

Protective Effects of Salidroside on Epirubicin-Induced Early Left Ventricular Regional Systolic Dysfunction in Patients with Breast Cancer

Hua Zhang,¹ Wei-sheng Shen,² Chun-heng Gao,¹ Li-chun Deng² and Dong Shen²

- 1 Department of Echocardiography, Affiliated Jiangyin Hospital of Southeast University Medical College, Jiangyin, Jiangsu, People's Republic of China
2 Department of Oncology, Affiliated Jiangyin Hospital of Southeast University Medical College, Jiangyin, Jiangsu, People's Republic of China

Abstract

Background: Salidroside [2-(4-hydroxyphenyl)ethyl- β -D-glucopyranoside], one of the most potent ingredients extracted from the plant *Rhodiola rosea* L., has been shown to have a cardiovascular protective effect as an antioxidant, and early treatment of epirubicin-induced cardiotoxicity has been the focus of clinical chemotherapy in patients with breast cancer. However, the cardio-protective effects of salidroside on epirubicin-induced cardiotoxicity, especially early left ventricular regional systolic dysfunction, have to date been sparsely investigated.

Objective: The aim of this study was to investigate the protective effects of salidroside in preventing early left ventricular regional systolic dysfunction induced by epirubicin.

Methods: Sixty patients with histologically confirmed breast cancer were enrolled. Eligible patients were randomized to receive salidroside (600 mg/day; n = 30) or placebo (n = 30) starting 1 week before chemotherapy. Patients were investigated by means of echocardiography and strain rate (SR) imaging. We also measured plasma concentrations of reactive oxygen species (ROS). All parameters were assessed at baseline and 7 days after each new epirubicin dose of 100 mg/m².

Results: A decline of the SR peak was observed at an epirubicin dose of 200 mg/m², with no significant differences between salidroside and placebo (1.35 \pm 0.36 vs 1.42 \pm 0.49/second). At growing cumulative doses of epirubicin, the SR normalized only with salidroside, showing a significant difference in comparison with placebo at epirubicin doses of 300 mg/m² (1.67 \pm 0.43 vs 1.32 \pm 0.53/second, p < 0.05) and 400 mg/m² (1.68 \pm 0.29 vs 1.40 \pm 0.23/second, p < 0.05). Moreover, a significant increase in plasma concentrations of ROS was found with placebo, but they remained unchanged with salidroside.

Conclusion: Salidroside can provide a protective effect on epirubicin-induced early left ventricular regional systolic dysfunction in patients with breast cancer.

Introduction

Epirubicin is one of the most effective drugs for treating breast cancer, and it is used in a wide spectrum of malignancies. However, recent clinical trials have shown that early left ventricular systolic dysfunction accompanied by high generation of reactive oxygen species (ROS) occurs during epirubicin chemotherapy.^[1] It is well established that oxidative stress plays an important role in the occurrence of epirubicin-induced cardiotoxicity.^[2] Recently, salidroside [2-(4-hydroxyphenyl)ethyl- β -D-glucopyranoside], one of the most potent ingredients extracted from the plant *Rhodiola rosea* L.,^[3] has been shown to exert cardiovascular protection as an antioxidant.^[4] In the present study, we investigated the protective effects of salidroside as an antioxidant on epirubicin-induced early left ventricular systolic dysfunction by strain rate imaging (SRI) derived from Doppler tissue imaging (DTI), and its potential mechanism.

Materials and Methods

Study Population and Methods

Sixty female patients (mean \pm SD age 54 ± 12 years) with histologically confirmed, previously untreated breast cancer were included in the study. The patients were all candidates for treatment with an epirubicin-based chemotherapy regimen (maximal cumulative dose 400 ± 40 mg/m²) according to the international standardized protocols for breast cancer.

At enrollment before randomization, all patients underwent echocardiographic analysis, a 12-lead electrocardiogram, and blood pressure measurement. The inclusion criteria were age between 18 and 68 years, and an echocardiographic left ventricular ejection fraction (LVEF) value $\geq 50\%$. Patients were not eligible if they had a history of coronary heart disease, hypertension, or diabetes mellitus, and/or had been previously

treated with chest irradiation. Our study was approved by the ethics committee of the Jiangyin People's Hospital, and written informed consent was obtained from all subjects.

In all subjects, blood samples were collected for the assessment of serum concentrations of ROS. The echocardiography and laboratory variables were assessed at baseline (t0) and 7 days after reaching an epirubicin dose of 100, 200, 300, and 400 mg/m² (t1, t2, t3, and t4, respectively). Both the subjects and the echocardiographic technicians were blinded to the treatment assignment. Salidroside with a purity of 99% was ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Shanghai, China).

The 60 enrolled patients were assigned as follows: 30 to the salidroside group and 30 to the placebo group. We performed a blind randomization with salidroside (600 mg/day) or placebo, beginning the therapy 1 week before the start of chemotherapy and continuing for the entire period of epirubicin administration. The clinical characteristics of the patients in each group are summarized in table I.

Table I. Clinical data of the two groups included in the study

Characteristic	Salidroside [n=30]	Placebo [n=30]
Patients		
Sex (n; male/female)	0/30	0/30
Age (years; mean \pm SD)	51 \pm 7	52 \pm 6
Body surface area (m ²)	1.84 \pm 0.26	1.86 \pm 0.17
Heart rate (beats/minute)	71 \pm 10	73 \pm 9
Tumor type		
Breast	30	30
Tumor stage		
I	15	14
II	8	9
III	5	6
IV	2	1

Table II. Conventional echocardiographic and strain rate imaging parameters in the two groups^a

Parameter and group	t0	t1	t2	t3	t4
Left ventricular ejection fraction (%)					
Placebo	66 ± 5	65 ± 4	65 ± 6	65 ± 5	66 ± 5
Salidroside	66 ± 7	65 ± 6	67 ± 6	65 ± 4	66 ± 6
Strain rate (/second)					
Placebo	1.69 ± 0.64	1.67 ± 0.19	1.35 ± 0.36*	1.32 ± 0.53*	1.40 ± 0.23*
Salidroside	1.68 ± 0.54	1.68 ± 0.23	1.42 ± 0.49*	1.67 ± 0.43 [†]	1.68 ± 0.29 [†]

a The data are reported as mean ± SD.

t0=baseline; t1=epirubicin 100 mg/m²; t2=epirubicin 200 mg/m²; t3=epirubicin 300 mg/m²; t4=epirubicin 400 mg/m²; *p<0.05 vs t0; [†]p<0.05 vs placebo.

Strain Rate Imaging (SRI) and Assessment of Oxidative Stress Markers

Conventional echocardiography and SRI were recorded using a commercially available system equipped with dedicated software (Qlab 5.0, Philips IE33). The LVEF was obtained from the apical 4- and 2-chamber views according to the Simpson rule and was considered abnormal if less than 50%. Myocardial SRI was derived from DTI. Strain rate (SR) data were recorded from the basal inter-ventricular septum (IVS), using standard apical views at a high frame rate (>90 frames/second). The region of interest (ROI) was constant at 5 mm² during the whole trial and was tracked automatically throughout the systole. SR data were stored in digital format and analyzed offline with dedicated software (Qlab 5.0, Philips IE33). SR data were averaged from 4–6 cycles. Our methodology for the myocardial SR has been described previously.^[5] In all subjects, the ROS serum concentrations were determined on fresh heparinized blood samples, using the free oxygen radicals test (FORT). The results are expressed as FORT units (FORT-U).^[6]

Statistical Analysis

The data are reported as mean ± SD. Intra-group differences between t0 values and values assessed at different epirubicin doses were calculated by a paired t-test. Differences between the salidroside group and the placebo group at the same epirubicin doses were calculated by a student's two-tailed t-test. The correlation between instrumental and laboratory variables was assessed

by Pearson correlation analysis. p-Values were considered significant when <0.05. To determine the reproducibility of the SR derived from DTI, SRI analysis was repeated by an additional investigator and by the same primary reader 1 day later. During these repeated analyses, the investigators were blinded to the results of both prior measurements.

Results

There were no appreciable differences in clinical characteristics between the salidroside and placebo groups at enrollment (table I). All patients reached the scheduled cumulative epirubicin dose of 400 mg/m². Chemotherapy associated with salidroside was well tolerated in all patients. Fifteen patients were randomly selected to undertake the intra- and interobserver reproducibility of the SR.

Conventional Echocardiography, SRI, and Laboratory Data

No significant abnormalities of the LVEF were found in either of the two groups throughout the entire treatment period (table II). However, we observed a reduction in the SR peak at t2 (p<0.05) at an epirubicin dose of 200 mg/m², with no significant differences between the salidroside and placebo groups (1.35 ± 0.36 vs 1.42 ± 0.49/second, p>0.05). With growing cumulative doses of epirubicin, the SR normalized only in the salidroside group, showing a significant difference in comparison with the placebo group at epirubicin doses

Table III. Serum concentrations of reactive oxygen species in the two groups^a

Group	Reactive oxygen species concentration (FORT-U)				
	t0	t1	t2	t3	t4
Placebo	498±41	502±36	849±15*	1023±75*	932±68*
Salidroside	498±30	500±31	519±12†	573±87†	542±98†

a The data are reported as mean ± SD.

FORT = free oxygen radicals test; **FORT-U** = FORT units; **t0** = baseline; **t1** = epirubicin 100 mg/m²; **t2** = epirubicin 200 mg/m²; **t3** = epirubicin 300 mg/m²; **t4** = epirubicin 400 mg/m²; * p < 0.05 vs t0; † p < 0.05 vs placebo.

of 300 mg/m² (1.67 ± 0.43 vs 1.32 ± 0.53/second, p < 0.05) and 400 mg/m² (1.68 ± 0.29 vs 1.40 ± 0.23/second, p < 0.05) [table II]. Furthermore, the ROS serum concentrations significantly increased at t2 in the placebo group (498 ± 41 vs 849 ± 15 FORT-U, p < 0.05), whereas they remained unchanged in the salidroside group (498 ± 30 vs 519 ± 12 FORT-U, p > 0.05) [table III]. We randomly selected 15 patients to undertake the intra- and interobserver reproducibility of the myocardial strain, and both intra- and interobserver variability were below 13% (table IV).

Correlations between Echocardiographic and Laboratory Data

We also correlated early impairment of significant echocardiographic parameters (calculated as a change in the SR [ΔSR] by subtracting the values from the baseline values) with an increase in serum concentrations of ROS after 200 mg/m² of epirubicin. We found modest correlations between the ΔSR and an increase in plasma concentrations of ROS (r = 0.49, p < 0.05).

Discussion

Although epirubicin is one of the most powerful antineoplastic agents, its clinical use is limited by dose-related cardiotoxicity.^[7] Epirubicin-induced myocardial dysfunction detected early by serial tissue Doppler echocardiography has been correlated with oxidative stress markers with an unchanged LVEF during epirubicin chemotherapy.^[8] DTI associated with SRI has shown its value in early detection of epirubicin-induced cardiotoxicity, and a measurable SR peak depression has

been regarded as the earliest sign of left ventricular regional systolic dysfunction in epirubicin-treated patients long before a clinical manifestation of heart failure.^[9] Our study also showed that a subtle systolic decline of the SR peak appeared after an epirubicin dose of 200 mg/m², but the LVEF remained unchanged in the salidroside and placebo groups between baseline and epirubicin chemotherapy.

Our findings also showed that a significant rise in ROS concentrations continued throughout epirubicin chemotherapy. Although the pathogenesis of epirubicin-induced cardiotoxicity remains controversial, the oxidative stress-based hypothesis has gained the widest acceptance.^[10] Robust generation of ROS is defined as oxidative stress, and significant increases in generation of ROS (a collective name for hydrogen peroxide, superoxide, and hydroxyl radicals) in cardiomyocytes, as well as serum concentrations, have been reported in epirubicin-induced cardiotoxicity.^[10,11] ROS are excessively generated from a likely mitochondrial source, then hasten lipid peroxidation and DNA damage, and consequently initiate cell apoptosis or necrosis.^[12,13] Accordingly, successful antioxidant interventions targeted to reduce ROS offer insights into preventing epirubicin-induced cardiotoxicity.

Rhodiola rosea has long been used as an adaptogen in traditional Tibetan medicine.^[14] Salidroside [2-(4-hydroxyphenyl)ethyl-β-D-glucopyranoside], the main active compound of *Rhodiola* plants, is reported to possibly play a central role in alleviation of mitochondrial-generated ROS and modulation of mitochondrial-related apoptosis signaling in multiple types of cells.^[15] More recently, *in vitro* analysis showed that pretreatment with salidroside exerted remarkable benefits in inhibition of ROS overgeneration as an antioxidant, and decreased mitochondrial superoxide concentrations.^[16] Salidroside supplementation

Table IV. Intra- and interobserver variability^a of the strain rate in 15 randomly selected patients

Intraobserver variability (%)	Interobserver variability (%)
10 ± 4	11 ± 3

a Intra- and interobserver variability values (%) = percentage of the mean of two repeated measurements ± SD.

could protect cultured cells against ultraviolet light, paraquat, and H₂O₂.^[17] In the present study, an early Δ SR derived from DTI parameters observed after an epirubicin dose of 200 mg/m² was accompanied in the placebo group by a significant increase in ROS serum concentrations, which seems to confirm the relationship between a ROS increase and epirubicin-induced early left ventricular systolic regional dysfunction.

Safety assessments of salidroside have been reported in our earlier study.^[18] Adverse events were spontaneously reported by the investigator at the end of the study. The investigator made the decision about whether an abnormality represented an adverse event. There were no clinical adverse events throughout the period of salidroside therapy.

The small number of patients enrolled and the short follow-up are some of the limitations of the present study. Moreover, DTI-derived strain measurements are dependent on the direction of the Doppler angle of incidence in relation to myocardial motion. This limitation could be overcome by a new measure of two-dimensional strain, using speckle tracking echocardiography, in a further study.

Recent studies have shown that salidroside induces cell-cycle arrest and apoptosis in human breast cancer cells and may be a promising candidate for breast cancer treatment.^[19] In a further study, we will focus on investigating (i) the uncertain effect of the salidroside-epirubicin compound; and (ii) the dose-related pharmacologic and probable toxicologic effects of salidroside.

Conclusion

Our preliminary study demonstrated that salidroside can provide a protective effect against epirubicin-induced early left ventricular regional systolic dysfunction in patients with breast cancer, and the protective effects provided by salidroside may be explained by its reduction of oxidative stress.

Acknowledgments

Hua Zhang and Wei-sheng Shen contributed equally to this study.

This project was supported by WuXi Health (grant no. ZX0806). None of the authors have any conflicts of interest that are directly relevant to the content of this article.

References

1. Minotti G, Menna P, Salvatorelli E, et al. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* 2004; 56: 185-229
2. Elliott P. Pathogenesis of cardiotoxicity induced by anthracyclines. *Semin Oncol* 2006; 33: S2-7
3. Zhou X, Wu Y, Wang X, et al. Salidroside production by hairy roots of *Rhodiola sachalinensis* is obtained after transformation with *Agrobacterium rhizogenes*. *Biol Pharm Bull* 2007; 30: 439-42
4. Wu T, Zhou H, Jin Z, et al. Cardioprotection of salidroside from ischemia/reperfusion injury by increasing N-acetylglucosamine linkage to cellular proteins. *Eur J Pharmacol* 2009; 613: 93-9
5. Mercurio G, Cadeddu C, Piras A, et al. Early epirubicin-induced myocardial dysfunction revealed by serial tissue Doppler echocardiography: correlation with inflammatory and oxidative stress markers. *Oncologist* 2007; 12: 1124-33
6. Mantovani G, Maccio A, Madeddu C, et al. Quantitative evaluation of oxidative stress, chronic inflammatory indices and leptin in cancer patients: correlation with stage and performance status. *Int J Cancer* 2002; 98: 84-91
7. Jensen BV, Skovsgaard T, Nielsen SL. Functional monitoring of anthracycline cardiotoxicity: a prospective, blinded, long-term observational study of outcome in 120 patients. *Ann Oncol* 2002; 13: 699-709
8. Mantovani G, Madeddu C, Cadeddu C, et al. Persistence, up to 18 months of follow-up, of epirubicin-induced myocardial dysfunction detected early by serial tissue Doppler echocardiography: correlation with inflammatory and oxidative stress markers. *Oncologist* 2008; 13: 1296-305
9. Jassal DS, Han SY, Hans C, et al. Utility of tissue Doppler and strain rate imaging in the early detection of trastuzumab and anthracycline mediated cardiomyopathy. *J Am Soc Echocardiogr* 2009; 22: 418-24
10. Ferreira AL, Matsubara LS, Matsubara BB. Anthracycline-induced cardiotoxicity. *Cardiovasc Hematol Agents Med Chem* 2008; 6: 278-81
11. Zweier JL, Talukder MAH. The role of oxidants and free radicals in reperfusion injury. *Cardiovasc Res* 2006; 70: 181-90
12. Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc Res* 2004; 61: 461-70
13. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species: its mechanism and measurement in mammalian systems. *FEBS Lett* 1991; 281: 9-19
14. Zhu YZ, Huang SH, Tan BKH, et al. Antioxidants in Chinese herbal medicines: a biochemical perspective. *Nat Prod Rep* 2004; 21: 478-89
15. Zhong H, Xin H, Wu LX, et al. Salidroside attenuates apoptosis in ischemic cardiomyocytes: a mechanism through a mitochondria-dependent pathway. *J Pharmacol Sci* 2010; 114: 399-408

16. Schriener SE, Abrahamyan A, Avanesian A, et al. Decreased mitochondrial superoxide concentrations and enhanced protection against paraquat in *Drosophila melanogaster* supplemented with *Rhodiola rosea*. *Free Radic Res* 2009; 43: 836-43
17. Schriener SE, Avanesian A, Liu YX, et al. Protection of human cultured cells against oxidative stress by *Rhodiola rosea* without activation of antioxidant defenses. *Free Radic Biol Med* 2009; 47: 577-84
18. Shen WS, Gao CH, Zhang H, et al. Effect of *Rhodiola* on serum troponin I, cardiac integral backscatter and left ventricle ejection fraction of patients who received epirubicin-contained chemotherapy. *Chin J Integr Trad West Med* 2010; 12: 1250-2
19. Hu X, Zhang X, Qiu S, et al. Salidroside induces cell-cycle arrest and apoptosis in human breast cancer cells. *Biochem Biophys Res Commun* 2010; 398: 62-7

Correspondence: Dr. *Chun-Heng Gao*, Department of Echocardiography, Affiliated Jiangyin Hospital of Southeast University Medical College, 163 Shou-Shan Road, Jiangyin, Jiangsu 214400, People's Republic of China.
E-mail: gaochh@yahoo.com