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Original Article

Plasma Concentrations of Trace Elements Selenium and Cobalt During and After Coronary Artery Bypass Grafting Surgery

Jia-Yi Zhou[a], Hai-Tao Hou[a], Shi-Fu Wang[b], Qin Yang[a], and Guo-Wei He[a,c,d]*

[a]*The Institute of Cardiovascular Diseases & Department of Cardiovascular Surgery,* [b]*Unit of Perfusion, Department of Cardiovascular Surgery, TEDA International Cardiovascular Hospital, Tianjin 300457, China,*
[c]*Drug Research and Development Center, Wannan Medical College, Wuhu 241002, China,*
[d]*Department of Surgery, Oregon Health and Science University, Portland, OR 97239, U.S.A.*

Trace elements selenium (Se) and cobalt (Co) are essential in the human body, and a correlation between Se and cardiac surgery has been suggested. We investigated the plasma concentrations of Se and Co during and after coronary artery bypass grafting (CABG) surgery under cardiopulmonary bypass (CPB). From December 2019 to January 2020, preoperative plasma samples from isolated first-time CABG patients (n = 20; 10 males, 10 females) were prospectively collected post-anesthesia and before CPB (T1), 45 min after CPB started (T2), 90 min after CPB started (T3), and postoperative days 1 (T4), and day 4 (T5). The plasma concentrations of Se and Co were measured. The Se concentration was significantly decreased at T2 (105.24 ± 4.08 vs. 68.56 ± 2.42 μg/L, $p < 0.001$) and T3 (105.24 ± 4.08 vs. 80.41 ± 3.40 μg/L, $p < 0.001$). The Co concentration was significantly decreased at T4 (0.35 ± 0.19 vs. 0.26 ± 0.13 μg/L, $p < 0.01$) and T5 (0.35 ± 0.19 vs. 0.23 ± 0.11 μg/L, $p < 0.001$). Five patients developed atrial fibrillation (AF); there was no other operative mortality or major morbidity. This is the first report of alterations of plasma Se and Co concentrations during and after CABG surgery. Our results may indicate that Se supplementation before or during CABG and Co supplementation after CABG may become necessary for patients undergoing CABG.

Key words: trace element, CABG, cardiopulmonary bypass, selenium, cobalt

Trace elements including selenium (Se) and cobalt (Co) are essential in the human body [1] and are involved in the body's metabolism during exercise [2]. In patients with coronary artery disease, the serum levels of trace elements may be changed [3]. Selenium may affect metabolic factors [4]. and Se deficiency is associated with several diseases [4, 5]. It has been shown that tuberculosis can change the concentrations of Se and Co [6] and that Se and Co deficiency are related to type 2 diabetes [7]. Selenium has also been reported to play an important role in the attenuation of injury in a variety of diseases such as heart failure, sepsis, multi-organ failure/dysfunction after cardiovascular surgery, PCI, and myocardial infraction [8].

A correlation between Se and cardiac surgery has been suggested [9]. In patients undergoing cardiac surgery with cardiopulmonary bypass (CPB), multi-organ damage is associated with a substantial decrease in the blood Se level [9]. A recent study suggested that the serum concentration of Se-binding protein l (SELENBP1) may be a quantitative marker for myocardial hypoxia [10], and it was proposed that the study of trace elements can improve the nursing level for cardiac surgery patients and reduce medical risks [10].

Most cardiac surgery procedures are performed with

Received March 26, 2021; accepted August 4, 2021.
*Corresponding author. Phone : +86-22-65209089; Fax : +86-22-65208732
E-mail : gwhezj@163.com (He. GW)

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CPB. The effect of CPB on trace elements has been studied [11, 12], and it was demonstrated that an intraoperative decrease in the plasma level of Se is associated with the postoperative development of multi-organ dysfunction in cardiac surgical patients and that plasma Se decreases significantly after CPB in children [13, 14]. Selenium pretreatment for the mitigation of ischemia/reperfusion injury in cardiovascular surgery may decrease the risk of acute organ damage and the inflammatory response [9].

It was also reported that hypoxia or hypoxia-mimetic agents (*i.e.*, cobalt chloride [$CoCl_2$] and deferoxamine) limit myocyte necrosis by upregulating the transcription factor hypoxia-inducible factor [15]. $CoCl_2$ attenuates myocardial apoptosis after hypothermic circulatory arrest, and it was reported that preconditioning with $CoCl_2$ before CPB and deep hypothermic circulatory arrest attenuates myocardial apoptosis and therefore protects the heart from ischemia-reperfusion injury [15]. It is thus possible that Co may protect the heart from ischemia-reperfusion injury during CABG surgery, but there are no data on the plasma Co concentration in CABG.

Coronary artery disease has a high mortality rate [16]. Coronary artery bypass grafting (CABG) is the major therapy for multi-vessel disease; it reduces the incidence of myocardial infarction and has significantly improved the survival rate of patients with coronary artery disease [17, 18]. During and after a CABG procedure, the body's levels of trace elements may be changed. As mentioned above, the plasma concentrations of trace elements may change during cardiac surgery [11, 12]. We recently observed that the plasma trace elements magnesium, copper, zinc, iron, and calcium are altered during and after CABG surgery [19]. However, little is known about the alteration of plasma Se during and after CABG, although it has been reported that there is a relationship between Se deficiency and post-operative atrial fibrillation (AF) after cardiac surgery [20]. In addition, the effects of Se supplementation on the metabolic status of patients undergoing CABG surgery were recently described [21]. To the best of our knowledge, there are no reports on the plasma Co concentration in CABG surgery.

We thus conducted the present study to investigate the effects of CPB and CABG surgery on the plasma Se and Co levels during and after CABG.

Patients and Methods

Patient selection. From December 2019 to January 2020, preoperative plasma samples from isolated first-time CABG patients were prospectively collected and frozen. Twenty patients, 10 males and 10 females, were included. There were no significant differences in age, gender, weight, or history of diseases between the male and female patients; an exception was the difference in their heights (Table 1).

This study was approved by the Institutional Review Board of TEDA International Cardiovascular Hospital ([2020]-1029-2), Tianjin, China, and informed consent was obtained from all patients recruited in this

Table 1 Patient characteristics

Gender	Male (n = 10)	Female (n = 10)	*p*-value
Age (years)[a]	62.4 ± 3.18	63.0 ± 1.97	0.874
Stature (cm)[a]	171.6 ± 1.17	161.1 ± 1.85	0.000
Weight (kg)[a]	79.7 ± 3.54	70.0 ± 4.40	0.104
BMI[a]	27.0 ± 1.05	26.9 ± 1.42	0.938
Hypertension[b]	5	8	0.350
Diabetes mellitus[b]	3	5	0.650
Smoking[b]	7	3	0.179
Ejection fraction moderate[a]	58.3 ± 3.27	59.5 ± 2.47	0.773
On-pump time (min)[a]	109.2 ± 7.75	93.9 ± 6.99	0.160
Cross-clamp time (min)[a]	87.6 ± 6.28	77.5 ± 7.16	0.303
Fluid balance[a]	576.0 ± 209.20	590.4 ± 148.90	0.956

The numbers are expressed as mean ± S.E.
BMI: body mass index.
[a]Unpaired *t* test.
[b]Chi-square test.

study.

Blood sample collection and analysis. The blood samples were collected at five time points: after anesthesia and before CPB (T1), 45 min after the start of CPB (T2), 90 min after the start of CPB (T3), postoperative day 1 (T4), and postoperative day 4 (T5). The blood samples were collected in 2-mL tubes containing lithium-heparin, centrifuged at 3,000 rpm for 10 min, separated, and stored at −80°C until the analyses. The separated plasma was used for this study.

Surgical techniques. Each CABG surgery followed the standard protocol at our hospital. All patients underwent on-pump CABG with CPB. The CPB was implemented with the use of a two-stage venous cannula of the right atrium and ascending aortic cannulation. The CPB technique followed the standard protocol at our hospital, including monitoring of the in-out water balance during and after the CPB. The nonpulsatile flow rate was 2 L/m^2/min and the mean systemic pressure was kept between 50 and 80 mmHg. The patient's body temperature was maintained at 32-34°C depending on the on-pump time. Blood cardioplegia was used. Retrograde cold-blood cardioplegia was initiated after each distal anastomosis. After each proximal anastomosis, retrograde cold-blood cardioplegia or cold blood was used. Magnesium sulfate (25% 10 mL = 2.5 g) was injected into the CPB circuit at 30 min and also on postoperative days 1 and 2 after the CABG surgery. Calcium chloride (usually 1 g) was added to the circuit after the heart was resuscitated if the concentration of calcium was lower than normal.

Patients with postoperative AF received anti-AF treatment. To ensure that the patients had never experienced AF preoperatively, all of the patients were screened by direct questioning and a 12-lead ECG examination before surgery. Other patient exclusion criteria included a history of nervous system disease, severe respiratory disease, renal or hepatic insufficiency, coagulation disorder, thyroid dysfunction, current treatment with antiarrhythmic drugs except beta-blockers, patients < 30 years or > 80 years old, those with a left ventricular ejection fraction (LVEF) < 35%, and emergency surgery.

Methods of determining Se and Co in plasma. We obtained the reference standard solutions of Se and Co with certified values comparable to the respective standard reference material of the National Institute of Standards and Technology (NIST) from high-purity standards (Dian Medical Laboratory CO, Ltd, Shanghai, China). Ultrapure-grade nitric acid was selected; the percentage varied from 68% to 70% according to the manufacturer selected by the laboratory. High-purity deionized water was obtained from a Milli-Q water purification (Milli-Q, Molsheim, France).

The working standard solutions of Se and Co were prepared from the respective standard solutions (Fig. 1) by dilution with 1% to 3.50% (v/v) nitric acid according to the lab protocol.

ICP-MS method and sample preparation. Inductively coupled plasma mass spectrometry (ICP-MS) analyses were performed on an NexION 2000-B system from Perkin Elmer in Dian Diagnostics (Shanghai, China). After each injection, the auto-sampler probe was cleaned using a standard procedure. Calibration was conducted according to the manufacturer's instructions (Fig. 1).

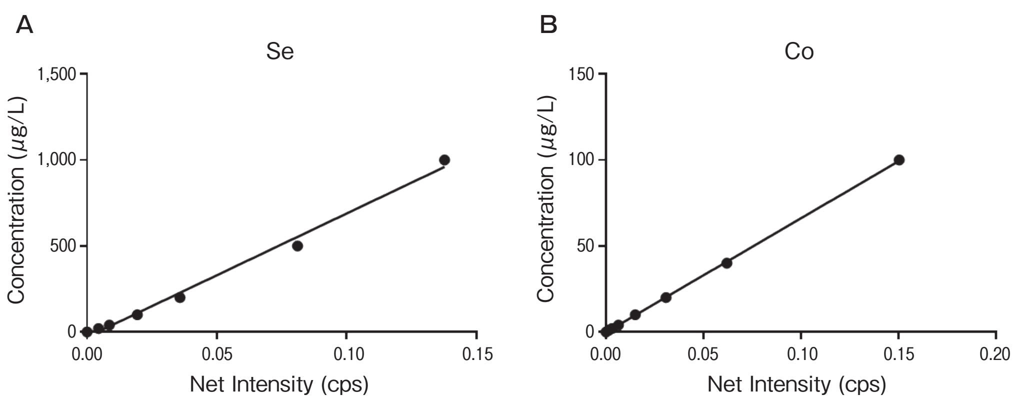

Fig. 1 The standard curves of measurement for selenium (Se) (**A**: r = 0.9999) and cobalt (Co) (**B**: r = 0.9960). Cps: counts per second for total ion flow.

The plasma sample preparation was performed as follows. A 200-μL aliquot of the serum sample was diluted with 4 mL of diluent. The diluent consisted of 5% (v/v) nitric acid, 0.05% (w/v) Triton X-100, and 1 μg/mL rhodium. The sample was vortexed and then ready for analysis.

Data analyses. The data were analyzed using SPSS 20.0 software (SPSS, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was performed for multiple comparisons. When the variance was equal, the Bonferroni test was used as a post hoc test. When the variance was unequal, the Games-Howell test was used as the post hoc test. The χ^2-test was used to compare categorical variables. The results are expressed as the mean ± SEM. Probability (p)-values <0.05 were considered significant.

Results

Alteration of plasma Se and Co during and after CABG. During the patients' CPB procedures, the fluid balance was 583.2± 124.98 (range −570-1,700 mL), and this did not include the blood loss during surgery. There was no significant difference in patient characteristics between the male and female patients (Table 1). During the CPBs, the plasma concentration of Se decreased significantly (Fig. 2A). At T2, the Se concentration decreased to 68.56 ± 2.42 μg/L compared to T1 before CPB (105.24 ± 4.08 μg/L, $p<0.001$, Bonferroni test). The concentration of Se remained low at T3 (80.41 ± 3.40 μg/L, $p<0.001$ compared to T1) (Fig. 2A) but recovered to the preoperative level on postoperative day 1 (T4) and remained normal on day 4 (T5) (Fig. 2A).

The plasma concentration of Co showed no significant changes during CPB (see T2 and T3 in Fig. 2B). However, it decreased on day 1 (0.26 ± 0.13 μg/L on T4 vs. 0.35 ± 0.19 on T1, $p<0.01$, Games-Howell test) and decreased further on T5 (0.23 ± 0.11 μg/L, $p<0.001$ compared to T1, Games-Howell test) (Fig. 2B).

Differences of Se and Co in male and female patients. The trend of the alterations of Se and Co remained for both the male and female patients (Fig. 3). In both the male and female patients, there were significant differences in the plasma Se concentration between T2 (males: 68.11 ± 2.99 vs. 107.28 ± 5.92 μg/L, $p<0.001$; females: 69.00 ± 3.97 vs. 103.19 ± 5.86 μg/L, $p<0.001$, Bonferroni test) and T3 (males: 83.59 ± 5.29 vs. 107.28 ± 5.92 μg/L, $p<0.05$; females: 77.23 ± 4.30 vs. 103.19 ± 5.86 μg/L, $p<0.01$, Bonferroni test).

Similar to the changes in all patients for Co (Fig. 2), there were no significant differences in Co during the CPB or after surgery. However, the change in the plasma concentration of Co between the male and female patients at the 5 time points differed by sex. In the male patients, the decrease of Co became significant only on day 4 (T5: 0.21 ± 0.01 vs. 0.35 ± 0.03 μg/L on T1, $p<0.05$, Games-Howell test, Fig. 4A), whereas in the female patients the decrease became significant earlier. Although the difference in Co values among the five time points was highly significant ($p<0.001$, one-way ANOVA), the difference between the T4 or T5 timepoints and T1 (0.34 ± 0.02 μg/L) did not reach significance. Compared to the Co level at the start of the operation (T2), Co was significantly decreased on postoperative day 1 (T4: 0.26 ± 0.02 vs. 0.37 ± 0.03 μg/L on T2, $p<0.05$, Bonferroni test) and day 4 (T5: 0.24 ± 0.02

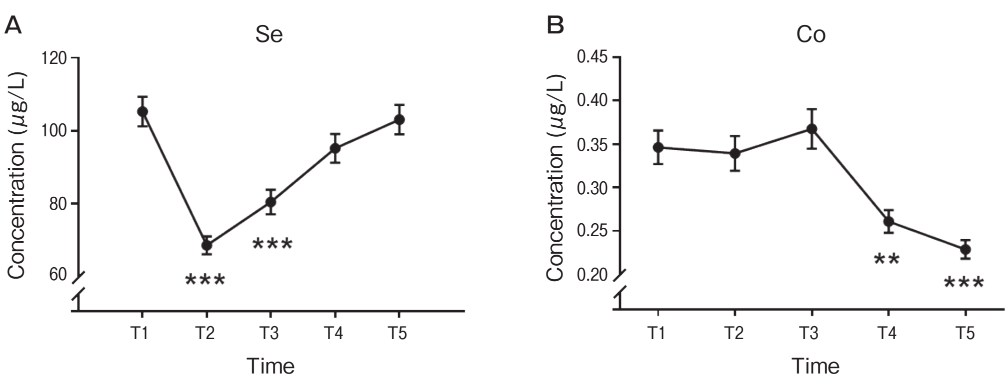

Fig. 2 Alterations of the plasma concentrations of Se and Co during and after CABG. T1: after anesthesia and before CPB. T2: 45 min after CPB started. T3: 90 min after CPB started. T4: postoperative day 1. T5: postoperative day 4. **A**, $p<0.001$, one-way ANOVA. ***$p<0.001$ vs. T1 (Bonferroni test); **B**, $p<0.001$, one-way ANOVA. **$p<0.01$, ***$p<0.001$ vs. T1 (Games-Howell test).

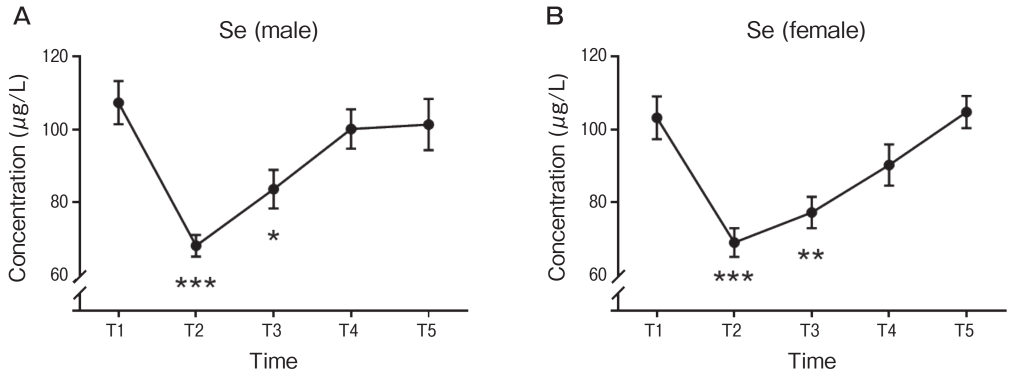

Fig. 3 Alteration of the plasma concentration of Se during and after CABG in the male and female patients. **A**, $p<0.001$, one-way ANOVA; **B**, $p<0.001$, one-way ANOVA. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. T1 (Bonferroni test).

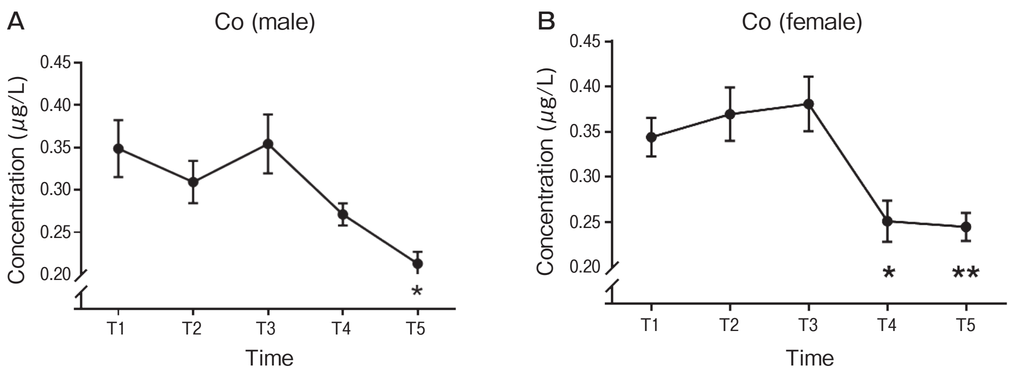

Fig. 4 Alteration of the plasma concentration of Co during and after CABG in the male and female patients. **A**, $p<0.01$, one-way ANOVA. $^{*}p<0.05$ vs. T1 (Games-Howell test); **B**, $p<0.001$, one-way ANOVA. $^{*}p<0.05$, $^{**}p<0.01$ vs. T2 (Bonferroni test).

vs. T2, $p<0.01$, Bonferroni test) (Fig. 4B).

Differences in Se and Co values between the male and female patients. There were no significant differences in the concentration of the two elements between the male and female patients at any time points (T1-T5) (Figs. 3, 4). Five patients developed AF after CABG (2 males and 3 females). No significant differences were detected in the concentration of Se or Co between the AF and sinus rhythm groups.

Discussion

This study provides the first demonstration that: (1) during or after CABG surgery, the plasma concentration of the trace elements Se and Co is altered; *i.e.*, Se is decreased during CABG and Co is decreased after CABG, and (2) the level of Se was recovered on day 5 after CABG but the level of Co remained low even after CABG surgery.

As mentioned earlier, trace elements play important roles in both physiological and pathological conditions. It has been reported that a low level of Se can affect patients' outcomes after open-heart surgery [22] and that pretreatment with a dietary surplus of Se and pretreatment with ebselen (a mimic of the selenoenzyme glutathione peroxidase) protected against acute ischemia-reperfusion injury-induced tissue damage during cardiovascular surgery requiring CPB and deep hypothermic circulatory arrest [9]. It was also demonstrated that the serum concentration of selenium-binding protein 1 was strongly associated with cardiac arrest and the duration of myocardial ischemia at an early timepoint during cardiac surgery [10]. However, those studies focused on a variety of cardiac surgeries, and little is known about the plasma concentration during and after CABG surgery, although it was reported that

Se deficiency is related to postoperative AF after CABG in intermediate-risk patients [20]. The plasma concentration of Se during CPB in CABG and after CABG has remained to be determined.

In addition, the plasma concentration of Co during CPB and after CABG has not been reported. Based on our previous study of the effect of CPB during the CABG procedure on trace elements [12,19], we designed the present study to investigate the plasma concentrations of Se and Co during and after CABG. Our findings demonstrated that the concentration of Se during CABG was significantly reduced after 45 min on CPB (Fig. 2A). This reduction was true for both the male and female patients (Fig. 3). The reduction of Se during CABG was recovered on day 1 (Fig. 3) and maintained before discharge. In contrast, the plasma concentration of Co was maintained during CPB (Fig. 4) but decreased after CABG on day 4.

These observations may indicate that Se supplementation during CPB in CABG surgeries may be necessary. A low level of Se was reported to be correlated with AF after CABG [20], and Se supplementation in patients undergoing CABG surgery was recently suggested [21]. The mechanism underlying the decrease in plasma Se during CABG is not yet clear, but we speculate that the decrease is related to the dilution of plasma and a possible transition of Se into cells. Further mechanistic studies are warranted.

We did not observed changes in the plasma concentration of Co during CPB (Fig. 4). However, the plasma Co decreased on day 1 after CABG and remained low on day 4 before discharge. This finding indicates that the plasma concentration of Co during CPB may not be significantly affected by blood dilution during CPB. It is unknown whether this is due to a sufficient reserve of Co in the body; it is also possible that there is a mechanism by which intracellular Co is released into the plasma. The deficiency of Co after CABG on day 1 in this study suggests that there may be a re-distribution of Co in the body after CABG. Our findings thus suggest that Co supplementation immediately after CABG may be necessary, although it may not be necessary during CPB. Further studies are necessary to uncover the mechanisms involved.

Hemodilution during CPB may affect the plasma concentrations of trace elements. Modified ultrafiltration during CPB may also affect these concentrations. The fluid balance was positive in the most patients in the present study as described above and in Table 1. However, the calculated fluid balance did not include the blood loss during surgery. As reported in another study [23], the total blood loss was 1,043 g during CABG surgery and the intraoperative blood loss was 640 g. In fact, the actual positive balance in this study (583 g) was less than that in the reference. Nevertheless, in the present study, only the Se concentration was reduced during the CPB. Our finding that the plasma concentration of Co did not change during CPB may suggest the minor role of hemodilution.

Another important question regarding trace elements is the correlations between the plasma concentrations of trace elements and the development of postoperative AF as mentioned above. Postoperative AF is a major complication of CABG [24,25], and the prevalence of post-CABG AF requiring anti-coagulant therapy after discharge from hospital is 25.7% [26] and 20-30% in our practice [27].

Study limitations. As the first study on the measurement of Se and Co at various timepoints during CABG under CPB, the sample size was limited, particularly for detecting the correlation between the concentrations of Se and Co and postoperative AF. Nevertheless, our results provide clear evidence of the changes in the concentrations of Se and Co during CABG under CPB.

In conclusion, this study for the first time identified the alteration of the plasma concentrations of Se and Co during and after CABG surgery. The findings suggest that Se supplementation before or during CABG and Co supplementation after CABG may become necessary for patients undergoing CABG. Further investigations are warranted to clarify the correlations of Se and Co and the development of postoperative AF.

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