model starting from the previous analysis which suggested that the concentration in blood is less affected by the transfer toward the dialysis fluid and the blood appears as a large reservoir of copper.

The model will become:

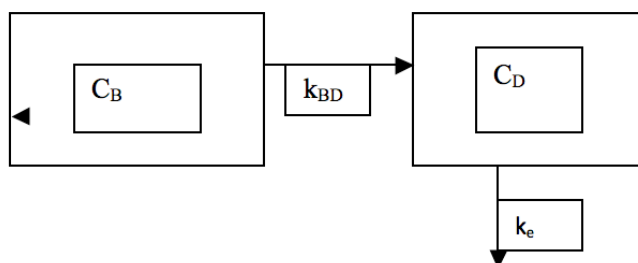


Figure 9
Copper dialysis model

For this model the solution for C_D is:

$$C_D(t) = \frac{C_B^0 k_{BD}}{k_{BD} - k_e} (e^{-k_e t} - e^{-k_{BD} t})$$

a so called absorption elimination curve.

If this is the model, since maximum concentration appears at the first measuring time and the decrease is slow, the curve of copper concentration suggests that the transfer from blood is much more rapid than the elimination. In fact, the elimination is determined by the flow of dialysis fluid.

Although two and three compartmental models are more effective in describing the time course of trace elements in dialysis fluid, they are much more complex than one-compartmental models, which justifies the large use of one-compartmental model [11,12].

Since data suggested that $k_{BD} > k_e$, the model can be simplified once again by neglecting $e^{-k_{BD}t}$ and it obtains:

$$C_D(t) = \frac{C_B^0 k_{BD}}{k_{BD} - k_e} e^{-k_e t} = \alpha e^{-k_e t}$$

If this is the case, the logarithm of concentration has to be linearly dependent on time:

$$\ln C_D(t) = \ln \alpha - k_e t$$

The examination of figure 8 suggests that the model could be considered reliable.

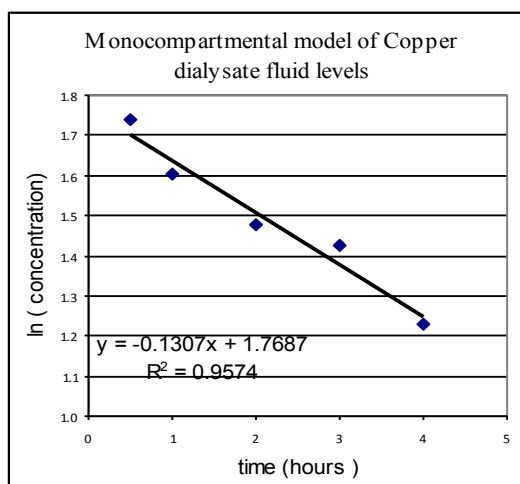


Figure 10

Time dependence of logarithm of concentration of copper in dialysis fluid

Another applicable model could be considered the model which retains only the transfer across the membrane, when the concentration on one side is much higher than the concentration on the other side, similar to what happens with the release of active substance from solid or semisolid media in sink conditions [13]. Such model leads to description of the transferred substances by square root laws, which also appeared compatible with our data.

The physiological approach took into consideration also blood flow limitations and heterogeneous flow distribution in the removal of solutes models were established by experiments on artificial kidney [14].

Later, based on physiologic data on organ perfusion and organ water content, urea transport during dialysis was described, not only by traditional two compartments representing sequestration of urea in various tissues, but

also to additional compartments representing heterogeneity of urea concentration in blood. [12][15 -16].

Parameter identification using radiolabeled urea and dialysis data could give information concerning the ratio of central to peripheral compartment volumes. [17] The within-blood differences between arterial and venous blood can be estimated as functions of dialyzer clearance (K_d), cardiac output (CO), access blood flow (Q_{ac}), and extracorporeal blood flow (Q_b). Cardiac output and access flow as well as the magnitude of arteriovenous and extra-intracorporeal gradients can be measured during dialysis, or mean values can be assumed for the dialysis population [19][20]. However, the variability is high and compartment volumes and time constants to characterize these blood-pool gradients are small and significant errors can result in dialysis.

Following the above difficulties personalization of dialysis based on pharmacokinetic parameters remains an elusive goal. Mono, bi or tricompartamental models like that applied by us in the case of copper are written but finally, in current practice, physicians look mostly to a monocompartamental model, otherwise recommended in dialysis devices utilization handbook.

Conclusions

Although the mean plasmatic copper concentration was lower after dialysis, there was no significant difference regarding the mean plasmatic concentration after dialysis and the one before.

In comparison with the control group, the mean copper concentration in patients was significantly lower.

Concentration of copper in dialysis fluid was ten times lower than the mean concentration in blood.

Copper pharmacokinetics in the tissue, blood, dialysis fluid is essentially tricompartamental.

Following the fact that transfer across membrane is slow, blood appears to be a reservoir, the transfer affecting less the plasma levels.

Time course of copper in dialysis fluid can be considered as lineary dependent on the square root of time. Dependence of logarithm of concentration on time could be considered also linear. In fact it is difficult to make a correct hierarchy between the models, since the number of points and the time interval are not large enough. But both models suggested the behaviour of blood as a reservoir, less affected by the dialysis.

Finally, the large used monocompartmental model for describing the clearance of urea in evaluation of dialysis efficacy, could be extended to trace elements also.

Acknowledgements

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