THE CARDIOVASCULAR EFFECTS OF SALIDROSIDE IN THE GOTO-KAKIZAKI DIABETIC RAT MODEL

Many factors, including hyperglycemia, hypertension, obesity, dyslipidemia, and a sedentary lifestyle, contribute to a high prevalence of cardiovascular disease. Specific vascular impairment treatments in the context of diabetes and vascular risk need to be improved. Salidroside is the primary active component of Rhodiola rosea and has documented antioxidative, cardioprotective, and vasculo-protective properties. The aim of this study was to test the hypothesis that salidroside has protective effects against hyperglycemia, hypertension, and vaso-dilation impairment in the Goto-Kakizaki (GK) rat model of diabetes. We evaluated cardiovascular parameters (e.g., daytime/nighttime systolic and diastolic blood pressure, heart rate, and activity), metabolic parameters (e.g., body weight, food and water consumption, serum fructosamine level, glucose tolerance), ENOS / phospho-eNOS expression level and in vitro vascular reactivity of aorta and second-order mesenteric arteries in Wistar-Kyoto (control) and GK (diabetic) rats treated with salidroside (40 mg/kg) or placebo (water) for 5 weeks. GK rats showed hypertension, marked glucose intolerance, and impaired endothelium-dependent and endothelium-independent vasodilation capacity. Salidroside showed beneficial effects on endothelial and non-endothelial vasodilation and likely acts on the endothelium and smooth muscle cells through the soluble guanylyl cyclase pathway. Despite its vaso-protective effects, salidroside had no effect on blood pressure and heart rate in GK and control rats, it did not improve glucose metabolism or limit hypertension in the GK model of type 2 diabetes.

**Key words:** type 2 diabetes, salidroside, telemetry, Rhodiola rosea, vascular function, glucose intolerance, nitric oxide, hypertension, guanylyl cyclase

INTRODUCTION

Many factors, including hyperglycemia, hypertension, obesity, dyslipidemia, and a sedentary lifestyle, contribute to the high prevalence of cardiovascular disease (CVD). The American Heart Association (AHA) considers diabetes mellitus and metabolic syndrome major risk factors for CVD (1).

Endothelial dysfunction, which is associated with decreased nitric oxide (NO) bioavailability and impaired vasodilation, is one of the earliest stages of cardiovascular events in diabetes. Impaired vasodilation has been noted in animal models of hyperglycemia and diabetes (2, 3), as well as in clinical studies for both type 1 and type 2 diabetes patients (4, 5). It has also been demonstrated that endothelial dysfunction plays an important role in the association of diabetes and hypertension (6).

Goto-Kakizaki (GK) rats are a unique non-obese rat model of polygenic type 2 diabetes that were developed by selective inbreeding of glucose-intolerant Wistar rats (7). GK rats are characterized by disturbed glucose-stimulated insulin release (8, 9) and islet blood flow (10), defective intracellular glucose metabolism (11), insulin resistance (12), and hyperinsulinemia (8, 10). They manifest stable pathological features that resemble human type 2 diabetes, including hyperglycemia, hypertension, and endothelial dysfunction (13). However, GK is a lean diabetes model, whereas most (but not all) patients with type 2 diabetes are overweight.

Salidroside (p-hydroxyphenethyl-β-D-glucoside) is the primary active component of Rhodiola rosea. This plant has been used for hundreds of years as a traditional medicine for different therapeutic purposes (14). Salidroside was found to have neuro-, cardio-, and hepato-protective activity (14). Salidroside and Rhodiola rosea extracts have also been tested in humans to improve mental performance and to combat depression (14).

Salidroside might have beneficial effects on vascular functions. The noted in vitro cardiovascular effects of salidroside include cardio-protection (15) and protection of endothelial cells against hypoxia (16).

In in vitro studies, salidroside stimulates glucose uptake in skeletal muscle cells by activating phosphorylation of AMP-activated protein kinase (17). These findings suggest the possibility of its potential therapeutic application as an antidiabetic therapy. However, most studies regarding the effects of salidroside on...
vascular function and glucose metabolism have been performed in vitro. In vivo studies are necessary to evaluate the potential therapeutic benefits of salidroside in the context of type 2 diabetes.

The aim of our study was to test the hypothesis that salidroside has protective effects against hyperglycemia, hypertension, and endothelial dysfunction in the rat GK model of diabetes.

MATERIALS AND METHODS

Animal protocols

Male Goto-Kakizaki (GK) rats (aged 12 weeks, n=26) were obtained from the academic laboratory UMRS 972 (Paris). Male Wistar Kyoto (WKy) control rats (aged 12 weeks, n=26) were obtained from Charles River Laboratories. Rats were synchronized to a 12 h light/12 h dark schedule, under controlled environmental conditions at an ambient temperature of 20–24°C with ad libitum food (standard chow SDS M20) and water. Food and water consumption was monitored. Daily oral gavage with salidroside (40 mg/kg - WKy Sal, GK Sal) or water (placebo - WKy placebo, GK placebo) was started in 20-week-old rats and continued for 35 days. The selected dose of salidroside was similar to that used by other researchers (18, 19).

For telemetric assessment of heart rate (HR), blood pressure (BP), and spontaneous locomotor activity, 12 WKy rats (6 WKy placebo, 6 WKy Sal) and 11 GK rats (6 GK placebo, 5 GK Sal) were implanted prior to gavage at 17 weeks of age. For in vitro vascular reactivity tests, non-implanted WKy rats (7 WKy placebo, 7 WKy Sal) and GK rats (8 GK placebo, 7 GK Sal) were used. A schematic of the protocol used in this study is shown in Fig. 1.

All manipulations with animals were performed in accordance with the United States National Institutes of Health guidelines and European Community standards on the Care and Use of Laboratory Animals (Ministere de l’Agriculture, France, Authorization No. 49072). The Ethics Committee for Animal Experimentation of Pays de la Loire approved this study (Protocol No. CEEA. 2010.40).

Salidroside

Salidroside was purchased from Shanghai Tauto Biotech Company, Ltd. (China). The identity of salidroside was verified using nuclear magnetic resonance analyses. The 1H spectrum was recorded in CD3OD on a Jeol GSX 270 MHz (Jeol Europe, Croissy-sur-Seine, France) spectrometer and corresponded to that of salidroside (20). Its purity was evaluated using high-performance liquid chromatography (HPLC). HPLC analysis was performed on a Waters 2695 apparatus (Waters, Guyancourt, France) consisting of a pumping system, vacuum degasser, and DAD detector, and assisted by the Empower 2 software (Waters). A 10 µL sample (1 mg/mL) was directly injected onto a Lichrospher 100 RP18 column (150 × 4.6 mm; 5 µm; Agilent Technologies) using a gradient acidic water/methanol solvent system. The flow rate was 1 mL/min with UV detection at 254 and 275 nm. Salidroside was 100% pure at both wavelengths.

Cardiovascular parameters

A telemetry system (Data Science International® - DSI, St Paul, MN, USA) was used to monitor BP, HR, and locomotor activity for conscious, freely-moving rats. After 2–3 weeks of acclimatization to laboratory conditions and daily handling, 12 WKy rats and 11 GK rats were surgically fitted with intraperitoneal radiotelemetry transmitters (TA11PA-C40, DSI) according to the recommendations of DSI as previously described (21). Surgery was performed under isoflurane anesthesia. Anesthetized rats received an intramuscular injection of Temgesic® (buprenorphine, 0.1 mg/kg) to provide analgesia during the surgery. A blood pressure catheter was placed in the lower abdominal aorta and secured with surgical glue (3 M Vetbond™, USA). Its placement was verified using a radio receiver. The transmitter was secured in the abdomen by suturing to the muscle wall with a non-absorbable suture. Rats recovered for 14 days and received three subcutaneous injections of antibiotic (streptomycin, 40 mg/kg/day). Post-operative analgesia was provided by 40 mg/kg of pediatric ibuprofen (Advil®) in the drinking water for 3 days post-surgery. Rats were housed individually in standard cages during the first 3–4 days of recovery. After this period, each rat was placed with a non-operated partner. Non-operated partners were animals from the same cages in which the operated rats were housed before surgery. WKy rats were randomly assigned to the WKy placebo (n=6) or WKy Sal (n=6) groups, and GK rats were randomly assigned to the GK placebo (n=6) or GK Sal (n=5) groups.

Cardiovascular parameters and activity were then recorded telemetrically from 19 to 24 weeks of age.

Oral glucose tolerance tests (OGTT)

OGTT were performed 6 days before treatment with salidroside or placebo and on day 28 of treatment. Blood glucose (sampled from v. caudale laterale) was measured after an overnight fast before and 30 min, 1 h, and 2 h after a glucose

---

**Fig. 1.** Diagram of the study protocol. OGTT, oral glucose tolerance test; D-20, D-6, and D-3 refer to 20, 6, and 3 days before starting oral gavage, respectively; D1, D28, and D35 indicate days of gavage.
bolus (2 g/kg diluted in 1 mL of water). Blood tests were performed under isoflurane anesthesia using the Accu-Check Performa system (Roche Diagnostics GmbH, Germany).

Fructosamine assessments

After the 5-week treatment period, all rats were sacrificed by CO₂ inhalation. Blood samples were collected by cardiac puncture in glass tubes, and serum was separated by centrifugation at 4°C for 15 minutes. Serum samples were stored at −79°C. Fructosamine levels were assayed by photometric analysis on a Cobas® 8000 Modular Analyzer System (Roche Diagnostics, Japan). Immediately following blood sampling, rats from the “telemetric” group were explanted, and vessels from rats in the “vascular reactivity” group were dissected and divided into several segments used for vascular reactivity tests and for Western blot analysis.

Vascular reactivity

Two segments of second-order mesenteric arteries and two segments of thoracic aorta (2 mm long) were dissected from each rat (WKy placebo n=7, WKy Sal n=7, GK placebo n=8, GK Sal n=7). Segments were mounted on a wire myograph (DMT, Aarhus, Denmark) in physiological salt solution maintained at 37°C and pH 7.4 as described previously (22). After wall-tension normalization and stabilization for 45 minutes, vessel viability was tested with potassium-rich solution (80 mM). Endothelium

Table 1. Metabolic and cardiovascular parameters in control (WKy) and diabetic (GK) rats treated with salidroside or placebo.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKy placebo</th>
<th>WKy Sal</th>
<th>GK placebo</th>
<th>GK Sal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight before treatment (week 20), g</td>
<td>436±6</td>
<td>437±3</td>
<td>366±8*</td>
<td>359±6</td>
</tr>
<tr>
<td>Body weight (week 24) g</td>
<td>463±7</td>
<td>462±6</td>
<td>380±7*</td>
<td>367±7</td>
</tr>
<tr>
<td>Mean food consumption for 5 weeks (from 20 to 24 weeks), g/kg/day</td>
<td>48±1</td>
<td>46±1</td>
<td>56±2*</td>
<td>55±3</td>
</tr>
<tr>
<td>Mean water consumption for 5 week (from 20 to 24 weeks), mL/kg/day</td>
<td>50±1</td>
<td>50±1</td>
<td>99±13*</td>
<td>95±11</td>
</tr>
<tr>
<td>Serum fructosamine for week 25 μmol/L</td>
<td>153±3</td>
<td>152±2</td>
<td>187±9*</td>
<td>199±12</td>
</tr>
<tr>
<td>Fasting blood glucose, before treatment mg/dL</td>
<td>100±3</td>
<td>98±3</td>
<td>156±12*</td>
<td>163±13</td>
</tr>
<tr>
<td>Fasting blood glucose, week 4 of treatment mg/dL</td>
<td>113±4</td>
<td>103±3</td>
<td>122±8</td>
<td>141±15</td>
</tr>
<tr>
<td>Blood glucose, before treatment T_120, mg/dL</td>
<td>124±3</td>
<td>118±4</td>
<td>301±23*</td>
<td>331±30</td>
</tr>
<tr>
<td>Blood glucose, week 4 of treatment T_120, mg/dL</td>
<td>125±3</td>
<td>121±2</td>
<td>278±20*</td>
<td>297±25</td>
</tr>
<tr>
<td>SBP day, before treatment mmHg</td>
<td>121±3</td>
<td>115±3</td>
<td>135±3*</td>
<td>134±4</td>
</tr>
<tr>
<td>SBP day, mean for 4 week of treatment mmHg (mean for 4 week of treatment)</td>
<td>121±3</td>
<td>117±2</td>
<td>138±4*</td>
<td>139±3</td>
</tr>
<tr>
<td>SBP night, before treatment mmHg</td>
<td>126±4</td>
<td>122±3</td>
<td>143±4*</td>
<td>143±4</td>
</tr>
<tr>
<td>SBP night, mean for 4 week of treatment mmHg</td>
<td>126±3</td>
<td>123±2</td>
<td>145±4*</td>
<td>146±3</td>
</tr>
<tr>
<td>DBP day, before treatment mmHg</td>
<td>85±3</td>
<td>82±2</td>
<td>96±2*</td>
<td>95±2</td>
</tr>
<tr>
<td>DBP day, mean for 4 week of treatment mmHg</td>
<td>85±3</td>
<td>83±2</td>
<td>98±2*</td>
<td>99±3</td>
</tr>
<tr>
<td>DBP night, before treatment mmHg</td>
<td>89±4</td>
<td>87±2</td>
<td>104±2*</td>
<td>104±2</td>
</tr>
<tr>
<td>DBP night, mean for 4 week of treatment mmHg</td>
<td>89±3</td>
<td>87±2</td>
<td>104±2*</td>
<td>105±3</td>
</tr>
<tr>
<td>HR day, before treatment bpm</td>
<td>290±4</td>
<td>280±3</td>
<td>272±2*</td>
<td>278±5</td>
</tr>
<tr>
<td>HR day, mean for 4 weeks of treatment bpm</td>
<td>294±5</td>
<td>284±4</td>
<td>279±3*</td>
<td>289±5</td>
</tr>
<tr>
<td>HR night, before treatment bpm</td>
<td>347±6</td>
<td>337±3</td>
<td>324±7*</td>
<td>339±15</td>
</tr>
<tr>
<td>HR night, mean for 4 weeks of treatment bpm</td>
<td>344±5</td>
<td>334±3</td>
<td>323±8*</td>
<td>339±14</td>
</tr>
<tr>
<td>Activity day, before treatment counts/min</td>
<td>1.0±0.2</td>
<td>1.0±0.1</td>
<td>0.8±0.05</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Activity day, mean for 4 weeks of treatment counts/min</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Activity night, before treatment counts/min</td>
<td>3.1±0.3</td>
<td>3.8±0.3</td>
<td>3.6±0.2</td>
<td>4.2±0.5</td>
</tr>
<tr>
<td>Activity night, mean for 4 weeks of treatment counts/min</td>
<td>2.8±0.3</td>
<td>3.4±0.3</td>
<td>2.9±0.1</td>
<td>3.5±0.4</td>
</tr>
<tr>
<td>Phospho-eNOS/eNOS aorta</td>
<td>1.45±0.21</td>
<td>1.30±0.27</td>
<td>1.61±0.30</td>
<td>1.59±0.36</td>
</tr>
<tr>
<td>Phospho-eNOS/eNOS mesenteric artery</td>
<td>0.94±0.13</td>
<td>0.88±0.08</td>
<td>0.90±0.07</td>
<td>0.94±0.13</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.; * P<0.05 WKy-placebo vs. GK placebo; No significant difference was observed for WKy placebo vs. WKy Sal; No significant difference was observed for GK placebo vs. GK Sal.
integrity was controlled by evaluating acetylcholine-induced relaxation as described previously (22). Endothelium-independent vasodilatory functions were assessed by CRC to sodium nitroprusside (SNP 10^{-9}–10^{-8} M) after pre-contraction with Phe 10^{-6} M. Between the curves, we applied a wash out and equilibration period of 20 min. The relaxant responses to acetylcholine and SNP were calculated as percentages of the precontraction by 10^{-6} M Phe.

Western blot analysis

Other segments of second-order mesenteric arteries and thoracic aorta were homogenized. Proteins (25 mg total protein from each sample) were separated by SDS-PAGE using a 4% stacking gel, followed by a 10% running gel. Proteins were detected with specific antibodies (eNOS 1:1,000, phospho-eNOS 1:500, and β-actin 1:1,000 in bovine serum albumin in Tris-buffered saline with Tween [T-TBS-BSA] 5%; Transduction Laboratories). Protein expression was visualized using the ECL Plus chemiluminescence kit (Amersham).

Statistical analysis

Data are presented as mean ± standard error (S.E.). For weight dynamics, food and water consumption, glucose tolerance, and telemetric variables, we used two-way analysis of variance (ANOVA), with time as the within-subject factor and with group as the between-subject factor. Statistically significant differences were further analyzed by pairwise comparisons. For studies on isolated vessels, analyses of dose-response curves were performed. Sensitivity (median effective concentration (EC50)) and maximal response were calculated from the respective dose-response equations. Comparison of differences was performed with one-way ANOVA, with a post hoc Bonferroni tests. Differences were considered statistically significant if P ≤ 0.05. Analysis was performed using GraphPad Prism 6.

RESULTS

General characteristics

The body weights of 20-week-old WKy rats were approximately 20% higher compared to those of GK rats of the same age. Weight gains for the 24th weeks of life were higher in WKy rats (approximately +25 g - WKy vs. approximately +10 g - GK) (Table 1). However, food consumption per unit of body weight was 15–20% higher in GK rats, and relative water consumption was twice higher in GK rats compared to WKy rats. Serum fructosamine concentrations at 25 weeks of age were approximately 30% higher in GK rats. Fasting glucose in GK rats slightly increased, and fed glucose levels during OGTT were significantly higher in GK rats compared to those in WKy rats (Table 1, Fig. 2).

Salidroside did not affect body weight, serum fructosamine concentration, or glucose tolerance (Table 1, Fig. 2).

Blood pressure, heart rate, and activity

Telemetric monitoring of cardiovascular parameters showed a significant increase in systolic and diastolic blood pressure in GK rats (approximately 20 mmHg higher for SBP and 15 mmHg higher for DBP), along with a reduction in heart rate. There was no difference in activity between the different groups (Table 1). Salidroside had no significant effect on blood pressure, heart rate, or activity in GK rats and control rats.

Vascular reactivity

In aortic rings from GK placebo rats, maximal vasodilation capacity to acetylcholine (ACH) was impaired. There was also a slight decrease in maximal SNP vasodilation capacity (Fig. 3, Table 2). Mesenteric artery segments from GK placebo rats showed relaxation was very slightly impaired (small decrease in log EC50 but maximal vasodilation to ACh was unchanged) in response to ACh, with a decrease in maximal vasodilation capacity to SNP (Fig. 4, Table 2).

Chronic salidroside treatment did not affect WKy rats; however, in GK rats, vasodilatory response to SNP was preserved both in mesenteric arteries and aortic rings. Salidroside treatment improved vasodilation in response to ACh (maximal response) in aortic rings from GK rats.

Western blot analysis

There was no difference in eNOS, phospho-eNOS, and the ratio of phospho-eNOS to eNOS expression level in the
mesenteric artery and aorta between the different groups (Fig. 5, Table 1). Chronic salidroside treatment had no significant effect on eNOS expression and activation in GK rats and control rats.

**DISCUSSION**

Our GK rat model data are consistent with previously published results in this model. In addition to severe glucose intolerance and diabetic status in the absence of obesity, GK rats are hypertensive and have impaired endothelial-dependent and endothelial-independent vasodilation. Chronic treatment with salidroside did not affect glucose tolerance or hypertension but did affect endothelial-dependent and endothelial-independent vasodilation.

**Cardiovascular impairment in the Goto-Kakizaki rat model**

Goto-Kakizaki rats aged 20 and 25 weeks displayed fasting hyperglycemia and enhanced glucose intolerance similar to previous reports in GK rats (23, 24). Although food consumption of GK rats was higher than WKy rats, body weight and weight
gain in WKy rats was higher compared to GK rats. As noted by Landersdorfer et al. (25), this might be explained by metabolic impairments specifically related to direct perturbations in insulin metabolism or by higher metabolic rates in GK rats compared to WKy rats. The increased food consumption in GK rats is related to leptin resistance (25).

Telemetric recordings demonstrate that GK rats are hypertensive even when fed a typical diet (0.25% sodium content). GK rats have significantly higher systolic and diastolic blood pressure than WKy rats, which might be due to reduced dilation capacity of vessels in response to endothelial dysfunction (13). We also observed decreased heart rates in GK rats compared to WKy rats.
to those in WKy rats. This finding may be related to changes in the autonomic nervous system or baroreflex sensitivity, or to inherited metabolic disorders, particularly those that alter insulin metabolism (26). Reduced heart rates were previously described in streptozotocin-induced diabetic rats, which are also characterized by hyperinsulinemia and hyperglycemia (27).

Studies with isolated vessels have demonstrated that endothelium-dependent dilation is impaired in GK rats. As observed in the current study, endothelium-dependent vasodilation is primarily impaired in large vessels, such as the aorta (28) or superior mesenteric arteries (29). However, it appears preserved in smaller arteries, such as second- or third-order mesenteric arteries (30-32). It has been shown that hyperglycemia promotes deregulation of endothelial NO synthase -eNOS (33), which alters blood vessel dilation via the NO pathway. Hyperglycemia induces an increase in NADPH oxidase, which is involved in the reactive oxygen species (ROS) intracellular pathway, thus inducing oxidative stress that likely impairs NO synthesis and bioavailability by endothelial cells (34) in GK rats. We found no difference between WKy and GK rats for eNOS and phospho-eNOS expression in the mesenteric artery and aorta. According to observations of other authors, vascular eNOS expression in GK rats is rather increased (35-36), and so is eNOS mRNA expression (28, 37). However, some researchers report reduced vascular eNOS expression in GK rats (38, 39). The level of vascular eNOS phosphorylation in GK rats may be reduced (39, 40) or unchanged (41). In general, it seems that endothelium-dependent vasodilation impairment in GK rats is not due to decline in eNOS expression (28). Impaired vasodilation capacity could also be related to changes in smooth muscle as suggested by the blunted response observed in response to SNP. Witte et al. (23) reported that soluble guanylyl cyclase activity is reduced in aorta from GK rats. Decreased activity of soluble guanylyl cyclase is expected to attenuate the relaxation response to both ACh and SNP. This decreased vasodilation capacity might also contribute to elevated blood pressure in the animal model. Hyperglycemia promotes ROS generation. The increase of ROS in vascular smooth muscle cells of GK rats could induce an oxidation of the prosthetic heme group of soluble guanylyl cyclase and thereby blunt sensitivity to NO (23). An attenuated vasodilation response to SNP in uterine arteries from GK rats has also been reported and is linked with soluble guanylyl cyclase-dependent mechanisms (42).

Vascular effects of salidroside

Diabetes is a very common chronic disease, for which efficient treatment needs to be improved. New ways for diabetes management are constantly looked for using various diabetic models. Sakr (43) had recently shown that oral gavage of sitagliptin, a dipeptidyl peptidase-4 inhibitor, for one month decreased blood glucose level and insulin resistance and improved cognitive functions in type 2 diabetic Sprague-Dawley rats. Similarly, Kang et al. (44) demonstrated that oral administration of xenoestrogens for 5 days protects from STZ-induced apoptosis of pancreatic islets, improves blood glucose and insulin level in type 1 diabetic mice. In the present study we aimed to test salidroside in GK spontaneous diabetes model.

Rhodiola and salidroside have neuro, cardio-, and hepato-protective activity that prevent or reduce stress-induced impairments. Salidroside has been proposed to be a potential compound for anti-diabetic therapy (17). In our study, salidroside did not affect glucose metabolism or hypertension; however, it affected the vascular vasodilatory capacity at the level of the aorta and mesenteric arteries.

Salidroside has potent anti-oxidant properties. Rhodiola and salidroside inhibit oxidative stress in rat hepatic stellate cells (45) as well as inhibit intracellular ROS production (46). Salidroside acts directly at the endothelial cell level. In vitro, salidroside protects cultured endothelial cells against hydrogen peroxide cytotoxicity (47).

To our knowledge, salidroside and *Rhodiola rosea* extracts have not been tested in *vitro* on smooth muscle cell vasodilatory function. We have shown in *vivo* that chronic treatment with salidroside prevents endothelium-independent vasodilation impairment in the context of type 2 diabetes. Salidroside did not change eNOS expression and the ratio of phospho-eNOS to eNOS in GK and control rats, suggesting that salidroside is not able to elicit eNOS activation in *vivo*. As far as we know, in *vivo* effects of salidroside on eNOS regulation were not tested earlier. However in *vitro* studies had shown that salidroside was able to normalize eNOS activation in cultured endothelial cells, when eNOS expression was attenuated by high glucose (48) or homocysteine (49), or enhanced by H$_2$O$_2$ (50).

We hypothesize that salidroside could thus mainly act through endothelium-independent mechanisms involving guanylyl cyclase. Soluble guanylyl cyclase oxidation impairs its function by modifying the heme group (51). Increased oxidative stress affects soluble guanylyl cyclase that becomes unresponsive to NO. Salidroside, by reducing oxidative stress, could also have beneficial effects on smooth muscle function and soluble guanylyl cyclase activity.

Surprisingly, salidroside did not reduce hypertension in GK rats. Thus, the mechanisms of hypertension in GK rats involve impaired vasodilatory capacity (13) as well as other impaired pathways (6).

In our study, salidroside had no remarkable effect on glucose tolerance and hypertension. However, in the study of Wang et al. (52) *Rhodiola crenulata* root (500 mg/kg/day for 4 weeks by oral gavage) improved glucose tolerance and ameliorated metabolic derangements in Zucker diabetic fatty rats. Furthermore, *Rhodiola rosea* whole extract inhibited carbohydrate-degrading enzymes and angiotensin-converting enzyme as shown by Kwon et al. (53), suggesting its potential utility in control of postprandial hyperglycemia and hypertension associated with diabetes. It can be that salidroside alone, being just one of the potentially active compounds isolated from *Rhodiola* species, should be not enough for more prominent and full therapeutically beneficial effects on diabetes and cardiovascular pathological manifestations. We should focus our further studies on whole plant extracts.

In summary, we observed impaired endothelial and non-endothelial vasodilation capacity in GK rats. Salidroside has beneficial effects on endothelial and non-endothelial vasodilation and likely acts on the endothelium itself but also on smooth muscle cells and soluble guanylyl cyclase. Despite its vascular effects, salidroside did not improve glucose metabolism or hypertension in the GK model of type 2 diabetes.

**Acknowledgements:** This work was supported by CNES, the French Space Agency. Asmaa Alameddine is the recipient of a Ph.D. Grant from CNES and the regional council “Region des Pays de la Loire”.

**Conflict of interest:** None declared.

**REFERENCES**


Received: October 9, 2014
Accepted: February 5, 2015

Author’s address: Dr. Nastassia Navasiolava, UMR CNRS 6214 - INSERM 1083, Université d’Angers, UFR Medecine, Rue Haute de Reculee 49045, Angers Cedex, France.

E-mail: nastassia.navasiolava@chu-angers.fr