RESVERATROL IMPROVES SYSTEMIC OXIDATIVE STRESS AND LOWER URINARY TRACT DYSFUNCTION IN OBSESE MICE

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INTRODUCTION

Overweight and obesity are common health conditions and their prevalence is increasing globally followed by its comorbidities. The most common comorbidities related to obesity are: cardiovascular diseases, diabetes, cancer and musculoskeletal disorders (1).

Urological disorders have been increasingly recognized as a complication of obesity in the last few years (2,3). A number of observational studies revealed that bladder dysfunction, including lower urinary tract symptoms (LUTS), overactive bladder (OAB), and stress and urge incontinence are associated with obesity (4-5).

The NO-soluble guanylate cyclase-cGMP signaling pathway plays a crucial role in the regulation of a variety of pathophysiologic processes in mammals. It is well established that drugs acting through the NO/cGMP pathway (or by increasing NO bioavailability) are able to relax the bladder smooth muscle. Therefore, pharmacological agents that stimulate or activate soluble guanylate cyclase (sGC) or PDE5 inhibitors have been reported to ameliorate LUTS and OAB (6).

The polyphenol resveratrol is produced in plants and is present in many plant-based foods, such as grapes, peanuts, or berries. Preclinical studies have identified a number of mechanisms and targets by which resveratrol exerts benefits against cardiovascular diseases, including vascular dysfunction, hypertension, inflammatory states, cardiac injury, platelet hyperaggregability and oxidative stress (7).

Resveratrol has been shown to activate lipolysis in rat and human adipocytes, reduces white adipose tissue and liver lipogenic activity, increases liver fatty acid oxidation, and enhances brown adipose tissue and skeletal muscle thermogenesis (7).

Resveratrol exerts anti-inflammatory and anti-oxidant effects in the bladder (8-9). However, the effects of resveratrol on obesity-associated OAB models have not been investigated. A recent study carried out in high-fat fed obese mice showed degradation of soluble guanylate cyclase and reduction of cGMP production in detrusor (10) and urethra smooth muscle (11).

OBJECTIVE

This study aimed to evaluate whether chronic oral intake with resveratrol prevents obesity-induced OAB.

EXPERIMENTAL DESIGN AND METHODS

To perform concentration-response curves to the relaxing agents BAY 60-2770 (sGC activator; 10µM – 30 µM), BAY 41-2272 (sGC stimulator; 100 µM - 300µM), S-nitrosoglutathione (SNOG; 1 nM - 100 µM), gliceryl trinitrate (GTN; 1nM – 100 µM) and acetylated sodium nitrite (1 nM - 300 µM) in urethral smooth muscle from obese and lean animals.

To perform molecular and biochemical studies (Western Blotting, cGMP assay and ROS measurement) in urethral tissues of obese and lean mice.

METHODS

C57BL/6 male mice fed for 12 weeks with standard chow (n=20) or high-fat diet (n=22) were used. Control and obese mice were treated with vehicle (water) or resveratrol (100 mg/kg/day for 2 weeks). The metabolic profile was assessed by measuring body weight, epididymal fat mass, fasting glucose, total cholesterol, LDL and HDL. The oxidative stress status was evaluated by using TBARS assay kit in serum.

In separate experiments, bladder and urethra smooth muscle from control and obese mice were mounted in 5-ml organ bath chambers containing Krebs solution (95% O2 / 5% CO2, pH 7.4, 37°C). Contractile responses to the muscarinic agonist carbachol (CCh; 0.01 - 100 µM) and to electrical-field stimulation (EFS; 1 - 32 Hz) were obtained in the bladder smooth muscle. Relaxant responses to the NO donor gliceryl trinitrate (GTN; 0.001 - 100 µM) were obtained in the urethra.

The experimental protocols were approved by the Animal Ethics Committee of UNICAMP (CEUA-IB/UNICAMP, 3510-1).

Metabolic profile measurements

- Body and epididymal fat measurements;
- Blood glucose (ACCU-CHEK® Performa glucometer);
- Lipid profile (Cayman Chemical, Ann Arbor, Michigan);

Tissue preparations and isometric force recording

- Urethral and bladder smooth muscles were mounted in myographs;
- Krebs Henseleit solution, pH 7.4, 37°C, bubbled with O2/CO2 (5/95%) gas mixture;
- Tension: 2 mN (urethra) and 5 mN (bladder);
- Contractile curves with the muscarinic agonist carbachol (CCh; 0.001 - 100 µM) and to electrical-field stimulation (EFS; 1 - 32 Hz) were obtained in the bladder smooth muscle. Relaxant responses to the NO donor gliceryl trinitrate (GTN; 0.001 - 100 µM) were obtained in the urethra;
- The potency (pEC50) and maximal response (Emax) values were determined;

Semen lipid peroxide level measurement

- TBARS-assay kit, oxidative stress estimation through reaction of malondialdehyde (MDA) with 2-thiobarbituric acid (TBA). (Cayman Chemical TBARS Assay Kit, Ann Arbor, MI, USA);

Statistical analysis

All data are expressed as means ± S.E.M. (n). The program Instat (GraphPad software) was used for statistical analysis. One-way analysis of variance and Student’s t test were used to evaluate the results. P<0.05 was accepted as significant.

RESULTS

Resveratrol treatment reduced epididymal fat mass, blood glucose and TBARS in obese mice

Obese mice showed increases in body weight, epididymal fat mass, fasting glucose, total cholesterol and HDL (P<0.05) compared to lean mice. Oral treatment with resveratrol reduced epididymal fat mass and blood glucose. There was no difference in LDL levels. TBARS assay showed a 71% higher lipid peroxidation in obese mice when compared to lean mice, which was reversed by resveratrol (Figure 1, Table 1).

Table 1. Body weight, epididymal fat mass, blood glucose, lipid profile (total cholesterol, LDL and HDL) and TBARS in lean and obese mice treated or not with resveratrol.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Epididymal fat mass (g)</th>
<th>Blood glucose (mg/dL)</th>
<th>Total cholesterol (mg/dL)</th>
<th>HDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean + Vehicle</td>
<td>30.3 ± 3.3</td>
<td>0.25 ± 0.01</td>
<td>103.6 ± 3.2</td>
<td>90.0 ± 1.2</td>
<td>48.9 ± 1.0</td>
</tr>
<tr>
<td>Lean + Resveratrol</td>
<td>31.5 ± 2.1</td>
<td>0.27 ± 0.01</td>
<td>103.0 ± 9.2</td>
<td>89.1 ± 1.2</td>
<td>44.8 ± 1.1</td>
</tr>
<tr>
<td>Obese + Vehicle</td>
<td>31.5 ± 2.1</td>
<td>0.27 ± 0.01</td>
<td>103.0 ± 9.2</td>
<td>89.1 ± 1.2</td>
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</tr>
</tbody>
</table>

*P<0.05 compared with lean + vehicle; #P<0.05 compared with obese + vehicle (One way ANOVA Tukey's).

Enhanced contractile responses to carbachol and EFS in detrusor smooth muscle from obese mice: Prevention by oral treatment with Resveratrol in obese mice.

The impaired urethral smooth muscle relaxations in obese mice are reverted by Resveratrol treatment.

CONCLUSION

Resveratrol treatment improved obesity-associated OAB probably due to improvement of systemic oxidative stress.

REFERENCES


Financial Support: