Phytoestrogenic Isoflavones Daidzein and Genistein Reduce Glucose-Toxicity-Induced Cardiac Contractile Dysfunction in Ventricular Myocytes

Kadon K. Hintz and Jun Ren*

Department of Pharmacology, Physiology and Therapeutics, University of North Dakota School of Medicine, Grand Forks, North Dakota, USA

ABSTRACT

Epidemiological evidence suggests a reduction in the incidence of coronary heart disease, cancer and osteoporosis in populations with a high dietary intake of plant estrogen or phytoestrogen. The clinical benefit of phytoestrogens in cereals, vegetables and medicinal plants is attracting increasing attention for the general public. In the present study, we examined the effect of phytoestrogenic isoflavones daidzein and genistein on glucose toxicity-induced cardiac mechanical malfunction simulating diabetic cardiomyopathy. Adult rat ventricular myocytes were isolated and maintained for 24 hours in normal (NG, 5.5 mM) or high glucose (HG, 25.5 mM) medium in the absence or presence of isoflavones daidzein (50 μM) or genistein (20 μM). Cardiac contractile indices were evaluated using an IonOptix® MyoCam system including peak shortening (PS), maximal velocity of shortening/relengthening (± dL/dt), time-to-PS (TPS) and time-to-90% relengthening (TR₉₀). Myocytes maintained in HG medium displayed altered mechanical function simulating in vivo diabetes including reduced PS, ± dL/dt and prolonged TR₉₀ associated with normal TPS compared to those from NG myocytes. Interestingly, these HG-induced mechanical dysfunctions were abolished by co-incubation of daidzein or genistein.

*Correspondence: Jun Ren, Associate Professor, Division of Pharmaceutical Sciences, University of Wyoming, Laramie, WY 82071, USA; E-mail: jren@uwyo.edu.
However, daidzein but not genistein itself depressed PS in NG myocytes. Neither daidzein nor genistein affected any other mechanical parameters tested in NG myocytes. Collectively, these data suggest that the phytoestrogenic isoflavones daidzein and genistein may reduce glucose toxicity-induced cardiac mechanical dysfunction and thus possess therapeutic potential against diabetes-associated cardiac defects.

**Key Words:** Phytoestrogen; Isoflavones; Daidzein; Genistein; Myocytes.

### INTRODUCTION

Phytoestrogens or “plant estrogen” are naturally occurring compounds found in cereals, vegetables, medicinal plants and many other foods. They share structural similarity with 17β-estradiol and possess estrogenic effects (1). Phytoestrogens are composed of several categories such as isoflavones, coumestans, lignans and resorcylic acid lactones, all of which have been identified in mammals to produce certain biological activity (1,2). Recently, phytoestrogens have become increasingly attractive to the general public courtesy of the epidemiological and clinical evidence that individuals with limited intake of dietary phytoestrogens especially soybean isoflavones (e.g., genistein, daidzein), display a much higher incidence of coronary heart diseases, atherosclerosis, osteoporosis and cancers of the breast, prostate, and colon (1–5). The mechanism through which phytoestrogens, especially isoflavones, may exert these beneficial effects seems to depend, at least in part, on their mixed estrogen agonist-antagonist properties, their ability to inhibit enzymatic activity, in particular protein kinases such as tyrosine kinase, or activation of an “orphan” receptor distinct from the estrogen type I receptor (3). What may be unique is that soybean phytoestrogens have no estrogen agonist effects on the reproductive system (1,3). It is therefore reasonable to explore the potential of these naturally occurring plant estrogens in the treatment of cardiovascular diseases in both genders.

Diabetes mellitus is associated with a high incidence of patient mortality largely due to the development of cardiac dysfunctions and heart failure independent of macro- and microvascular coronary diseases (6,7). Several cellular defects have been proposed to contribute to the impaired ventricular function including impaired glucose transport/metabolism, elevated free radical production and malfunction of intracellular Ca\(^{2+}\)-regulating proteins such as Na\(^+/\)Ca\(^{2+}\) exchanger and sarco(endo)plasmic reticulum (SR) Ca\(^{2+}\)-ATPase (8–10). While many of these defects may contribute to cardiac damage and dysregulated ventricular function in diabetes, the role of estrogen in the clinical application of diabetic cardiomyopathy has been somewhat undefined. It has been shown that postmenopausal diabetic women display a significant increase in mortality compared to age-matched diabetic men (11). Several cardiac risk factors may be favorably influenced by estrogen replacement therapy in postmenopausal diabetic women (11), indicating a possible benefit of estrogen in the treatment of diabetic cardiomyopathy. However, the cancer promoting and female reproductive effects of estrogen make it somewhat risky or inapplicable for estrogen therapy (12). The aim of the present study was to examine the impact of the phytoestrogenic isoflavones daidzein and genistein on glucose toxicity-elicited cardiac mechanical dysfunction in...
ventricular myocytes. Earlier studies from our laboratory have established that normal ventricular myocytes may display diabetes-like phenotype of cardiac mechanical dysfunctions simulating in vivo diabetes after only 12–24 hours of culture in a serum-free high glucose medium (7,13,14). The use of this myocyte model should allow precise control of the extracellular milieu in which ventricular myocytes reside, thus eliminating interference from fibroblasts, endothelial metabolism and diffusional barriers (14).

**METHODS**

**Isolation and Culture of Ventricular Myocytes**

The experimental procedures described in this study were approved by the Animal Use and Care Committee at University of North Dakota (Grand Forks, ND, USA). Briefly, adult male Sprague-Dawley rats (225–250 g) were anesthetized with ketamine/xylazine (5:3, 1.32 mg/kg i.p.). Hearts were rapidly removed and perfused in a retrograde manner through a Langendorff apparatus with Krebs–Henseleit bicarbonate (KHB) buffer (mM: NaCl 118, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, N-[2-hydro-ethyl]-piperazine-N'[2-ethanesulfonic acid] (HEPES) 10, glucose 11.1 at pH 7.4 and 37°C for 3–5 min. The heart was then perfused for 20 min with KHB buffer containing 176 U/ml collagenase II (Worthington Biochemical Corp., Freehold, NJ, USA) and 0.5 mg/ml hyaluronidase (Sigma Chemical, St. Louis, MO, USA). After perfusion, the left ventricle was removed and minced. The tissues were further digested with 0.02 mg/ml trypsin (Sigma) before being filtered through a nylon mesh (300 μm). Extracellular Ca2+ was added incrementally back to 1.25 mM. Dispersed myocytes were plated on glass coverslips pre-coated with laminin (10 μg/ml), and maintained in a defined medium consisting of Medium 199 with Earle’s salts containing HEPES (25 mM) and NaHCO3 (25 mM), supplemented with albumin (2 mg/ml), L-carnitine (2 mM), creatine (5 mM), taurine (5 mM), insulin (100 nM), D-triiodothyronine (0.1 nM), penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (5 μg/ml). The medium 199 contained either normal glucose (NG: 5.5 mM) or high glucose (HG: 25.5 mM) supplemented with or without the phytoestrogenic isoflavones daidzein (50 μM) or genistein (20 μM). Both isoflavones were obtained from Sigma Chemicals. Cardiac myocytes were maintained in respective medium for 24 hours at 37°C before being studied (14).

**Cell Shortening/Relengthening**

Mechanical properties of ventricular myocytes were assessed using an IonOptix® MyoCam optical detection system (IonOptix Corporation, Milton, MA, USA) (14). In brief, cells were superfused at 30°C with a contractile buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl2, 1 MgCl2, 10 glucose, 10 HEPES, at pH 7.4. The cells were field stimulated at 0.5 Hz with super-threshold voltage through a pair of platinum wires. The myocyte was displayed on the computer monitor using an IonOptix® MyoCam camera, which rapidly scans the image area at every 8.3 msec such that the amplitude of shortening/relengthening is recorded with good fidelity.
Statistical Analyses

For each experimental series, data are presented as Mean ± SEM. Statistical significance (p < 0.05) for each variable was estimated by analysis of variance (ANOVA).

RESULTS

Culturing ventricular myocytes for 24 hours with HG medium had no overt effect on cell phenotype such as resting cell length or presence of distinct striations. The resting cell length was similar between NG and HG groups. However, co-incubation of

\[ \text{Mechanical property of ventricular myocytes cultured for 24 hours in serum-free medium with normal glucose (NG: 5.5 mM), high glucose (HG: 25.5 mM), NG or HG with daidzein (50 \mu M) or genistein (20 \mu M). Mechanical indices of myocyte shortening include the following. (A): Resting cell length; (B): Peak shortening amplitude normalized to resting cell length; (C): Maximal velocity of shortening (+dL/dt); and (D): Maximal velocity of relengthening (-dL/dt). Mean ± SEM, n = 30–40 cells/group, *p < 0.05 vs. all other groups, **p < 0.05 vs. NG group, #p < 0.05 vs. HG group.} \]
the isoflavones daidzein (50 μM) and genistein (20 μM) significantly increased the resting cell length in myocytes maintained in HG but not NG medium, indicating potential growth promoting effects of the isoflavones under high glucose environment. Consistent with our previous reports (13,14), cardiac myocytes maintained in HG medium for 24 hours exhibited reduced peak shortening amplitude (PS, normalized to resting cell length) and maximal velocity of shortening/relengthening (± dL/dt) compared to those of NG group (Fig. 1). HG treatment also prolonged time-to-90% relengthening (TR_{90}) without affecting time-to-PS (TPS) (Fig. 2), also consistent with our earlier reports (13,14). Interestingly, 24 hours co-incubation of either daidzein (50 μM) or genistein (20 μM) significantly reduced glucose toxicity-induced mechanical dysfunction in HG myocytes. The HG-induced mechanical dysfunctions in ± dL/dt and TR_{90} were abolished by both isoflavones (Fig. 1 and Fig. 2). In addition, genistein protected diminished PS in response to HG exposure. However, daidzein itself depressed PS in NG myocytes although it did significantly restore the depressed PS in HG myocytes (Fig. 1). Neither daidzein nor genistein elicited any effect on ± dL/dt, TPS and TR_{90} in NG myocytes after 24 hours of incubation.

Figure 2. Mechanical property of ventricular myocytes cultured for 24 hours serum-free medium with normal glucose (NG: 5.5 mM), high glucose (HG: 25.5 mM), NG or HG with daidzein (50 μM) or genistein (20 μM). (A): Time-to-shortening (TPS); (B): Time-to-90% relengthening (TR_{90}); Mean ± SEM, n = 30–40 cells/group, *p < 0.05 vs. all other groups.
DISCUSSION

Results from the present study revealed that the phytoestrogenic isoflavones daidzein and genistein effectively antagonized glucose toxicity-induced mechanical malfunction in ventricular myocytes. These data suggest that phytoestrogenic isoflavones may be favorably considered in the treatment regimen of diabetic and possibly other heart diseases. Such a notion is somewhat consistent with the observation that estrogen replacement therapy may reduce certain cardiac risk factors in postmenopausal diabetic women (11). It is also in line with the clinical finding that isoflavones from other origins (e.g., red clover) may favorably regulate blood pressure and endothelial function in postmenopausal type 2 diabetic women (15).

Hyperglycemia is a key factor for the development of diabetic cardiomyopathy, which is characterized by reduced cardiac contractility, depressed maximal velocity and prolonged duration of contraction/relaxation (7,8,10,16). This study confirmed our earlier observations that normal ventricular myocytes maintained in HG medium develop abnormal mechanics such as depressed cardiac contractility and maximal velocity of contraction/relaxation, associated with prolonged contraction/relaxation cycle, similar to in vivo diabetes (7,13,14).

Although it is beyond the scope of our present study, several possible mechanisms of action may be speculated for the phytoestrogenic isoflavones-elicited cardiac protection against glucose toxicity. First, isoflavones may exert their cardiac protective effects through direct action on cardiac excitation-contraction coupling. It has been indicated that cardiac protection elicited by phytoestrogenic isoflavones may be related partly to their ability to act as natural Ca²⁺ channel antagonists (17,18). Ca²⁺ channel antagonists are used clinically in the treatment of heart dysfunction in diabetes (16). This Ca²⁺ channel blocking scenario seems to be supported by our observation that daidzein inhibited peak myocyte shortening in NG myocytes. Acute genistein exposure was reported to enhance SR Ca²⁺ load and cardiac contraction through inhibition of tyrosine kinase (18). The negative finding of genistein on myocyte shortening (PS) after 24 hours of incubation may suggest that concurrent effects such as inhibition of Ca²⁺ channel and Na⁺/Ca²⁺ exchanger may be triggered by the isoflavones and thus neutralize the stimulatory effect of genistein on SR Ca²⁺ load and cardiac contraction (18). In addition, the beneficial effect of isoflavones on cardiac excitation-contraction coupling may be achieved through their ability to improve myofilament Ca²⁺ sensitivity (18,19), which is known to be diminished by diabetes (9).

Secondly, it is possible that the phytoestrogenic isoflavones daidzein and genistein may effectively reduce the enhanced oxidative stress induced by glucose toxicity. Isoflavones have been shown to inhibit glucose autoxidation, scavenge superoxide and alleviate enhanced oxidative stress (20–22). Elevated extracellular glucose level is known to initiate glucose autoxidation, superoxide production, shifted redox status and oxidative stress (9). Shift in redox status or oxidative stress has been proven to directly induce cardiac mechanical defects manifested as prolonged duration of contraction/relaxation and depressed cardiac contractility (23,24). Further study is warranted to determine the precise effect of isoflavones on redox status and oxidative stress.

Thirdly, isoflavones may exert beneficial hypolipidemic and antidiabetic effects through activation of the peroxisome-proliferator activated receptors (PPAR) (25). Dysregulation of PPAR pathway has been demonstrated to directly contribute to the
diabetes-associated cardiac dysfunction, which also provides a rationale for serum lipid-lowering strategies in the treatment of diabetic cardiomyopathy (26). Last but not least, it is quite unlikely that the protective effect of isoflavones against glucose toxicity-induced mechanical dysfunction observed in our present study is mediated through inhibition of tyrosine kinase since daidzein is an inactive analog of the tyrosine kinase inhibitor genistein. Our earlier study revealed that higher concentration of genistein (50 μM) blocked the anti-diabetic drug metformin-induced cardiac protection against glucose toxicity (14), indicating that tyrosine kinase inhibition may not favor for normal cardiac mechanical function.

Replacement of dietary animal protein with soy protein has long been known to prevent or delay the development of a number of devastating cardiovascular diseases including coronary heart diseases, atherosclerosis and hypercholesterolemia (3). Although a number of studies have given much credit of such cardiovascular protection to the ability of isoflavones to improve blood lipid levels and reduce arterial fatty streaks (2,3), the physiological mechanism by which soy isoflavones (genistein, daidzein and glycitein are the three main isoflavones found in soybeans) improve blood lipid profiles has been the subject of speculation with unknown certainty. Although the current in vitro experimental milieu does not favor alteration in lipid profile and lipid metabolism as major contributing factors for either glucose-induced mechanical dysfunction or isoflavones-elicited beneficial effects, the contribution of improved lipid metabolism to isoflavones-induced cardiac protection remains obscure and should not be excluded as a possible mechanism of action.

In summary, our study revealed beneficial effects of phytoestrogenic isoflavones daidzein and genistein against glucose toxicity-induced cardiac mechanical dysfunction. Considering the distinctive role and potential of phytoestrogenic isoflavones in the treatment and prevention of ischemic and oxidative cardiac injury (4,5), learning and understanding the mechanism of action as well as safety and effective use for phytoestrogenic isoflavones is essential in the clinical application of these natural plant products.

ACKNOWLEDGMENTS

The authors wish to thank North Dakota Experimental Program to Stimulate Competitive Research (EPSCoR) and Max Baer Heart Fund for research support. KKH was a recipient of the United States North Dakota EPSCoR science bound award.

REFERENCES


