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ORIGINAL ARTICLE

Characteristics of a nickel–albumin binding assay for assessment of myocardial ischaemia

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Abstract

Background: The aim of this study was to describe a method to measure ischaemia-induced alterations of the binding capacity of serum albumin to exogenous nickel.

Methods: We measured the levels of cardiac troponin I (cTnI), serum albumin, ischaemia-modified albumin (IMA) measured by a cobalt–albumin binding assay (CABA), and a nickel–albumin binding assay (NABA) in the following groups: myocardial infarction ($n = 32$) and non-ischaemic chest pain ($n = 64$).

Results: IMA, cTnI and NABA levels were higher in the myocardial infarction group. NABA presented a higher ability to discriminate myocardial ischaemia than CABA.

Conclusions: Patients with myocardial infarction have reduced nickel binding to human serum albumin, and NABA may have an important role as an early marker of myocardial ischaemia.

Keywords: *Myocardial ischaemia; serum albumin; nickel; cobalt; oxidative stress*

Introduction

Myocardial infarction (MI) is defined as myocardial cell death due to prolonged ischaemia (Joint European Society of Cardiology/American College of Cardiology Committee 2000). Although clinical presentation and electrocardiogram (ECG) are critically important for its diagnosis, biochemical markers including myoglobin, creatine kinase-MB, and particularly cardiac troponin I (cTnI) and T (cTnT) play a fundamental role in the diagnosis, risk stratification, and therapeutic management of acute coronary syndrome (ACS) patients (Christenson & Azzazy 1998, Christenson et al. 2001, Moresco et al. 2005). Myocardial ischaemia results from the lack of an adequate blood perfusion to the myocytes leading to a deficiency of oxygen and nutrients, eventually compromising their vital functions.

During ischaemia, the generation of reactive oxygen species (ROS) influences the metal binding capacity of albumin for transition metals (Roy et al. 2006, Cichota et al. 2008, Duarte et al. 2009). Recently, a novel biochemical method measuring ischaemia-induced alterations of the binding capacity of human serum albumin (HSA) to exogenous cobalt was reported (Bar-Or et al. 2000). Also, ischaemia-modified albumin (IMA) has been shown to be a rapid rising and sensitive biochemical marker especially for the diagnosis of myocardial ischaemia (Bar-Or et al. 2000, Sinha et al. 2004). IMA is considered to be a marker of myocardial ischaemia in contrast to cardiac enzymes that are released when cardiac necrosis occurs (Sbarouni et al. 2006). Admission measurement of IMA can be used for early classification of patients presenting to the emergency department (ED) to assist in patient triage (Collinson et al.

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2006) as negative IMA has a high negative predictive value to exclude acute coronary syndromes (Peacock et al. 2006). However, some authors do not support the use of IMA as an effective risk stratification tool for patients with chest pain in the ED, in particular because of its low specificity (Keating et al. 2006). Thus, considering the importance of biochemical markers of ischaemia, the purpose of this study was to describe a method for measuring ischaemia-induced alterations of the binding capacity of serum albumin to exogenous nickel, and to evaluate its association with an albumin–cobalt binding assay. We investigated the diagnostic characteristics of these assays for the assessment of myocardial ischaemia, as well as whether the type of metal (cobalt and nickel) influences the performance of the test.

Methods

Study population

Patients from the Hospital de Caridade, Santa Maria-RS, Brazil were enrolled prospectively in this study. Ninety-six patients with chest pain were divided into two groups as follows: the MI group, 32 patients (age, mean ± SD, 73.4 ± 9.9 years) presenting to the ED and definitively diagnosed with MI, and the non-ischaemic chest pain (NICP) group, 64 patients (age, mean ± SD, 64.9 ± 14.5 years). The final diagnosis of MI was based on cTnI elevation in conjunction with the ECG and clinical findings according to the criteria of European Society of Cardiology/American College of Cardiology Joint Committee definition (Joint European Society of Cardiology/American College of Cardiology Committee 2000). Patients were classified as NICP if: (1) a reported non-cardiac mechanism was confirmed as the cause of pain; (2) negative cTnI results on serial sampling; and (3) presence of normal ECG. Patients presenting chronic kidney disease were excluded from this study. This study protocol was approved by the local ethics committee (number 0180.0.243.000-08).

Biochemical determinations

All blood samples were collected at the time the patient first presented and within 12 h following the onset of acute symptoms. After obtaining blood samples in Vacutainer® tubes (BD Diagnostics, Plymouth, UK) without anticoagulants, specimens were routinely centrifuged for 15 min at 2500 *g* within 1 h of collection, and aliquots of serum samples were stored at -20°C for a maximum of 4 weeks before IMA measurement. Serum levels of cTnI were measured by use of a chemiluminescence immunoassay on Immulite 2000® (Siemens Healthcare Diagnostics, Los Angeles, CA, USA), and albumin levels were measured by use of the bromocresol green method on a Cobas Mira® analyser (Roche Diagnostics, Basel, Switzerland). Serum IMA was measured by a colorimetric cobalt–albumin binding assay (CABA) previously described (Bar-Or et al. 2000). This method involved adding 50 µl of 0.1% cobalt chloride ($CoCl_2.6H_2O$; Sigma) in H_2O to 200 µl of serum, gently mixing, and waiting 10 min for adequate cobalt–albumin binding. Fifty microlitres of dithiothreitol (DTT) (1.5 mg ml^{-1} H_2O; Sigma) was added as a colourizing agent and the reaction was quenched 2 min later by adding 1.0 ml of NaCl 0.9%. Using a spectrophotometer Hitachi U-2800A® (Hitachi High-Technologies Corporation, Japan) at 470 nm, the colour development with DTT was compared with a serum–cobalt blank without DTT and results were reported in absorbance units (ABSU).

A colorimetric method based on nickel–albumin binding was described to measure the ischaemia-induced alterations of the binding capacity of HSA to exogenous nickel. This assay was developed based on biochemical properties of albumin to bind transition metals (Bar-Or et al. 2000, 2001). The nickel–albumin binding assay (NABA) involved adding 50 µl of 0.1% nickel sulfate ($NiSO_4.6H_2O$; Sigma) in H_2O to 100 µl of patient serum, gently mixing, and waiting 10 min at 37°C for adequate nickel–albumin binding. Then, 100 µl of DTT (1.5 mg ml^{-1} H_2O; Sigma) was added as a colourizing agent and the reaction was quenched 2 min later by adding 500 µl of NaCl 0.9%. Using a spectrophotometer Hitachi U-2800A® (Hitachi High-Technologies Corporation) at 470 nm, the colour development with DTT was compared with a serum–nickel blank without DTT and results were reported in absorbance units (ABSU). The colorimetric assay format quantitatively measures unbound nickel remaining after nickel–albumin binding has occurred. Thus, with reduced nickel–albumin binding, there is more unbound nickel, resulting in elevated assay levels.

Statistical analysis

Student's *t*-test was used to evaluate the differences between groups. Pearson's correlation was assessed to evaluate the association between NABA and CABA. A receiver operator characteristic (ROC) curve was performed to quantify the overall ability of NABA and CABA to discriminate among those individuals with myocardial ischaemia and those without myocardial ischaemia. The sensitivity and specificity of these assays were assessed by ROC curve. Statistical significance was assumed at $p < 0.05$.

Results

Baseline characteristics of study patients are shown in Table 1. The levels of cTnI were higher in the MI group in comparison to the NICP group. No significant differences were observed for serum albumin levels between the two

Table 1. Baseline characteristics of study patients.

	Non-ischaemic chest pain group	Myocardial infarction group
n	64	32
Male (%)	48.4	53.1
Age (years)	64.9 ± 14.5	73.4 ± 9.9**
Albumin (g l^{-1})	35.30 ± 4.78	33.56 ± 5.22
cTnI (ng ml^{-1})	<0.20	58.38 ± 21.45***
CABA (ABSU)	0.369 ± 0.202	0.479 ± 0.197*
NABA (ABSU)	0.384 ± 0.165	0.555 ± 0.171***

Data are expressed as mean ± SD. *p <0.05, **p <0.01, ***p <0.001. cTnI, cardiac troponin I; CABA, cobalt-albumin binding assay; NABA, nickel-albumin binding assay; ABSU, absorbance units.

groups. The levels of IMA measured by CABA were higher in patients with MI (0.479 ± 0.197 vs 0.369 ± 0.202 ABSU, p <0.05). We also observed higher levels of NABA in the MI group (0.555 ± 0.171 vs 0.384 ± 0.165 ABSU, p <0.001), as shown in Table 1. We used an albumin-adjusted correction (Lippi et al. 2007) to evaluate the influence of albumin levels on CABA and NABA values in study subjects, and differences between groups remained significant after correction, as follows: CABA (MI: 0.430 ± 0.178 ABSU vs NICP: 0.358 ± 0.209 ABSU, p <0.05) and NABA (MI: 0.511 ± 0.176 ABSU vs NICP: 0.363 ± 0.171 ABSU, p <0.001). In addition, significant correlations between NABA and CABA were reported for the NICP group (r = 0.5776, p <0.001), and for the MI group (r = 0.3991, p <0.05), as shown in Figure 1. Intra-assay and interassay coefficients of variation (CVs) were 2.6% and 4.6% for NABA, and 4.3% and 5.2% for CABA, respectively.

A ROC curve was employed to quantify the overall ability of CABA and NABA to discriminate among those individuals with myocardial ischaemia and those without myocardial ischaemia. Areas under the curve for CABA and NABA were 0.6582 (95% confidence interval (CI) 0.5444–0.7720, p <0.05), and 0.7800 (95% CI 0.6785–0.8816, p <0.001), respectively. Thus, both CABA and NABA have the ability to discriminate myocardial ischaemia, as shown in Figure 2. CABA levels <0.450 ABSU demonstrated a sensitivity of 61.7% (95% CI 48.7–73.6%) and a specificity of 53.1% (34.7–70.9%) for the assessment of myocardial ischaemia, and the negative and positive predictive values (NPV and PPV) were 72.7% and 41.5%, respectively. NABA levels <0.500 ABSU demonstrated a sensitivity of 78.1% (95% CI 66.0–87.5%) and a specificity of 65.6% (46.8–81.4%) for the assessment of myocardial ischaemia, and the NPV and PPV were 82.0% and 60.0%, respectively.

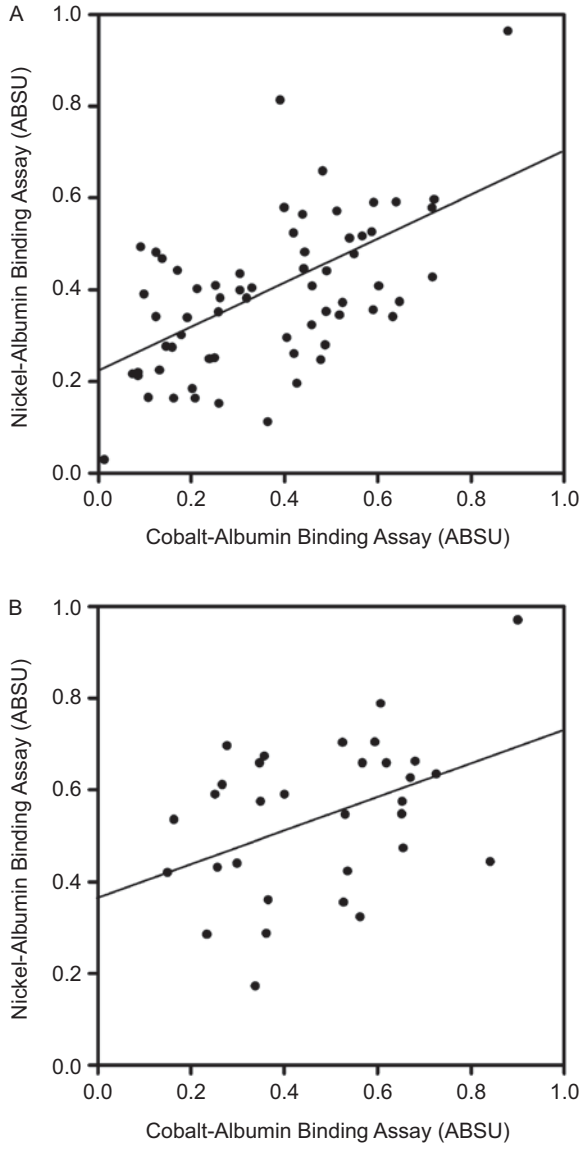

Figure 1. Significant correlations between the nickel–albumin binding assay (NABA) and the cobalt–albumin binding assay (CABA) for (A) the non-ischaemic chest pain group (r = 0.5776, p <0.001), and (B) the myocardial infarction group (r = 0.3991, p <0.05). Results are expressed as absorbance units.

Discussion

Currently, available MI markers, such as CK-MB, myoglobin and troponin, appear to be released from myocyte sources only after irreversible cellular damage and disruption of cell membrane integrity. Furthermore, shorter episodes of ischaemia do not consistently result in elevated blood levels of these markers (Brogan et al. 1997, Bar-Or et al. 2000). In those cases when abnormal MI marker results are reported following myocardial ischaemia, there is usually a delay of several hours after the onset of symptoms before abnormal levels can be detected (Bar-Or et al. 2000). Bar-Or et al. (2000) first suggested that myocardial ischaemia may alter the metal binding capacity of circulating serum albumin and proposed a new blood assay based on ischaemia-induced alterations of the binding capacity of HSA to exogenous cobalt.

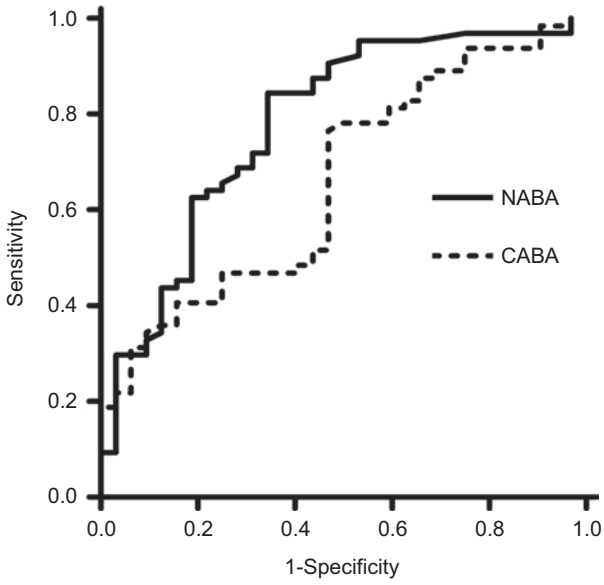

Figure 2. Receiver operator characteristic (ROC) curves of the cobalt–albumin binding assay (CABA) and the nickel–albumin binding assay (NABA) for the assessment of myocardial infarction. Areas under the curve for CABA and NABA were 0.6582 (95% confidence interval (CI) 0.5444–0.7720, $p < 0.05$), and 0.7800 (95% CI 0.6785–0.8816, $p < 0.001$), respectively.

Although the nickel–HSA binding has been described previously (Bar-Or et al. 2001), to our knowledge, this is the first report to demonstrate the characteristics of a nickel–HSA binding assay for the assessment of myocardial ischaemia. This study indicates that patients with MI have reduced nickel binding to HSA, thus a blood assay based on this mechanism was proposed. Biochemical properties of albumin to bind transition metals such as nickel and cobalt have been reported previously (Bar-Or et al. 2001). Nickel(II) and copper(II) deserve special consideration among the metals because most mammalian albumins bind them more tightly and more specifically than other cations. Cysteine participates in the uptake of copper, and copper binds preferentially to mercaptalbumin and in time forms an albumin–copper–cysteine complex. Nickel(II) binds to the amino terminus in a similar manner. The nickel ion chiefly participates in a square-planar chelate ring like copper, but about 30% of the ligand is said to be held in an octahedral structure, which is less stable (Peters 1995).

NABA values were higher in patients with MI. NABA measures unbound nickel remaining after nickel–albumin binding occurred. Thus, with reduced nickel–albumin binding there is more unbound nickel, resulting in elevated NABA levels. Ischaemia may alter the metal binding capacity of circulating serum albumin, and biochemical mechanisms involved in the *in vivo* alterations to metal–albumin binding during either ischaemia or reperfusion may include hypoxia, acidosis, free radical damage, membrane energy-dependent sodium and calcium pump disruptions, free iron, and copper ion exposure (Berenshtein et al. 1997, Bar-Or et al. 2000, Christenson et al. 2001, Cichota et al. 2008). Most of these conditions occur *in vivo* within minutes after the onset of acute myocardial ischaemia (Reimer et al. 1977, Cobbe & Poole-Wilson 1980, Bar-Or et al. 2000). The first three amino acids in the N-terminus, Asp-Ala-His, constitute a specific binding site for transition metals and the most susceptible region for degradation compared with other regions of albumin (Sbarouni et al. 2006, Cho et al. 2007, Duarte et al. 2009). Generation of ROS can transiently modify the N-terminal region of albumin and produce an increase in IMA levels (Roy et al. 2006). The cobalt–HSA binding assay mechanism of action is an interplay among multiple variables including the proportion of intact N-terminus of HSA, HSA concentration, plasma cysteine/cystine ratio, plasma pH, and the state of oxidation of cys34 of HSA (and potentially others). These factors all interact with added cobalt and DTT (Bar-Or et al. 2008). Furthermore, it is known that HSA is the primary binder of fatty acids, commonly known as free fatty acids (FFA), and that plasma concentrations of FFAs are increased during myocardial ischaemia. Bhagavan et al. (2009) reported that changes in IMA values during acute MI are likely caused by reversible conformational changes in HSA associated with FFA fluxes (Bhagavan et al. 2009). Likewise, we suggest that these variables may interact also with added nickel and DTT on NABA.

We suggested that NABA reflects ischaemia, but it can also reflect necrosis due to high concentrations of cTnI observed in MI group. Thus, total acute occlusion of the culprit artery causes acute tissue necrosis that prevails over ischaemia limiting access of the modified HSA to the systemic circulation. Therefore, with myocardial necrosis, less albumin will be exposed to circulating free radicals resulting in less IMA production. Because of the difficulty of pinpointing the exact time of onset of an ischaemic event, there is always the possibility that IMA was initially raised but had already decreased below the diagnostic cut-off at the time of the blood draw. These intriguing findings do not detract from the fact that IMA is a sensitive marker of ischaemia (rather than necrosis) (Sinha et al. 2004).

Results of this preliminary study indicate that emergency patients with MI have reduced nickel and cobalt binding to HSA when measured by colorimetric assay. We also describe a significant correlation between CABA and NABA. ROC curve analysis of CABA and NABA were consistent with these observations. The sensitivity and NPV of NABA were higher than those of CABA, indicating that NABA may have a rule-out role better than that of CABA. However, the discriminatory power of NABA as a rule-out test would not be sufficient for clinical application and further studies are required. The specificity and PPV of CABA and NABA were low because the

metal binding capacity of albumin for transition metals could be influenced by various factors, including several ischaemic conditions (Lee et al. 2007). The binding of transition metals to the N-terminal region of albumin has been studied. However, the exact biochemical mechanism that causes altered nickel(II) binding to albumin during ischaemia is not understood. We suggest that differences on clinical characteristics of assays reported in this study could be attributed in part due to a variety of factors including chemical characteristics of each metal, molecular weight, as well as their abilities of binding to HSA.

In summary, the present study reported a method to measure ischaemia-induced alterations of the binding capacity of serum albumin to exogenous nickel and its diagnostic characteristics. We conclude that altered nickel binding to HSA may prove to be an early marker of ischaemia, and the assay described here could have an important role in reducing the inappropriate admission of low-risk patients. However, its diagnostic specificity was low, indicating that NABA should be performed in combination with markers of necrosis. Thus, the discriminatory power of NABA as a rule-out test would not be sufficient for clinical application and further studies are required to investigate the power of this test in a larger population.

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Declaration of interest

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

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