Selenium Attenuates Adriamycin-Induced Cardiac Dysfunction via Restoring Expression of ATP-Sensitive Potassium Channels in Rats

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Abstract The possible mechanism of adriamycin (ADR) and/or selenium (Se) deficiency-induced cardiac dysfunction, and cardioprotective effects of Se against ADR-induced cardiac toxicity were investigated in this study. Cardiac function was evaluated by plasma brain natriuretic peptide level and echocardiographic and hemodynamic parameters. Cardiac glutathione peroxidase (GPx) activity was assessed spectrophotometrically. Expression of ATP-sensitive potassium channels (K_{ATP}) subunits—SUR2A and Kir6.2—were examined by real-time PCR and Western blotting. The results showed that cardiac function and cardiac GPx activity decreased remarkably after administration of ADR or Se deficiency; more dramatic impairment of cardiac function and cardiac GPx activity were observed after co-administration of ADR and Se deficiency. Mechanically, it is novel for us to find down-regulation of K_{ATP} subunits gene expression in cardiac tissue after administration of ADR or Se deficiency, and more significant inhibition of cardiac K_{ATP} gene expression was identified after co-administration of ADR and Se deficiency. Furthermore, cardiac toxicity of ADR was found alleviated by Se supplementation, accompanied by restoring of cardiac GPx activity and cardiac K_{ATP} gene expression. These results indicate that decreased expression of cardiac K_{ATP} is involved in adriamycin and/or Se deficiency-induced cardiac dysfunction; Se deficiency exacerbates adriamycin-induced cardiac dysfunction by future inhibition of K_{ATP} expression; Se supplementation seems to protect against adriamycin-induced cardiac dysfunction via restoring K_{ATP} expression, showing potential clinical application in cancer chemotherapy.

Keywords ATP-sensitive potassium channel · Adriamycin · Selenium · Cardiac dysfunction

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Introduction

Chemotherapy is now a major regimen of cancer for its effectiveness which significantly increases survival rate in patients with cancer. However, the cardiotoxicity after chemotherapy administrations which is presented as cardiac dysfunctions draws our attention. Both early diastolic and later systolic cardiac dysfunction were observed in clinical practice and animal models [1]. Pathologically, sacroplasmic reticulum injury, mitochondrial damage, and perimyocytic matrix loss were observed in heart after chemotherapy administrations [2].

It is suggested that heart failure is associated with lower circulating selenium (Se) level. Se deficiency is believed as an admitted cause of reversible heart failure such as Keshan Disease [3]. Epidemiological data showed that low Se concentration was associated with cardiovascular death in patients with acute coronary disease [4]. Lowered circulating Se level has been recognized as an indicator of increasing risk of cardiovascular diseases [5]. Thus, patients with low Se status may have already suffered from potential or

subclinical cardiovascular diseases before observation of marked cardiac dysfunction. As an antioxidant inducer, the cardiac protective role of Se has been described in previous studies [6, 7]. It is interesting for us to investigate whether the low Se status exacerbates cardiac dysfunction on one hand, and on the other hand, if Se supplement attenuates cardiac dysfunction during chemotherapy administration.

Expression of inward rectifier K^+ channels (K_{ATP}) has been indentified in cardiac muscle at high density [8]. Cardiac K_{ATP} is described molecularly composed of two subunits: regulatory sulfonylurea receptors 2A (SUR2A) and inward rectifying K^+ channel 6.2 (Kir6.2), which locate on intracellular membranes of cardiomyocytes [9]. As a metabolism activity-gated biological sensor [10], the most important role of K_{ATP} is to adjust potential-dependent functions to accommodate cell energy demand under physiological and pathological conditions [11]. There are immediate and delayed regulations of K_{ATP} channels. The former means temporal regulation of channel activities after instant metabolic changes [12], while the later means postponed but more profound regulation of K_{ATP} channels by modulating the gene expressions [13]. Activation and up-regulated expression of K_{ATP} were described cardioprotective in previous studies [14, 15].

Thus, mechanically, it is logical for us to assume that the regulation of K_{ATP} channels may be involved in chemotherapy and/or low-selenium-induced cardiac dysfunction; and supplementation of Se could attenuate chemotherapy-induced cardiac dysfunction via modulating expression of K_{ATP} channel. In order to demonstrate this hypothesis, the present study employed adriamycin and/or low-selenium-induced cardiac dysfunction animal models. Cardiac function and expression of K_{ATP} channel were assessed before and after supplementation of Se in animals.

Materials and Methods

Animals

Fifty-one male weaning (3-week-old) Sprague–Dawley rats (SPF class, body weight 75 ± 10 g) were raised in ten polypropylene cages under controlled environment conditions (12-h light–dark cycle, temperature 25 ± 1 °C, humidity 65 ± 4 %). Standard diet (containing 0.2 mg selenium/kg food) [16] was produced by Animal Experimental Center of Xi'an Jiaotong University and low-selenium diet (<0.02 mg selenium/kg food) was produced by Trophic Animal Feed High-tech Co. (Jiangsu, China) according to AIN-93M formula. Diets were stored at 4 °C and fresh tap water was allocated continuously. All animal experimental procedures followed protocols approved by Medical Animal Research Ethics Committee at Xi'an Jiaotong University.

Groups and Treatments

Animals were assigned randomly into five groups: control ($n=10$), ADR ($n=10$), LS ($n=10$), LS + ADR ($n=11$), and ADR + SS ($n=10$). In control, animals were fed with standard diet for 14 weeks and then treated by intraperitoneal injection of physiological saline everyday for 21 days. In ADR, animals were fed with standard diet for 14 weeks and then treated by four equal intraperitoneal injections of adriamycin (Pharmacia & Upjohn, UK) once a week during 4 weeks. Each injection contained 4 mg/kg bodyweight adriamycin, for a total dose of 16 mg/kg bodyweight (modified from previous studies [17]). In LS, animals were fed with low-selenium diet for 14 weeks and then treated by intraperitoneal injection of physiological saline every day for 21 days. In ADR + LS, animals were fed with low-selenium diet for 14 weeks and then treated by four equal intraperitoneal injections of adriamycin (4 mg/kg bodyweight) once a week during 4 weeks; In ADR + SS, animals fed with standard diet for 14 weeks and then treated by four equal intraperitoneal injections of adriamycin (4 mg/kg bodyweight) once a week during 4 weeks, then intraperitoneal injection of sodium selenite (0.05 mg/kg bodyweight; Sigma-Aldrich, USA) was administrated as selenium supplementation every day for 21 days [18].

Echocardiographic and Hemodynamic Parameters

Echocardiography

Echocardiographic tests were performed according to protocols described in other previous studies [19, 20]. Animals were anesthetized with chloral hydrate (10 %, 0.03 ml/kg bodyweight) intraperitoneally 10 min before imaging. In order to maintain an optimal image quality, the hair of anterior chest wall was removed by 7 % sodium sulfite solution and rats were placed left laterally. Vivid 7 dimension (GE Healthcare, USA) with probe (12L) working at 10 MHz was utilized. The probe was placed parallel to left margin of sternum, adjusting image depth ranging from 2.0 to 4.0 cm. Regurgitant jets were assessed by two-dimensional color and continuous Doppler. M-mode tracings were applied to determine left ventricular end-systole diameter (LVESD) and left ventricular end-diastole diameter (LVEDD). At last, functional parameters such as left ventricular fractional shortening (LVFS%) which stands for percentage of blood ejected by left ventricle each heart beat and left ventricular ejection fraction (LVEF%) which measures the change of left ventricular diameter from each diastole to systole were calculated respectively: LVFS%=[(LVEDD−LVESD)/LVEDD]%; LVEF%=[(LVEDV−LVESV)/LVEDV]%.

Hemodynamic Parameters

According to methods described in a previous study [21], hemodynamic determination was conducted. Animals were anesthetized with chloral hydrate (10 %, 0.03 mL/kg bodyweight) intraperitoneally. A catheter connected to Powerlab 4/25 biological analysis system (AD Instrument, Australia) was intubated from right carotid artery into left ventricle. Left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) were measured. Left ventricular developed pressure (LVDP) was calculated by subtracting LVEDP from LVSP (LVDP=LVSP−LVEDP). Heart rate (HR) was measured by electrocardiogram connected to Powerlab 4/25 biological analysis system.

Selenium Concentration Detection

Samples of whole blood and hearts harvested from rats were wet-washed by mixed acids (nitric acid and perchloric acid) in borosilicate reaction tubes. After removal of excess acids, samples were titrated to 10 ml by ultra pure water (Aqulix 5 water purification system, Merck Millipore, USA). Selenium concentration was then determined by flameless atomic absorption spectrophotometry method using Z-5000 spectrophotometer (Hitachi, Japan) with cathode lamp of Se (resonance line, 196.0 nm; Photron, Australia). Standard selenium solutions were used to calibrate the results.

Plasma BNP Assay

After femoral artery puncture, whole blood samples were collected using EDTA-Na$_2$ vacuum blood collection tubes. Supernatant was collected after centrifuged at 1,500 rpm for 20 min. Samples were processed by Triage brain natriuretic peptide (BNP) assay (Biosite, USA) within 1 h after collection at room temperature according to the manufacturer's instructions.

Tissue Preparation

Heart was removed immediately after sacrifice, trimmed on ice, then transferred to −80 °C refrigerator after flash-frozen in liquid nitrogen. Collected tissue was then processed for real-time PCR and Western blotting.

Real-Time PCR

According to manufacturer's instructions, total RNA was extracted from tissue using RNAfast 200 Kit (Fastagen, Shanghai, China). Reverse transcription was performed by using PrimeScript RT Master Mix (TaKaRa, Japan). SYBR Premix Ex TaqTMII (TaKaRa, Japan) was employed to perform real-time PCR, using oligonucleotide primers for SUR2A and Kir6.2 [6] (Table 1). Relative expression of mRNA was analyzed and compared using Bio-Rad IQ5 software (Version 2.0, BioRad, USA). $\triangle$cycle threshold ($\triangle Ct = Ct_{Target} - Ct_{\beta-actin}$) was employed when β-actin expression was introduced as endogenous reference gene to normalize mRNA expression of SUR2A and Kir6.2.

Western Blotting

Frozen cardiac tissue was homogenized in RIPA lysis buffer system (Santa Cruz, USA) with PMSF (Santa Cruz, USA). All procedures followed manufacturer's instructions. Sample protein concentration was detected by using BCA protein assay kit (Santa Cruz, USA). Sample protein was boiled in 1× SDS-PAGE loading buffer and separated by electrophoresis in 10 % SDS–polyacrylamide gel, then transferred to a PVDF membrane. Antibodies against Kir6.2 (ab79171, Abcam, USA) and SUR2A (ab84229, Abcam, USA) were applied to incubate the bolts at 4 °C overnight. Tris-buffered saline (containing 0.02 % Tween 20) was used to wash the membranes which were then incubated with goat polyclonal secondary antibody to rabbit IgG conjugated to HRP (ab6721, Abcam, USA). Membranes were developed using Super Signal West Pico chemiluminescence reagent (Thermo Scientific, USA) and then visualized on X-ray films.

Cardiac Tissue Glutathione Peroxidase Activity Assay

GPx activity in heart extract was assessed by spectrophotometrical method using Glutathione Peroxidase Cellular Activity Assay Kit (Sigma-Aldrich, USA), following the

Table 1 Primers for real-time PCR

Gene primer	Sequence	Size (bp)
SUR2A	Forward 5′-GCTGAAGAATATGGTCAAATCTC-3′ Reverse-5′-CGGAGTGTCGTATTCCAAAATA-3′	451
Kir6.2	Forward 5′-TCCAACAGCCCGCTCTAC-3′ Reverse 5′-GATGGGGACAAAACGCTG-3′	167
β-Actin	Forward 5′-TGGCACCCAGCACAATGAA-3′ Reverse 5′-CTAAGTCATAGTCCGCCTAGAAGCA-3′	186

manufacturer's instructions. The assay is based on the reaction in which oxidation of glutathione (GSH) to oxidized glutathione (GSSG) is catalyzed by GPx. As NADPH is consumed when GSSG is recycled back to GSH, the decrease in NADPH absorbance at 340 nm (Tecan Sunrise Absorbance Reader, Tecan, Austria) could be utilized to calculate the activity of GPx indirectly.

Statistical Analysis

Data obtained was input into Excel (version 12.0, Microsoft, USA) and analyzed by SPSS (version 17.0, SPSS, USA). Results were presented as (mean ± SD). Among groups, comparisons of differences were analyzed by one-way ANOVA, and then a LSD test was carried out. $P<0.05$ was considered statistically significant.

Results

Se Concentration in Cardiac Tissue and Blood Samples

Se concentration in cardiac tissue and blood is shown in Table 2. Significant changes of Se concentration were observed in low-Se dietary and/or Se intraperitoneal injection-treated animals. Compared with control, Se concentration in blood and/or cardiac tissue decreased significantly in LS and ADR + LS groups. Se intraperitoneal injection was proved to increase Se concentration in blood and cardiac tissue in ADR + SS group compared with ADR group.

Table 2 Selenium concentrations in blood and cardiac tissue of rats in different groups

Groups	Se (blood), mg/L	Se (cardiac tissue), mg/kg, wet tissue
Control	0.134±0.008	0.335±0.008
ADR	0.131±0.009	0.311±0.012
LS	0.024±0.005[a(#)]	0.162±0.001[a(*)]
ADR + LS	0.022±0.004[a(**),b(#)]	0.154±0.004[a(**),b(**)]
ADR + SS	0.277±0.008[c(*)]	0.494±0.005[c(*)]

Se concentrations in blood and cardiac tissue of rats were analyzed spectrophotometrically. Se deficit diet was applied to LS and ADR + LS; intraperitoneal injection of sodium selenite was applied to ADR + SS as Se supplementation. Results were expressed as mean ± SD ($n=10$ for control, ADR, LS, and ADR + SS, $n=11$ for ADR + LS group)

[a] Values are significantly different compared with control [*$P<0.05$; **$P<0.01$; #$P<0.001$]

[b] Values are significantly different compared with ADR. *$P<0.05$; **$P<0.01$; #$P<0.001$]

[c] Values are significantly different compared with ADR. [*$P<0.05$; **$P<0.01$; #$P<0.001$]

Effects of Se Deficiency and/or Se Supplementation on ADR-Induced Cardiac Dysfunctions
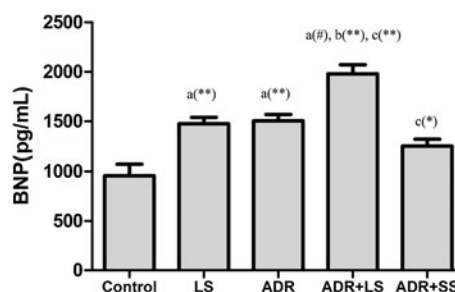
Plasma BNP

As demonstrated in Fig. 1, it was showed by Triage BNP assay that plasma BNP level increased significantly in LS or ADR group compared with control. A more dramatic increase of plasma BNP was found in ADR + LS group. After Se supplementation by intraperitoneal injections, a drop of plasma BNP level in ADR + SS group was indentified compared with ADR.

Echocardiographic and Hemodynamic Determination

Echocardiographic and hemodynamic parameters including LVEDD, LVESD, LVEF, LVFS, LVSP, LVDP, and HR were detected and calculated in this study as shown in Tables 3 and 4. An overall increase of LVEDD, LVESD, and LVDP accompanied by an overall decrease of LVEF, LVFS, LVSP, and HR were found in LS, ADR, and ADR + LS groups. However, it appeared to be more significant increase of LVEDD, LVESD, and LVDP, decrease of LVEF, LVFS, LVSP, and HR in ADR + LS group compared with LS or ADR groups. Significant recovery of cardiac function marked by echocardiographic and hemodynamic parameters was found in ADR + SS group compared with ADR group, which was evidenced by restoring of LVEDD, LVESD, LVEF, LVFS, LVSP, LVDP, and HR after Se supplementation.

Effects of ADR, Se Deficiency, and Se Supplementation on Expression of K_ATP Subunits

Real-time PCR and Western blotting were employed to detect the changes of expression of Kir6.2 and SUR2A in the present study. As shown in Figs. 2a and 3a, mRNA and protein expression of SUR2A in cardiac tissue was impaired

Fig. 1 Effects of Se deficiency/supplementation and/or adriamycin on plasma BNP level. Plasma BNP concentration (pg/ml) was examined by Triage BNP Assay. *Columns* indicate BNP concentrations in control, LS, ADR, ADR + LS, and ADR + SS, respectively. Values are presented as mean ± SD. *a* Values are significantly different from control; *b* value is significantly different from LS ($P<0.01$); *c* value is significantly different from ADR [*$P<0.05$; **$P<0.01$; #$P<0.001$]

Table 3 Echocardiographic parameters of rats in different groups

Group	No.	LVEDD (mm)	LVESD (mm)	LVEF (%)	LVFS (%)
Control	10	4.14±0.38	1.13±0.77	95.60±11.02	75.64±8.30
LS	10	5.29±0.26[a(*)]	2.77±0.35[a(**)]	78.93±10.92[a(*)]	50.44±5.73[a(*)]
ADR	8	5.13±0.27[a(*)]	2.68±0.25[a(**)]	74.79±8.45[a(**)]	48.20±3.84[a(**)]
ADR + LS	7	6.72±0.43[a(#),b(**),c(**)]	3.90±0.65[a(#),b(**),c(**)]	68.24±12.37[a(#),b(**),c(**)]	41.93±1.28[a(#),b(**),c(**)]
ADR + SS	8	4.87±0.82[c(*)]	1.94±0.29[c(**)]	84.56±8.89[c(*)]	61.07±9.24[c(*)]

Cardiac functions (left ventricular functions) were assessed by echocardiographic determination. All results are presented as mean ± SD

LVEDD stands for left ventricular end-diastole diameter, *LVESD* stands for left ventricular end-systole diameter, *LVEF* stands for left ventricular ejection fraction, *LVFS* stands for left ventricular fractional shortening

[a] Values are significantly different from control ($P<0.05$) [*$P<0.05$; **$P<0.01$; #$P<0.001$]

[b] Values are significantly different from LS ($P<0.05$) [*$P<0.05$; **$P<0.01$; #$P<0.001$]

[c] Values are significantly different from ADR ($P<0.05$). [*$P<0.05$; **$P<0.01$; #$P<0.001$]

by treatment of Se deficiency and/or adriamycin in LS, ADR, and ADR + LS groups. Co-administration of Se deficiency and ADR resulted in more dramatic decrease of SUR2A gene expression in ADR + LS group compared with LS or ADR group. mRNA and protein levels in cardiac tissue of rats received injection of Se in ADR + SS group were significantly recovered compared with those in ADR group. Similarly, as demonstrated in Figs. 2b and 3b, expression at transcriptional and translational levels of another subunit of cardiac K_{ATP}, Kir6.2, decreased significantly in LS, ADR, and ADR + LS groups compared with control group. Decreasing expression of Kir6.2 in ADR + LS group was enhanced by co-administration of Se deficiency and adriamycin compared with ADR or LS group. However, it seemed that supplementation of Se reversed the decreasing expression of Kir6.2 in ADR + SS group compared with ADR group.

Effects of ADR, Se Deficiency, and Se Supplementation on GPx Activity

As shown in Fig. 4, activity of GPx in LS, ADR, and ADR + LS group decreased significantly compared with control. It

was observed that in ADR + LS group, activity of GPx dropped more apparently compared with LS or ADR group. However, a noticeable recovery of GPx activity in ADR + SS group was confirmed by GPx activity assay when compared with ADR group.

Discussion

As a widely used chemotherapy agent, ADR is also well-known for its cardiac toxicity [22]. In this study, impaired cardiac function was confirmed in ADR-treated rats. Se deficiency is considered as an indicator for cardiovascular diseases [23]; measurements performed in the present study revealed cardiac dysfunctions in rats fed with low-Se diet. Considering these two cardiac harmful factors together, it motivated us to investigate whether heart is more susceptible to ADR-induced damage during Se deficiency, and if Se supplementation which plays a role in cardiac protection [24] alleviates ADR-induced cardiac toxicity. Mechanically, K_{ATP} has been proved cardioprotective in K_{ATP} disrupted animals [25], so it is logical for us to speculate that down-

**Table 4** Hemodynamic parameters of rats in different groups

Group	No.	LVSP (mmHg)	LVDP (mmHg)	HR (bmp)
Control	10	130.61±10.86	2.31±0.96	368.14±38.74
LS	10	82.49±5.40[a(#)]	7.39±0.50[a(#)]	294.68±27.49[a(*)]
ADR	8	80.46±3.03[a(#)]	7.73±0.70[a(#)]	278.33±25.30[a(*)]
ADR + LS	7	65.37±2.11[a(#),b(*),c(*)]	14.83±1.11[a(#),b(*),c(*)]	233.00±19.89[a(#),b(*),c(*)]
ADR + SS	8	73.80±4.47[c(*)]	8.50±0.48[c(*)]	255.07±21.44[c(*)]

Cardiac functions (left ventricular functions) were assessed by hemodynamic tests using a cardiac intubation method. All results are presented as mean ± SD

LVSP stands for left ventricular systolic pressure, *LVDP* stands for left ventricular developed pressure, *HR* stands for heart rate

[a] Values are significantly different from control ($P<0.05$); [*$P<0.05$; **$P<0.01$; #$P<0.001$]

[b] Values are significantly different from LS ($P<0.05$); [*$P<0.05$; **$P<0.01$; #$P<0.001$]

[c] Values are significantly different from ADR. [*$P<0.05$; **$P<0.01$; #$P<0.001$]
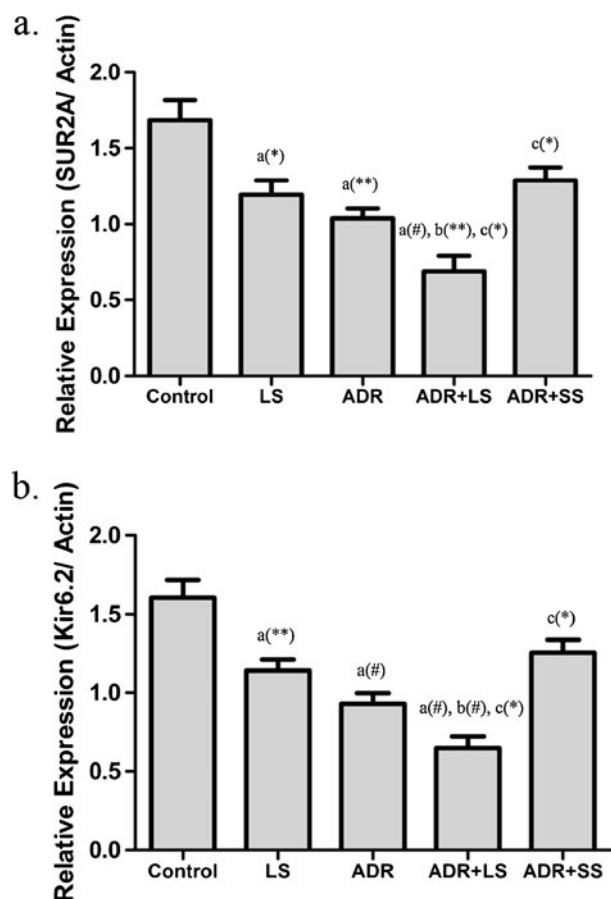
Fig. 2 Effects of Se deficiency/supplementation and/or adriamycin on mRNA expression of K_{ATP} subunits in cardiac tissue. Total mRNA was extracted from cardiac tissue and then detected by real-time PCR with designed primers for K_{ATP} subunits SUR2A and Kir6.2 in Table 1. mRNA expression of β-actin was introduced to normalize mRNA expression of SUR2A and Kir6.2. a Analysis of expression of SUR2A normalized by β-actin. Columns in graph present the ratio of SUR2A/β-actin as mean ± SD (n=6) of control, LS, ADR, ADR + LS, and ADR + SS, respectively. b Analysis of expression of Kir6.2 normalized by β-actin. Columns in graph present the ratio of Kir6.2/β-actin as mean ± SD (n=6) of control, LS, ADR, ADR + LS, and ADR + SS, respectively. a Indicates significant differences when compared with control; b indicates significant differences when compared with LS; c indicates significant differences when compared with ADR [*$P<0.05$; **$P<0.01$; #$P<0.001$]

regulation of K_{ATP} maybe accounted for impaired cardiac function in Se deficiency and/or ADR-treated rats, and correspondingly, up-regulation of K_{ATP} may be the mechanism of recovery of cardiac function after Se supplementation.

In this study, cardiac function was measured by echocardiographic and hemodynamic determination. An overall increase of LVEDD, LVESD, and LVDP accompanied by an overall decrease of LVEF, LVFS, LVSP, and HR indicate that both left ventricular systolic and diastolic functions are impaired in rats [21]. In response to pressure overload and/or volume expansion stimulation, BNP is released from cardiac ventricles dramatically. In patients with left ventricular
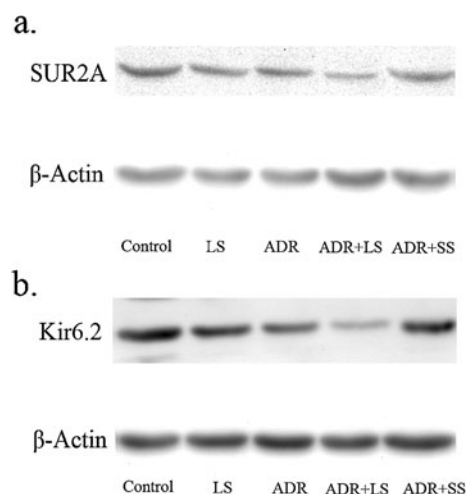
Fig. 3 Effects of Se deficiency/supplementation and/or adriamycin on protein expression of K_{ATP} subunits in cardiac tissue. Sample protein was extracted, separated, and detected by specific antibodies against K_{ATP} subunits SUR2A and Kir6.2. The expression of SUR2A and Kir6.2 was normalized by β-actin in the same sample. Darkness of immunoblots stands for expression level of protein. a Western blotting of SUR2A. Immunoblots stands for control, LS, ADR, ADR + LS, and ADR + SS from left side to right side, respectively. b Western blotting of Kir6.2. Immunoblots stands for control, LS, ADR, ADR + LS, and ADR + SS from left side to right side, respectively

dysfunction, elevation of plasma BNP level is usually used to evaluate the severity and clinical prognosis [26]. It is considered that plasma BNP level is reliable in monitoring cardiac function [27].

Adriamycin (doxorubicin) which belongs to anthracycline drug family has been shown to be an effective antitumor chemotherapeutic agent [28]. Even nowadays, adriamycin is still being widely used in treatment of many kinds of
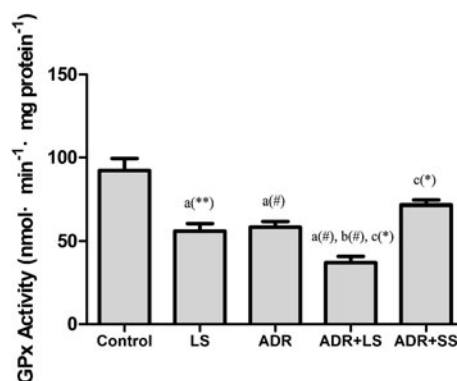
Fig. 4 Effects of Se deficiency/supplementation and/or adriamycin on GPx activity in cardiac tissue. Spectrophotometrical method was utilized to examine the activity of GPx in cardiac tissue. Values indicated by columns in graph are presented as mean ± SD (n=6). Columns from left side to right side stand for control, LS, ADR, ADR + LS, and ADR + SS, respectively. a Indicates significant differences when compared with control; b indicates significant differences when compared with LS; c indicates significant differences when compared with ADR [*$P<0.05$; **$P<0.01$; #$P<0.001$]

hematologic malignances and solid tumors such as leukemia, multiple myeloma, breast cancer, lung cancer, and so on. Though adriamycin's strong anti-tumor therapeutic effects have been confirmed, it was described early in clinical practice that cardiac dysfunction was one of the complications after administration of adriamycin [29]. In 1,697 patients with non-Hodgkin's lymphomas, 147 (3.2 %) patients who received an adriamycin-based treatment developed acute cardiac complications, including acute heart failure, myocardial infarction, atrial fibrillation, and myocarditis [30]. In the present study, after intrapertoneal injection of adriamycin, measurements confirmed adriamycin-induced cardiac dysfunction in ADR group. While adriamycin binds to DNA, released electrons convert the drug from quinone from to semiquinone from. During the reaction, superoxide anions are produced, which induce further reactive oxygen species (ROS) generation [31]. It is proposed that formation of ROS is responsible for cardiac injury during chemotherapy [32]. As a major peroxide scavenging enzyme, decreased GPx activity has been described under condition of oxidative stress [33]. In the present study, GPx activity in cardiac tissue treated by adriamycin decreased significantly, indicating that adriamycin-induced cardiac dysfunction was associated with oxidative stress.

In this study, we demonstrated decreased cardiac function in rats received low-Se diet by plasma BNP and echocardiographic and hemodynamic determinations. Se deficiency or low Se status is always mentioned crucial in development of heart diseases. Early population-based clinical study showed that low selenium level was associated with cardiovascular outcomes [34]. Low selenium status, which existed before observation of clinical cardiac events, was amplified by acute coronary syndrome [35]. In a recent prospective cohort study of patients with coronary artery disease, individual baseline serum selenium concentration was inversely associated with mortality of acute coronary syndrome. Low serum selenium was considered independent of classic risk factors including biomarkers of necrosis, LVEF, and so on of acute coronary syndrome [4]. In an animal study, Se deficiency was proved associated with pronounced worsening of cardiac functional parameters and cardiac hypertrophy, associated with remarkable decrease of GPx activity in cardiac tissue [36]. These results are in accordance with ours: increasing serum BNP level, LVEDD, LVESD, and LVDP, decreasing LVEF, LVFS, LVSP, and HR accompanied by decreasing GPx activity was found in low-Se-diet-treated rats.

It seems that administration of low-Se diet and adriamycin share the same pathway of impairment of GPx activity in worsening cardiac functions. On one hand, cancer patients are found lack of micronutrients including Se. It was mentioned that the serum Se concentration in patients with urinary or larynx cancer was lower than normal control [37]. On the other hand, low serum Se concentration is an independent risk

factor for coronary artery disease [4]. Thus, it is interesting and of clinical significance to investigate whether chemotherapy exacerbates cardiac function in patients with low Se status which indicates potential or existing heart diseases. In this study, co-administration of low-Se diet and intraperitoneal injections of adriamycin was applied to rats to mimic such certain clinical situation. Results in this study showed a more significant worsening of cardiac function in ADR + LS than in ADR or LS. Further, GPx activity assay confirmed more significant impairment of GPx activity after co-administration of adriamycin and low-Se diet, proposing that stimulated oxidative stress which depresses GPx activity was exerted by both Se deficiency and adriamycin. These results indicate that Se-deficient rats are more sensitive to cardiac toxicity of adriamycin, suggesting when cardiac toxic chemotherapy agents are employed, cancer patients with low Se status should be treated more cautiously.

As a regimen, intraperitoneal administration of Se was applied to rats receiving treatment of adriamycin which was proved cardiac toxic in ADR group in the present study. Cardiac function examination of BNP and echocardiographic and hemodynamic parameters showed that the adriamycin-induced cardiac dysfunction was attenuated significantly by supplementation of Se. And simultaneously, decreased GPx activity was restored by supplementation of Se, indicating that Se alleviated cardiac dysfunction by increasing activity of GPx which was one of the major ROS scavengers [38]. This result is coincided with previous studies on association between Se and cardiac dysfunctions [6, 24, 36, 39, 40]. Se is essential in biosynthesis of selenoenzymes including six groups of GPx (GPx 1–6), which play an important role in protecting cells against oxidative injury caused by ROS in certain pathological conditions [41]. Se supplementation may promote GPx activity by providing substrate for synthesis of GPx. Therefore, in chemotherapy of patients with cancer, Se containing drugs or compounds may be able to be used to enhance resistance to chemotherapy-induced cardiac toxicity.

Furthermore, this study explored possible mechanism of cardiac dysfunction induced by adriamycin and/or low-Se diet. Molecularly, K_{ATP} expressed in cardiomyocytes is a heteromultimer with octameric conformation. Data from animal models have indentified cardiac K_{ATP} is composed of tow subunits—Kir6.2 (encoded by $KCNJ11$) and SUR2A (encoded by $ABBC9$) [42, 43]. Generally, it is well accepted that K_{ATP} has a cardioprotective property in certain pathological conditions such as myocardial ischemia [44]. 5-HD, a K_{ATP} channel blocker, was applied in a number of studies to testify the role of K_{ATP} in ischemic cardiac dysfunctions. These studies showed that the cardiac protective effects were inhibited after administration of 5-HD [45]. Decreased expression of cardiac K_{ATP} was proposed as one of the

mechanisms for cardiac dysfunction in diabetic rats. Similar down-regulation of K_{ATP} expression was indentified in high-glucose incubated myocytes in the same study [46]. In the present study, decreasing expression of Kir6.2 and SUR2A were detected by real-time PCR and Western blotting in cardiac tissue from rats received treatment of adriamycin and/or Se deficiency. Therefore, our results suggest that decreased expression of cardiac K_{ATP} impairs cardioprotective effects against adriamycin and/or Se deficiency, leading to cardiac dysfunction.

Interestingly, whether in myocardial ischemia, hyperglycemia, adriamycin-, or Se-deficiency-induced cardiac dysfunction, oxidative stress always plays an important part. Studies have revealed malfunctions of ion channels in status of oxidative stress. Administration of H_2O_2 which is a strong oxidant-induced cytosolic Ca^{2+} influx in neuronal PC12 cells [47] and TRPM2 (transient receptor potential melastain 2) current in ovary cells [48]. In high-glucose-treated myocytes, previous study has illustrated that cardiac injury is caused by excessively increasing production of ROS [49]. Clearing of ROS is seemed to protect cell from injury, especially for myocyte which is more susceptible to oxidative stress. After application of Se as a free radical scavenger, this study demonstrated that the activity of GPx elevated significantly, indicating that supplement of Se inhibited oxidative stress in myocytes; we also found that the expression of Kir6.2 and SUR2A was restored to perform cardioprotective effects in adriamycin-treated rats after supplementation of Se. These results suggest that Se attenuates adriamycin-induced cardiac dysfunction by restoring expression of K_{ATP} via elevating activity of GPx to depress ROS generation. However, the specific mechanism(s) of inhibiting K_{ATP} expression by ROS and induction of K_{ATP} expression by Se (or other anti-oxidants) is still unclear. More studies are necessary to illustrate the change of K_{ATP} currents, structure, and signal transduction in cardiac dysfunction in the very near future.

Conclusions

This study may provide a new recognition of possible mechanism of Se deficiency and/or adriamycin-induced cardiac dysfunction and cardiac protective effects of Se supplementation:

1. Decreased expression of K_{ATP} is involved in adriamycin- and/or Se-deficiency-induced cardiac dysfunction.
2. Se deficiency exacerbates adriamycin-induced cardiac dysfunction by future inhibition of K_{ATP} expression.
3. Se supplementation seems to protect against adriamycin-induced cardiac dysfunction via restoring K_{ATP} expression.

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