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Effects of Calcium (II), Magnesium (II), Copper (II) and Iron (II) Ions on Ischemia Modified Albumin

[İskemi Modifiye Albumin Üzerine Kalsiyum (II), Magnezyum (II), Bakır (II) ve Demir (II) İyonlarınn Etkisi]

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ABSTRACT

Ischemia modified albumin is altered albumin by free radicals generated from ischemic tissues. Albumin cobalt binding assay is an indirect colorimetric assay of ischemia modified albumin. In this study we investigated effects of calcium (II), magnesium (II), iron (II), and copper (II) concentrations on the albumin cobalt binding assay. These cations were added into serum pools by increasing initial concentrations of 20, 50 and 100 %. Ischemia modified albumin was measured five times in these pools. Percent differences of absorbance values from the baseline absorbance values of ischemia modified albumin which were accepted 100 %. Percent differences from the baseline for calcium (II)- magnesium (II)- and copper (II)-spiked pools were as follows, respectively: at 20 % concentration increase: 115, 101, and 103 %; at 50 % concentration increase: 104, 101, and 125 %; at 100 % concentration increase: 107, 102, and 107 %. These deviations from the baseline were 97, 120, 109, 96 and 94 % at increased iron (II) concentration of 50, 100, 200, 300, and 400 %, respectively. In conclusion, albumin cobalt binding assay is not significantly affected by calcium (II), magnesium (II) and iron (II) concentrations but affected by copper (II).

Key Words: Ischemia modified albumin, albumin cobalt binding test, acute coronary syndrome.

ÖZET

İskemi modifiye albümin, iskemik dokulardan üretilen serbest radikaller ile değişikliğe uğrayan albümini, albümin kobalt bağlama testi ise iskemi modifiye albüminin indirekt kolorimetri yöntemi ile ölçümünü tanımlar. Çalışmamızda kalsiyum (II), magnezyum (II), demir (II) ve bakır (II) konsantrasyonunun albümin kobalt bağlama testi üzerine etkisini araştırdık. Başlangıç serum havuzlarına konsantre katyon çözeltileri eklenerek katyon konsantrasyonlarında % 20, 50 ve 100'lük artışlar sağlandı. Bu havuzlarda eş zamanlı olarak beşer kez iskemi modifiye albümin ölçümü yapıldı. Başlangıç havuzlarının absorbans değerleri ortalaması temel alınarak absorbans değerlerindeki yüzde değişim hesaplandı. Kalsiyum (II), magnezyum (II) ve bakır (II) için % 20 konsantrasyon artışı sırasıyla % 115, 101 ve 103; % 50 konsantrasyon artışı % 104, 101 ve 125; % 100 konsantrasyon artışı % 107, 102 ve 107 değişime neden oldu. Demir (II) için % 50, % 100, % 200, % 300, % 400 konsantrasyon artışı sağlanan çözeltilerde değişim sırasıyla % 97, % 120, % 109, % 96 ve % 94 idi. Sonuç olarak, albümin kobalt bağlama testi sonuçları kalsiyum (II), magnezyum (II) ve demir (II) konsantrasyonundan anlamlı ölçüde etkilenmezken; bakır (II) konsantrasyonundan etkilenmektedir.

Anahtar Kelimeler: İskemi modifiye albümin, albümin kobalt bağlama testi, akut koroner sendrom

INTRODUCTION

Currently, one of the biochemical markers for detection of myocardial ischemia is ischemia-modified albumin (IMA) (1). IMA is defined as albumin modified by the free radicals produced in ischemic tissues (2). N-terminal region of normal human serum albumin including Asp-Ala-His-Lys amino acid sequence constitutes a strong binding site for transition metal ions such as Co^{++}, Cu^{++}, and Ni^{++}. The modification site caused by ischemia is in this N terminal amino acid region of albumin (3,4). Endothelial and extracellular hypoxia, acidosis, free radical injury, sodium and calcium pump disturbations induced by ischemic events within minutes induce changes in the N-terminal region of albumin (2,5).

Indirect detection of IMA in the serum samples is firstly developed by Bar-Or et al (3,4). The test uses binding of cobalt ions to albumin and the method depends on the indirect colorimetry. When cobalt solution is added to serum, cobalt binds to the albumin and the concentration of free cobalt decreases. In the blood of patients with ischemia, modified albumin levels increase. When the same concentration of cobalt is added into the serum, less cobalt ions bind to IMA and so the concentration of free cobalt ions to be higher in the serum. Dithiothreitol (DTT) is used for color formation in the test. DTT reacts with free cobalt and creates a brown color detectable by spectrophotometer at 470 nm. Thus, intensity of color is directly proportional to IMA level in the serum (5). This test is cleared as an albumin cobalt-binding test (ACB) by FDA (6).

Serum Ca^{++}, Mg^{++}, Cu^{++} and Fe^{++} concentrations are highly variable in patients with acute coronary syndrome. Ca^{++} and Mg^{++} concentrations are significantly decreased during the acute ischemic event, and change in post-infarction period (7). Cu^{++} and Fe^{++} concentrations are significantly increased during acute myocardial ischemia (8,9,10). Alterations of serum concentrations of these ions may effect cobalt binding on albumin molecule and consequently albumin cobalt binding test and IMA results. However, currently there is not adequate information on this matter. From this point of view, we aimed to investigate the effects of Ca^{++}, Mg^{++}, Cu^{++} and Fe^{++} ions on IMA results. We chose ion concentrations ranges, which can be met in clinical practice of patients with acute coronary syndrome.

MATERIALS AND METHODS

DTT, NaCl, $CaCl_2$, $MgCl_2.6H_20$, $CuSO_4.5H_20$ and $FeSO_4.7H_20$ were supplied from Merck (Darmstadt, Germany), $CoCl_2.6H_20$ from Monplet & Esteban SA (Barcelona, Madrid). The cation and albumin concentrations of the prepared pools were measured in duplicate with a Modular D+P analyzer (Roche Diagnostics). Ca^{++}, Mg^{++}, and Fe^{++} levels were determined by the manufacturer's original reagents for the analyzer. Cu^{++} levels were measured by a Randox (Randox Laboratories Ltd, UK) kit on the same analyzer. ACB test was performed with Shimadzu UV 120-01 spectrophotometer.

Two serum pools, one containing lower levels of Ca++, Mg++ and Cu++ and the other containing lower level of Fe++ with normal albumin levels were prepared from the residual sera in routine chemistry studies. Initial cation concentrations of the pools were as following: Ca++: 1.85 mmol/L; Mg++: 0.8 mmol/L; Fe++: 7.46 µmol/L; Cu++: 16.75 µmol/L. Addition of cations was performed by considering serum concentration range which can be met in clinical laboratories. In order to increase Ca++, Mg++, and Cu++ levels, solutions of $CaCl_2$ (94.1 mmol/L), $MgCl_2.6H_2O$ (40.8 mmol/L), and $CuSO_4.5H_2O$ (854.25 mmol/L) prepared in deionized water were used. These solutions were added into the serum pools of 10 mL assigned for each cation in a manner to increase the baseline Ca++, Mg++, and Cu++ concentrations by 20 %, 50 %, and 100 %, without changing the albumin concentrations.

We prepared a second serum pool to obtain low serum iron. In order to evaluate the effect of Fe^{++}, a solution of $FeSO_4.7H_20$ (1.499 mmol/L) in deionized water was added into the serum pool in a manner to increase the initial Fe^{++} concentration by 50 %, 100 %, 200 %, 300 %, and 400 %. No dilution effects of these additions were observed on albumin concentrations. Maximal volume of stock cation solutions added to 10 mL of serum pool each were < 200 µL. We measured albumin concentrations of the pools before and after the addition of cation solutions and we observed no difference between these albumin levels.

ACB test was performed as defined by Bhagavan et al (11): 200 µL of sample and 50 µL of cobalt chloride (4.2 mmol/L) were added to the control and test tubes, the tubes were mixed and kept at room temperature for 10 minutes. Then 50 µL of DTT solution (97 mmol/L) was added to the test tubes and after two minutes 1 mL of NaCl solution (0.154 mol/L) was added. A sample blank was prepared in the same way omitting DTT addition. The absorbances were measured at 470 nm. ACB test result was estimated as the difference of absorbance unit (ΔA) between test absorbance (the absorbance value of the DTT added tubes) and control (blank, the absorbance value of the tubes without DTT addition).

In the reproducibility studies of the method, two serum pools were prepared with the albumin concentrations of 597.4 µmol/L (39.7 g/L) and 779.4 µmol/L (51.8 g/L). ACB test was studied in these two serum pools in different working days (n=38).

Statistical Analysis

Mean and minimum-maximum values of ΔA were calculated and the effects of each given cation were analyzed by repeated measures ANOVA and *post hoc* Student t-test. Accepting the mean of initial ΔA values as 100 %, deviation from this value due to cation addition was determined. Statistical analyses were made by a "SPSS for Windows Ver. 13.0" packaged statistics program.

RESULTS

Percent absorbance changes from the baseline were 115, 101, and 103 at 20 % concentration increase; 104, 101, and 125 at 50 % increase; 107, 102, and 107 at 100 % increase for Ca^{++}, Mg^{++}, and Cu^{++}, respectively. Percent absorbance differences from the baseline were 97, 120, 109, 96, and 94 at increasing Fe^{++} concentrations of 50 %, 100 %, 200 %, 300 %, and 400 %, respectively. Increased Ca^{++}, Mg^{++}, and Fe^{++} concentrations did not cause a significant difference as compared to initial ΔA values of each pool (Figure 1A, 1B and 1C). When ΔA values of the pools with increased Cu^{++} concentrations were compared with those values of the initial pool; at the 20 % increase there was no significant difference; but at 50 % and 100 % increments, the results were significantly changed (Figure 1D).

Reproducibility Study

Between-day imprecisions of ACB test for the pools with lower and higher albumin were 10.9 % and 11.1.

DISCUSSION

According to the results of this study, Ca^{++}, Mg^{++} and Fe^{++} concentrations that commonly encountered in clinical laboratories do not show significant effect on ACB test. Our hypothesis was that the increase in the cation concentrations at the constant albumin concentration might yield increased free cobalt ions and consequently increased ΔA values. But, in our study it was determined that the increased Ca^{++}, Mg^{++} and Fe^{++} concentrations did not effect the ACB test results. The reason for this situation could be binding of these cations on a region other than the N-Asp-Ala-His-Lys of albumin molecule. There is limited information in the literature about the region of albumin that bind these metal ions (12,13). Binding of Cu^{++} and Zn^{++} ions to the albumin has been reviewed by Masuoka et al. (13) and the authors state that there is contradictory information on the affinity and number of binding regions (13). It was shown that zinc binds to a more interior region than the N-terminal region that copper binds to (14). It was reported that zinc ion can bind to imidazol, thiol, carboxyl and peptide oxygen and copper ion can bind to imidazol, α-amino or peptide nitrogen (14). In the study of Sadler et al., which employed by H-NMR spectroscopy, it was notified that Cu^{++}, Ni^{++} and Co^{++} metal ions bind to the N-terminal amino acid residues (Asp-Thr-His-Lys for bovine albumin and Asp-Ala-His-Lys for human albumin) and that Al^{3+} and Cd^{++} bind to a different region of the protein (15).

The ions that have the highest binding capacity on the N terminal region of albumin are Cu^{++} ions (Ka=1016 L/mol) (16,17). In our study, the raise of IMA absorbance values in the pools in which the Cu^{++} concentrations were

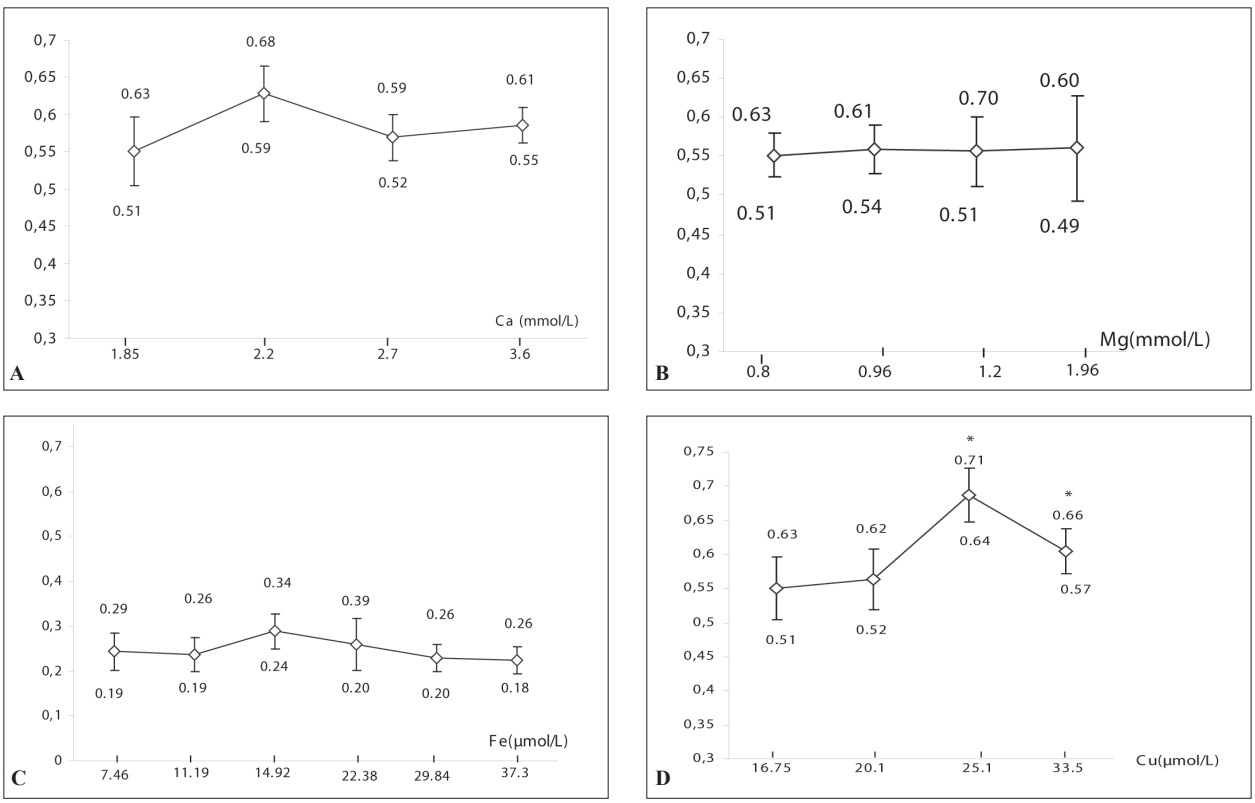

Figure 1. Effects of (A) Ca, (B) Mg, (C) Fe, and (D) Cu on IMA. The serum pool used for Ca, Mg and Cu was different from the pool used for Fe. For each pool, cation concentrations were shown on the x-axis; ΔA values were shown on the y-axis; (Δ): minimum- maximum ΔA; (*): ($p<0.05$)

increased by 50 % and 100 % verifies our hypothesis. The absorbance values could be increased because of the elevated Cu^{++} concentrations. Increased concentrations of Cu^{++} can remove cobalt ions from the region it binded or prevent the binding of cobalt ions to albumin molecule. However, Cu^{++} interference on IMA was not concentration dependent. Lower interference from Cu^{++} ions at ~34 µmol/L (100 % increase) than 25 µmol/L of Cu^{++} (50 % increase) remained unclear.

Bhagavan et al. reported that individuals with myocardial ischemia had 0.63 ± 0.25 ΔA whereas nonischemic groups had a lower mean, 0.43 ± 0.10 ΔA (mean ± 2SD) (11). In the present study, the mean ΔA values of pools with 50 % and 100 % increased Cu^{++} were 0.687 ± 0.039 and 0.605 ± 0.033, respectively. Baseline mean ΔA was 0.551 ± 0.046. Therefore, difference from the baseline ΔA values at 25 µmol/L (50 % increase) and ~34 µmol/L (100 % increase) of Cu^{++} seem to be clinically significant.

As a result, ACB test results are not affected by Ca^{++}, Mg^{++} and Fe^{++} concentrations but by Cu^{++} concentrations. Higher Cu^{++} concentrations, especially in a region close to upper reference limit can give rise to erroneously higher IMA results. Although ACB test has a relatively high imprecision, the erroneously higher IMA results can affect clinical decision.

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