

Analysis of Mg, Ca and Sr distribution between blood plasma and blood cells

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For the first time to our knowledge the distribution of magnesium and calcium between blood plasma and blood cells in hepatitis C infected samples has been analyzed. The character of the distribution depends very much on the metal and the duration of the infection, suggesting that there is a variety of physico-chemical forms of Mg and Ca in the blood of the patients infected with hepatitis C. The achieved results have also demonstrated that the proposed analytical procedures can be successfully applied for a rapid and accurate determination of the distribution of Mg and Ca in human blood plasma and blood cells.

Key words: blood analysis, plasma, cells, Mg and Ca determination, FAAS

INTRODUCTION

Blood is a circulating tissue composed of fluid plasma and cells (red blood cells, white blood cells and platelets). The main function of blood is to supply nutrients (oxygen, glucose) and constitutional elements to tissues and to remove waste products. Blood also enables cells and different substances (amino acids, lipids, hormones) to be transported between tissues and organs. On the other hand, blood is considered to be a connective tissue holding everything together. Problems with blood composition or circulation can lead to a downstream tissue dysfunction [1]. The physiological importance of metals for a human organism, especially for blood cells, blood plasma or blood serum has been shown by many publications. At low concentrations, metals play an important role in metabolism and biological processes as enzyme activators, stabilizers, functional components of proteins, etc. Toxic doses of metals and their compounds can lead to serious health problems [2–5].

Moreover, the distribution of metals between blood plasma and blood cells serves as very important clinical information as well [6–8]. Different metals present in the composition of blood can form different complexes with amino acids, fatty acids, albumin, glucose, fibrino-

gen, cholesterol and many other organic compounds and biomolecules, which have been found to be the main constituents of blood; also with an enormous amount of different chelating ligands (orally and intravenously active chelators) that can be introduced into the blood of patients as various therapeutical reagents [9–11].

There is an increased interest in the role of IIA group metals in clinical medicine, nutrition and physiology [12–14]. For instance, a deficiency of magnesium in blood (hypomagnesemia) among other disorders causes cardiac arrhythmia and increased irritability of the nervous system with tetany. On the other hand, excess of calcium within a cell may damage it or even cause it to undergo apoptosis [15–17].

The information about the changes of the concentration of Mg and Ca in a human body could be also useful to make different diagnoses such as cancer [8, 18]. The aim of the present study was to investigate the distribution of Mg, Ca and Sr between blood plasma and blood cells in the blood samples from the patients infected with hepatitis C.

EXPERIMENTAL

The amount of metals in the blood samples from the patients infected with hepatitis C was determined by flame atomic absorption spectroscopic method (FAAS)

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using a Hitachi 170-50 spectrometer [19]. The instrumental parameters were adjusted according to the manufacturer's recommendations. The following conditions for metal determination by the FAAS method were used: (i) absorption line (285.2 nm Mg, 422.7 nm Ca and 406.7 nm Sr), (ii) electric current of the lamp (10 mA Mg, Sr and 15 mA Ca), (iii) flame – acetylene, (iv) gas pressure (2.45×10^6 Pa (Mg) and 2.94×10^6 Pa (Ca, Sr)), and (v) pressure of air – 1.47×10^7 Pa. Double-distilled water and analytical-grade reagents were used for the preparation of stock standard solutions of metals, which were used to obtain calibration solutions.

The blood samples were taken from 15 volunteer patients infected with hepatitis C. The determination of metals in the blood specimens was performed directly without any preconcentration. For the analysis of the metal distribution between blood plasma and blood cells, the immediate separation of plasma from cells was performed by centrifugation (spin speed 8000 min^{-1}) prior to the FAAS determination. For the determination of metals in the blood samples, the specimens to be analyzed were burnt in an ordinary furnace at $600 \text{ }^\circ\text{C}$. The obtained residuals were dissolved in 10 ml of nitric acid (1:1), transferred into a 25-ml volumetric flask and diluted with double-distilled water.

RESULTS AND DISCUSSION

For the evaluation of metal distribution between blood plasma and blood cells, the blood samples were immediately spun to separate the plasma from the cells. It is interesting to note, that according to the FAAS analysis data, Sr was not found neither in blood plasma nor in blood cells of the blood samples infected with hepatitis C, or its concentration was lower than the detection limit ($0.2 \text{ } \mu\text{g/g}$) determined for this element. Therefore, only the distribution of Mg and Ca was analyzed in the further experiments. The dependences of the recovery of magnesium and calcium in an accidentally taken blood sample on the duration of centrifugation are shown in Figs. 1 and 2, respectively. As it is seen, full separation of plasma from cells was achieved after 6–8 minutes. Therefore, the duration of eight minutes for the separation of plasma from cells in all analytes infected with hepatitis C has been selected.

The fifteen patients infected with hepatitis C were chosen by chance. A random distribution of magnesium in plasma and cells is evident from the results presented in Fig. 3. As seen, the concentrations of Mg determined in different parts of blood are rather similar. However, calcium, the second element from the same group in the periodic system of elements shows a little different distribution (Fig. 4). After the separation, the most part of Ca remained in blood plasma. A higher amount of calcium in blood cells was determined only in the infected blood analytes 4 and 5. The results presented in Figs. 3 and 4 clearly demonstrate that both metals exist in the blood in different physico-chemical forms.

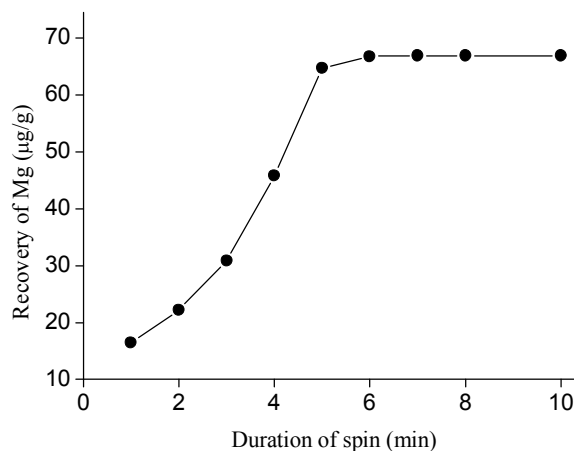


Fig. 1. Dependence of the distribution of Mg between blood plasma and blood cells on the duration of centrifugation

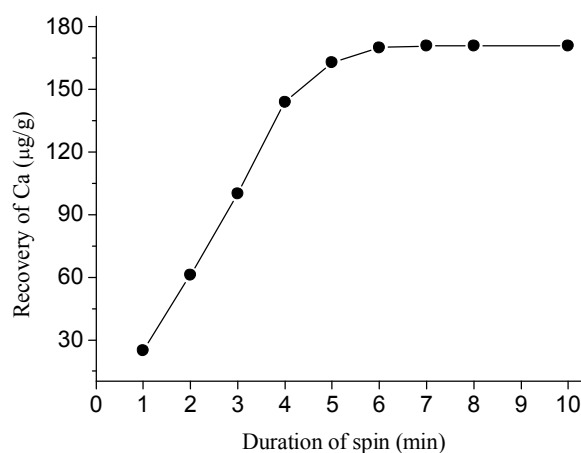


Fig. 2. Dependence of the distribution of Ca between blood plasma and blood cells on the duration of centrifugation

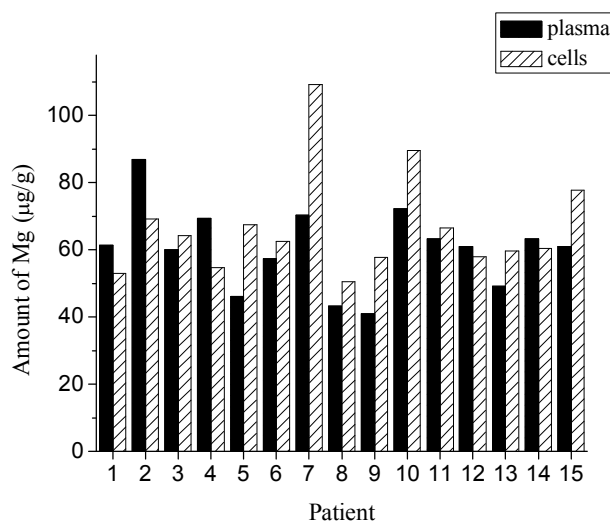


Fig. 3. Distribution of Mg between plasma and cells in blood samples from different patients

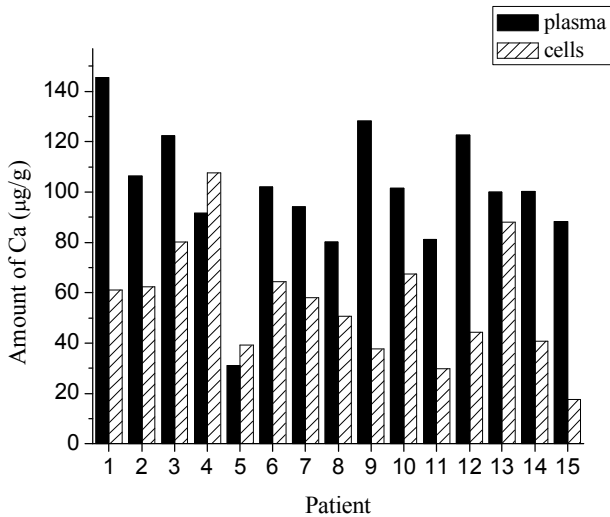


Fig. 4. Distribution of Ca between plasma and cells in blood samples from different patients

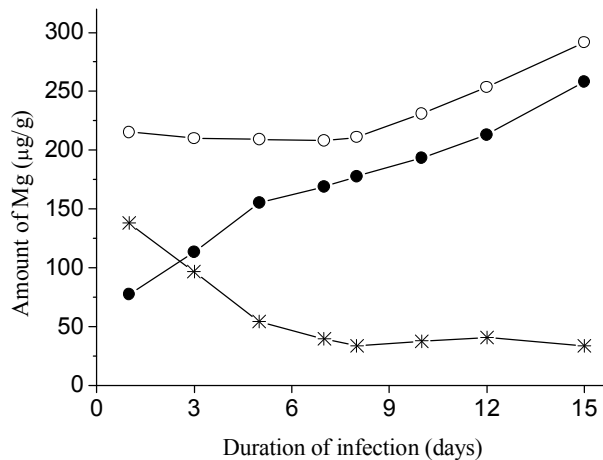


Fig. 5. Dependence of Mg content in blood from different patients on the duration of infection: (○) total amount, (●) in plasma, and (*) in cells

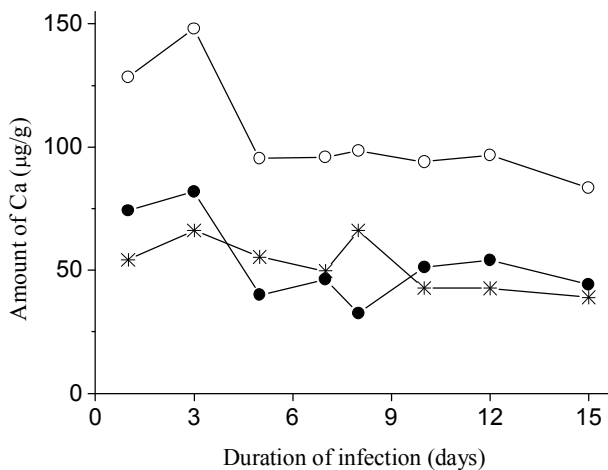


Fig. 6. Dependence of Ca content in blood from different patients on the duration of infection: (○) total amount, (●) in plasma, and (*) in cells

Possibly, the metal ions bound to different chelators or proteins and not bound ions distribute in the plasma and cells differently during centrifugation. It is interesting to note that a random distribution of both elements (magnesium and calcium) in the plasma and cells was observed for a control group (not infected with hepatitis C) of patients.

The distribution of magnesium and calcium levels depending on the duration of the infection was also investigated. For this purpose, blood samples from eight patients differently infected with hepatitis C were checked. Figure 5 shows the distribution of magnesium between plasma and cells in the patients having a slightly different background of the infection. The results showed that magnesium concentrations in the blood plasma and cells differ significantly in differently sick patients. As seen, the Mg content in the blood plasma increases with the increasing duration of the illness. Moreover, the amount of magnesium in the blood plasma predominates in the samples from long-time infected patients. On the contrary, almost no change was observed in the magnesium concentration in the blood cells of the same patients. These results suggest a new hypothesis that the amount of Mg in the blood plasma of untreated hepatitis C patients could be related to the occurrence or progression of the infection. As it was mentioned, magnesium levels in the blood plasma were found to be clearly increased, whereas almost no change was observed in calcium levels in the patients infected with hepatitis C as compared to the duration of illness (see Fig. 6). Such different behaviour of Mg and Ca is very interesting and rather unexpected, since the complex formation ability of these two metals with chelating ligands or proteins should be very similar.

The distribution of magnesium and calcium concentrations between blood plasma and blood cells were also estimated in female and male patients infected with hepatitis C. Absolutely no tendency of the distribution of magnesium in different parts of blood between female and male patients was detected. Figure 7 represents a distribution diagram for magnesium. As it is seen, the blood samples from the male patients contain a bit higher amount of magnesium. Besides, the content of magnesium slightly prevails in blood plasma for both female and male patients infected with hepatitis C. A very similar situation was identified in the distribution of calcium in the constituents of blood of female and male patients. Almost equal distribution of calcium in both plasma and cells was observed (Fig. 8).

In fact, too little patients were investigated in this study. Medical conclusions could be made only after a careful and systematic investigation of numerous patients having different health troubles. However, the results of the distribution of magnesium and calcium between blood plasma and blood cells in patients infected with hepatitis C are promising for further medical observation.

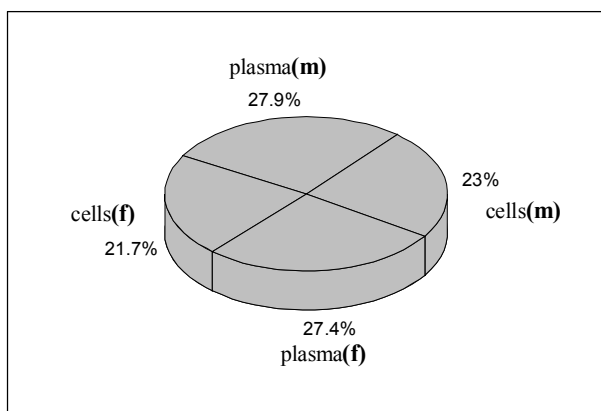


Fig. 7. Distribution diagram for magnesium: (f) – female, (m) – male

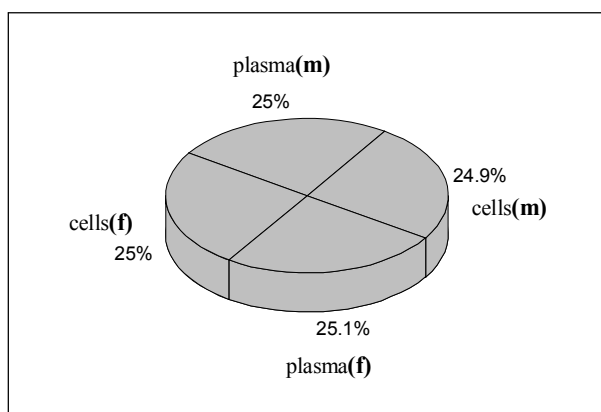


Fig. 8. Distribution diagram for calcium: (f) – female, (m) – male

CONCLUSIONS

The distribution of Mg, Ca and Sr in the blood samples from the patients infected with hepatitis C were examined by the method of flame atomic absorption spectrometry. Interestingly, strontium was not detected neither in the blood plasma nor in blood cells of the investigated samples. The obtained results also showed that magnesium and calcium are differently distributed between plasma and cells depending on the patient. Such behaviour could be related to the existence of a variety of physico-chemical forms of metals in the human blood infected with hepatitis C. The distribution of magnesium and calcium levels depending on the duration of the infection was also investigated. The results revealed that the magnesium concentrations in the blood plasma and cells differ significantly in differently sick patients. The magnesium levels in the blood plasma infected with hepatitis C increased with an increasing duration of the illness. It was determined, however, that the calcium levels are independent of the duration of the infection. Besides, no tendency of the distribution of magnesium of the different parts of blood (plasma and cells) between female and male patients was detected.

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Mg, Ca IR Sr PASISKIRSTYMO KRAUJO PLAZMOJE IR KRAUJO KŪNELIUOSE TYRIMAS

Santrauka

LAAS metodu ištirtas Mg, Ca ir Sr pasiskirstymas tarp kraujo plazmos ir kūnelių žmonių, infekuotų hepatito C virusu, kraujyje. Įdomu pažymėti, kad stroncio nebuvo aptikta nė viename iš tirtų kraujo pavyzdžių. Nustatyta, kad magnio ir kalcio pasiskirstymo kraujo plazmoje ir kūneliuose priklausomybė yra nevienoda skirtingų ligonių atveju. Tačiau skirtingos lyties žmonių kraujyje magnio ir kalcio pasiskirstymas tarp plazmos ir kūnelių yra gana tolygus.