Published in final edited form as:

Mol Cell Endocrinol. 2015 January 5; 399: 330–335. doi:10.1016/j.mce.2014.10.011.

Dietary phytoestrogens present in soy dramatically increase cardiotoxicity in male mice receiving a chemotherapeutic tyrosine kinase inhibitor

Pamela Ann Harvey¹ and Leslie Anne Leinwand¹

¹Department of Molecular, Cellular, and Developmental Biology & BioFrontiers Institute, University of Colorado at Boulder, Boulder, Colorado, USA 80309

Abstract

Use of soy supplements to inhibit cancer cell growth is increasing among patients due to the perception that phytoestrogens in soy inhibit carcinogenesis via induction of apoptosis. Genistein, the most prevalent phytoestrogen in soy, is a potent endocrine disruptor and tyrosine kinase inhibitor (TKI) that causes apoptosis in many cells types. Chemotherapeutic TKIs limit cancer cell growth via the same mechanisms. However, TKIs such as Sunitinib cause cardiotoxicity in a significant number of patients. Molecular interactions between Sunitinib and dietary TKIs like genistein have not been examined in cardiomyocytes. Significant lethality occurred in mice treated with Sunitinib and fed a phytoestrogen-supplemented diet. Isolated cardiomyocytes co-treated with genistein and Sunitinib exhibited additive inhibition of signaling molecules important for normal cardiac function and increased apoptosis compared to Sunitinib alone. Thus, dietary soy supplementation should be avoided during administration of Sunitinib due to exacerbated cardiotoxicity, despite evidence for positive effects in cancer.

Keywords

genistein; Sunitinib; tyrosine kinase inhibitors; cardiotoxicity

Introduction

Naturally occurring phytoestrogens such as genistein and daidzein that are present in soy have potent estrogenic and antioxidant cellular effects (1), and are key regulators of ion channel activity in the heart (2). Like other endocrine disruptors, genistein induces biphasic cellular responses. In cardiomyocytes, for example, concentrations of genistein present in

© 2014 Elsevier Ireland Ltd. All rights reserved.

Corresponding author and person to whom reprint requests should be addressed: Leslie A. Leinwand, PhD, Department of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder UCB 354, Boulder, CO USA 80309, Phone: +1 (303) 492-7606, Fax: +1 (303) 492-8907, leslie.leinwand@colorado.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosure Statement: The authors have nothing to disclose.

the plasma of individuals taking soy supplements produce both cardioprotection as well as toxicity (3–5). At lower concentrations ($<1\mu\text{M}$), genistein binds to estrogen receptors (6), producing results that are thought to be largely beneficial though the cardioprotective effects of genistein and soy remain controversial (7). By contrast, $1\text{--}10\mu\text{M}$ genistein potentially inhibits TKs, abrogating cardioprotective effects of preconditioning in ischemia/reperfusion models and inducing cardiomyocyte death via apoptosis (8, 9). At these higher concentrations, the compound competitively binds the ATP-binding site of many membrane and cytosolic tyrosine kinases (10). Thus, although genistein has been shown to be cardioprotective in numerous animal models, higher plasma concentrations ($1\text{--}10\mu\text{M}$) that can be achieved through soy supplementation can induce cardiotoxic effects (11, 12). Recently, our lab reported the direct molecular effect of genistein on phosphoproteins in adult cardiomyocytes and exacerbation of genetic cardiomyopathy by genistein (13). In light of genistein's ability to inhibit multiple TKs in cardiomyocytes and to induce cardiac dysfunction in a genetic model of cardiomyopathy, we asked whether interactions with pharmaceutical TKIs might negatively affect cardiac function in patients with cancer.

Inappropriate activation of receptor-associated tyrosine kinases (RTKs) can lead to uncontrolled cell growth, abnormal angiogenesis, and inhibition of apoptotic pathways, all hallmarks of cancer (14, 15). Small molecule inhibitors of the ATP binding sites on RTKs successfully interrupt kinase activity and reduce uncontrolled cell growth in several forms of cancer (16). Second generation small molecule TKIs such as Sunitinib were designed to inhibit multiple RTKs including platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and stem cell factor (c-kit), which each have known roles in the growth and survival of tumor cells as well as in angiogenesis (17). However, Sunitinib, like many other TKIs inhibit far more RTKs than originally thought (18). Sunitinib was approved by the US Food and Drug Administration in 2006 for treatment of three aggressive cancers (metastatic renal cell carcinoma, Imatinib-resistant gastrointestinal stromal tumor, and pancreatic cancer) (19–21). However, as with other TKIs used for cancer treatment, retrospective studies revealed a significant number of patients developed cardiotoxicity during or immediately following administration of Sunitinib (22, 23).

Importantly, not all patients receiving Sunitinib develop cardiotoxicity, suggesting that environmental factors such as diet may modulate its effects. Molecular interactions between prescription drugs and dietary compounds represent one of the major challenges to healthcare providers in determining appropriate doses of drugs. In fact, up to one-fifth of the US population takes herbal dietary supplements that have known interactions with prescription medications, including Sunitinib (24). Indeed, soy and dietary soy supplements continue to be used by patients with cancer because of the perception that soy may halt progression of cancers (25). As discussed above, the favorable effects of high soy intake have recently been disputed, particularly with regard to cardiovascular health, independent of its use in combination with other TKIs (7). Phytoestrogens present in soy such as genistein may increase the TK inhibitory effects of Sunitinib. Retrospective and prospective clinical studies of patients receiving Sunitinib have not examined the role of dietary soy supplementation on the development of cardiotoxicity. Here, we present data supporting the

detrimental cardiac effects of the dietary phytoestrogen, genistein, combined with oral administration of Sunitinib.

Material and Methods

Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Colorado at Boulder. Nine- to twelve-month-old male mice were fed *ad libitum* a casein-based diet (AIN-76A, Research Diets) supplemented with 227mg genistein (LC Laboratories) and 205mg daidzein (LC Laboratories). Amounts of the phytoestrogens genistein and daidzein and nutrients were equivalent to those present in standard laboratory rodent diets (Sterilizable Rodent Diet 8656, Harlan Teklad) (26). Sunitinib (40mg/kg/day) (22) or vehicle [dimethyl sulfoxide (DMSO)] was administered daily via oral gavage for 28 days. Individual doses were calculated from weekly body mass measurements. On day 29, mice were deeply anesthetized using inhaled isoflurane and rapidly sacrificed via cervical dislocation.

Neonatal rat ventricular myocytes isolation (NRVMs)

NRVMs were isolated from one-day-old Sprague-Dawley rat ventricles, as previously described (27).

Echocardiography

Digital images were obtained from mice in a prone position using 10MHz-phased array transduced VingMed System Five (GE Medical Systems, Milwaukee, WI) echocardiography machine and analyzed using EchoPAC version 6 software (GE Medical Systems, Milwaukee, WI), as previously described (26).

Caspase activity measurements

NRVMs were plated at 100 cells/mL on 60mm plastic cell culture plates. After 36 hours of the appropriate treatment, cellular protein lysates were incubated with a fluorogenic caspase-3/7-specific substrate (Ac-Asp-Glu-Val-Asp-AMC; Calbiochem, Darmstadt, Germany) and fluorescent intensity was measured, as previously described (26).

RTK antibody arrays

Mouse phospho-RTK arrays or human phospho-kinase arrays (R&D Systems, Minneapolis, MN) were performed according to the manufacturer's protocol. 75–100µg protein lysates from NRVMs treated for 36 hours with ethanol (vehicle), 150ng/mL Sunitinib or 10µM genistein were incubated individually with arrays overnight.

Statistical analyses

Data are reported as mean ± standard error of the mean (SEM). Differences between groups were evaluated for statistical significance using Student's *t*-test or analysis of variance followed by Tukey's post-hoc test for studies involving more than two groups. *P* values < 0.05 were considered statistically significant.

Results

In light of the theoretical potential for increased TK inhibition by the combination of Sunitinib and phytoestrogens present in soy, we tested the cardiac effects of dietary phytoestrogen supplementation with oral Sunitinib administration. Male mice were fed a phytoestrogen-supplemented diet containing genistein and diadzein, the most abundant phytoestrogens present in soy, and treated with a 28-day course of 40mg/kg/day Sunitinib (22). The formulation of the phytoestrogen-based diet was nutritionally similar to that of the standard soy-based laboratory rodent chow but eliminated the complex effects of whole soy protein (26). 60% of the phytoestrogen-fed animals died after administration of Sunitinib within approximately one week (Figure 1). 3/5 remaining mice treated with Sunitinib exhibited ocular discharge and/or corneal opacity, 4/5 exhibited yellowing and depigmentation of the skin or fur, and 2/5 developed severe skin and peritoneal muscle lesions. These effects are consistent with the symptoms of TKI overdose, which are characterized by eye discharge, skin yellowing and degradation, and loss of coordination in patients (28).

Surviving mice were subjected to echocardiography to measure cardiac function and morphometric parameters. No change in percent fractional shortening, left ventricular interior diameter (LVID;s) and volume (LV Vol;s) during systole was observed when mice treated with vehicle were compared to Sunitinib (Figure 2A-C, light gray bars). These data are consistent with echocardiography measurements in older mice, which are significantly lower compared to younger mice (29); we report the effect of dietary phytoestrogens and Sunitinib in mice that are aged 9–12 months because the cancers for which Sunitinib is approved do not occur in young adults. The average age of onset is approximately 55–65 years old for renal cell carcinoma, gastrointestinal stromal tumor, and pancreatic neuroendocrine tumor (30). However, relative to mice fed a casein-based diet, mice receiving vehicle or Sunitinib and fed a phytoestrogen-based diet had decreased cardiac function and echocardiographic evidence of ventricular dilation (Figure 2A-C, dark gray bars).

Both Sunitinib and genistein potently inhibit multiple RTKs (10, 18). To measure possible additive effects of Sunitinib and genistein on kinase activation that could account for cardiac dysfunction, NRVMs were treated with plasma-relevant doses of Sunitinib (75–300ng/mL) (31) alone or with 10–100 μ M genistein, a concentration that is physiologically relevant to people who take soy supplements (32). *In vitro* studies focused on genistein only due to its unique TKI properties compared to other isoflavones. NRVMs stained with crystal violet to measure cell viability (33) revealed a significant loss of cells with increasing doses of Sunitinib or with physiological or supraphysiological concentrations of genistein (10 μ M or 100 μ M) (13). However, cell loss was further increased when NRVMs were co-treated with Sunitinib and Genistein (Figure 3A). A caspase activity assay revealed that the cell loss was due to increased apoptosis, such that increased caspase 3 activity was observed in response to either Sunitinib or genistein; combined treatment resulted in significantly increased caspase activity relative to either treatment alone (Figure 3B). In fact, combined treated of Sunitinib and genistein in NRVMs produced caspase 3 activity that was not significantly

different from that of Staurosporine, a microbial alkaloid that is a potent protein kinase inhibitor known to induce apoptosis via caspase 3 activation (34).

To examine the precise inhibitory actions of genistein on cardiomyocytes, activation states of 29 RTKs was measured in NRVMs treated with 10 μ M genistein. 21/29 (72.4%) RTKs were inhibited by genistein with an average inhibition of 65% across all RTKs compared to vehicle-treated NRVMs. (Figure 4A, Supplemental Figure 1). Genistein-treated NRVMs shared all 21 inhibited RTKs with Sunitinib. Sunitinib alone inhibited 27/29 (93.1%) RTKs tested, in agreement with other reports that describe promiscuity of RTK inhibition with Sunitinib (18). Co-treatment with genistein and Sunitinib inhibited 26 RTKs, all of which were shared with Sunitinib treatment alone (Figure 4B). Importantly, 16 of them were inhibited more with combined treatment than with Sunitinib alone, including molecules important to cardiac function including ErbB2, PDGFR β , insulin-like growth factor receptor 1 (IGF-1 R), and Flt3 (35, 36) (Figure 4C).

Discussion

Despite the fact that phytoestrogens have a controversial role in reducing the risk of cardiovascular disease as well as anti-cancer effects, use of phytoestrogen supplementation continues to be prevalent among patients with cancer (25). The phytoestrogen genistein has kinase inhibitory effects on many cells types in the cardiovascular system; and competitively inhibits the ATP catalytic sites on RTKs (10). Genistein also inhibits the activity of intracellular signaling pathways through interactions with estrogen receptors, activation of G-protein-mediated signaling (37) as well as MAP kinases (38). In the studies presented here, the complex effects of soy phytoestrogens on intracellular signaling in cardiomyocytes had lethal consequences in mice receiving the TKI Sunitinib, likely due to additive effects of Sunitinib and genistein on these pathways.

Combinatorial effects of chemotherapies and genistein have been observed in several types of cancer cells. For example, co-administration of genistein with amrubicin induced synergistic inhibition of small cell lung cancer cells via Akt inhibition (39). The pro-apoptotic effects of other chemotherapeutics such as cisplatin, doxorubicin, and docetaxel are also potentiated by genistein both *in vitro* and *in vivo* (40). Although tempting to exploit the additive effects of genistein and Sunitinib to induce apoptosis in cancer cells, caution is warranted due to the cardiac and potentially additive systemic effects the compounds exert with oral administration. We demonstrate for the first time potent interactions between Sunitinib and the dietary phytoestrogen, genistein, in mice; inhibition of TKs required for normal cardiac function by Sunitinib is increased with exposure to genistein in cardiomyocytes. Data presented here contribute to the body of knowledge associated with dietary intake of phytoestrogen-containing foods such as soy in patients receiving Sunitinib and other TKI chemotherapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH 2R01HL050560 and Marisco Chair of Excellence to L.A. Leinwand, and NIH Postgraduate Training Grant in Cardiovascular Research T32 HL-07822 to P.A. Harvey. The authors thank Kelly Ambler for performing echocardiography on the mice presented in this study.

Abbreviations

TKI	tyrosine kinase inhibitor
RTK	receptor tyrosine kinase
NRVM	neonatal rat ventricular myocyte
LVID;s	left ventricular interior diameter during systole
LV Vol;s	left ventricular volume during systole

References

1. Lissin LW, Cooke JP. Phytoestrogens and cardiovascular health. *J Am Coll Cardiol.* 2000; 35:1403–1410. [PubMed: 10807439]
2. Hool LC, Middleton LM, Harvey RD. Genistein increases the sensitivity of cardiac ion channels to beta-adrenergic receptor stimulation. *Circ Res.* 1998; 83:33–42. [PubMed: 9670916]
3. Dang ZC, Lowik C. Dose-dependent effects of phytoestrogens on bone. *Trends in endocrinology and metabolism: TEM.* 2005; 16:207–213. [PubMed: 15922618]
4. El Touny LH, Banerjee PP. Identification of a biphasic role for genistein in the regulation of prostate cancer growth and metastasis. *Cancer Res.* 2009; 69:3695–3703. [PubMed: 19351854]
5. Liew R, Williams JK, Collins P, MacLeod KT. Soy-derived isoflavones exert opposing actions on Guinea pig ventricular myocytes. *J Pharmacol Exp Ther.* 2003; 304:985–993. [PubMed: 12604673]
6. Zava DT, Duwe G. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. *Nutrition and cancer.* 1997; 27:31–40. [PubMed: 8970179]
7. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. *Circulation.* 2006; 113:1034–1044. [PubMed: 16418439]
8. Fryer RM, Schultz JE, Hsu AK, Gross GJ. Pretreatment with tyrosine kinase inhibitors partially attenuates ischemic preconditioning in rat hearts. *Am J Physiol.* 1998; 275:H2009–H2015. [PubMed: 9843799]
9. Okubo S, Tanabe Y, Takeda K, Kitayama M, Kanemitsu S, Kukreja RC, Takekoshi N. Pretreatment with tyrosine kinase inhibitor attenuates the reduction of apoptosis 24 h after ischemic preconditioning. *The Japanese journal of physiology.* 2004; 54:143–151. [PubMed: 15182421]
10. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem.* 1987; 262:5592–5595. [PubMed: 3106339]
11. Si H, Liu D. Genistein, a soy phytoestrogen, upregulates the expression of human endothelial nitric oxide synthase and lowers blood pressure in spontaneously hypertensive rats. *The Journal of nutrition.* 2008; 138:297–304. [PubMed: 18203895]
12. Xu X, Cang H, Li C, Zhao ZK, Li H. Quartz crystal microbalance sensor array for the detection of volatile organic compounds. *Talanta.* 2009; 78:711–716. [PubMed: 19269417]
13. Haines CD, Harvey PA, Luczak ED, Barthel KK, Konhilas JP, Watson PA, Stauffer BL, Leinwand LA. Estrogenic compounds are not always cardioprotective and can be lethal in males with genetic heart disease. *Endocrinology.* 2012; 153:4470–4479. [PubMed: 22778230]

14. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol*. 1995; 19:183–232. [PubMed: 7612182]
15. Jones SM, Kazlauskas A. Growth factor-dependent signaling and cell cycle progression. *Chem Rev*. 2001; 101:2413–2423. [PubMed: 11749380]
16. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer*. 2009; 9:28–39. [PubMed: 19104514]
17. Zhang J, Cao R, Zhang Y, Jia T, Cao Y, Wahlberg E. Differential roles of PDGFR- α and PDGFR- β in angiogenesis and vessel stability. *FASEB J*. 2009; 23:153–163. [PubMed: 18827023]
18. Hasinoff BB, Patel D, O'Hara KA. Mechanisms of myocyte cytotoxicity induced by the multiple receptor tyrosine kinase inhibitor sunitinib. *Mol Pharmacol*. 2008; 74:1722–1728. [PubMed: 18815214]
19. Joensuu H. Sunitinib for imatinib-resistant GIST. *Lancet*. 2006; 368:1303–1304. [PubMed: 17046443]
20. Stadler WM, Szmulewitz RZ. Sunitinib--a new standard of care for metastatic renal cell carcinoma. *Nat Clin Pract Oncol*. 2007; 4:458–459. [PubMed: 17593923]
21. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Horsch D, Hammel P, Wiedenmann B, Van Cutsem E, Patyna S, Lu DR, Blanckmeier C, Chao R, Ruzniewski P. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *N Engl J Med*. 2011; 364:501–513. [PubMed: 21306237]
22. Chu TF, Rupnick MA, Kerkela R, Dallabrida SM, Zurakowski D, Nguyen L, Woulfe K, Pravda E, Cassiola F, Desai J, George S, Morgan JA, Harris DM, Ismail NS, Chen JH, Schoen FJ, Van den Abbeele AD, Demetri GD, Force T, Chen MH. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet*. 2007; 370:2011–2019. [PubMed: 18083403]
23. Kerkela R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, Walters B, Shevtsov S, Pesant S, Clubb FJ, Rosenzweig A, Salomon RN, Van Etten RA, Alroy J, Durand JB, Force T. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med*. 2006; 12:908–916. [PubMed: 16862153]
24. Tsai HH, Lin HW, Simon Pickard A, Tsai HY, Mahady GB. Evaluation of documented drug interactions and contraindications associated with herbs and dietary supplements: a systematic literature review. *International journal of clinical practice*. 2012; 66:1056–1078. [PubMed: 23067030]
25. Moon W, Balasubramanian SK, Rimal A. Perceived Health Benefits and Soy Consumption Behavior: Two-Stage Decision Model Approach. *JARE*. 2005; 30:315–332.
26. Stauffer BL, Konhilas JP, Luczak ED, Leinwand LA. Soy diet worsens heart disease in mice. *J Clin Invest*. 2006; 116:209–216. [PubMed: 16395406]
27. Maass AH, Buvoli M. Cardiomyocyte preparation, culture, and gene transfer. *Methods Mol Biol*. 2007; 366:321–330. [PubMed: 17568133]
28. Pfizer. NY, NY 10017. 2013. Sutent (sunitinib malate) labeling.
29. Piell KM, Qipshidze Kelm N, Caroway MP, Aman M, Cole MP. Nitrite treatment rescues cardiac dysfunction in aged mice treated with conjugated linoleic acid. *Free radical biology & medicine*. 2014; 72:66–75. [PubMed: 24721151]
30. National Cancer Institute hscgs. Surveillance, Epidemiology, and End Results Program. 2014. In:
31. Lankheet NA, Blank CU, Mallo H, Adriaansz S, Rosing H, Schellens JH, Huitema AD, Beijnen JH. Determination of sunitinib and its active metabolite N-desethylsunitinib in sweat of a patient. *Journal of analytical toxicology*. 2011; 35:558–565. [PubMed: 22004675]
32. deVere White RW, Tsodikov A, Stapp EC, Soares SE, Fujii H, Hackman RM. Effects of a high dose, aglycone-rich soy extract on prostate-specific antigen and serum isoflavone concentrations in men with localized prostate cancer. *Nutrition and cancer*. 2010; 62:1036–1043. [PubMed: 21058191]
33. Joshi MS, Tong L, Cook AC, Schanbacher BL, Huang H, Han B, Ayers LW, Bauer JA. Increased myocardial prevalence of C-reactive protein in human coronary heart disease: direct effects on

- microvessel density and endothelial cell survival. *Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology*. 2012; 21:428–435. [PubMed: 22285194]
34. Herbert JM, Seban E, Maffrand JP. Characterization of specific binding sites for [3H]-staurosporine on various protein kinases. *Biochem Biophys Res Commun*. 1990; 171:189–195. [PubMed: 2393390]
35. Pfister O, Lorenz V, Oikonomopoulos A, Xu L, Hauselmann SP, Mbah C, Kaufmann BA, Liao R, Wodnar-Filipowicz A, Kuster GM. FLT3 activation improves post-myocardial infarction remodeling involving a cytoprotective effect on cardiomyocytes. *J Am Coll Cardiol*. 2014; 63:1011–1019. [PubMed: 24184252]
36. Fontana L, Vinciguerra M, Longo VD. Growth factors, nutrient signaling, and cardiovascular aging. *Circ Res*. 2012; 110:1139–1150. [PubMed: 22499903]
37. Lin AH, Leung GP, Leung SW, Vanhoutte PM, Man RY. Genistein enhances relaxation of the spontaneously hypertensive rat aorta by transactivation of epidermal growth factor receptor following binding to membrane estrogen receptors-alpha and activation of a G protein-coupled, endothelial nitric oxide synthase-dependent pathway. *Pharmacological research : the official journal of the Italian Pharmacological Society*. 2011; 63:181–189. [PubMed: 21111822]
38. Dubey RK, Jackson EK, Gillespie DG, Zacharia LC, Imthurn B, Keller PJ. Clinically used estrogens differentially inhibit human aortic smooth muscle cell growth and mitogen-activated protein kinase activity. *Arterioscler Thromb Vasc Biol*. 2000; 20:964–972. [PubMed: 10764660]
39. Ueda Y, Igishi T, Hashimoto K, Suyama H, Araki K, Sumikawa T, Takeda K, Nakazaki H, Matsunami K, Kodani M, Shigeoka Y, Matsumoto S, Shimizu E. Synergistic cell growth inhibition by the combination of amrubicin and Akt-suppressing tyrosine kinase inhibitors in small cell lung cancer cells: implication of c-Src and its inhibitor. *International journal of oncology*. 2009; 34:689–696. [PubMed: 19212674]
40. Sarkar FH, Li Y. Using chemopreventive agents to enhance the efficacy of cancer therapy. *Cancer Res*. 2006; 66:3347–3350. [PubMed: 16585150]

Highlights

- Sunitinib and dietary phytoestrogens produced decreased cardiac function in mice.
- 60% of mice receiving oral Sunitinib and dietary phytoestrogens died.
- Sunitinib and genistein induced additive TK inhibition in cardiomyocytes.
- Dietary soy supplementation should be urgently assessed in patients receiving TKIs.

Figure 1.

Survival curves of male 9–12 month old mice fed a phytoestrogen-supplemented (black lines) or casein-based (red) diet. Mice received either vehicle (DMSO, solid lines) or 40mg/kg/day Sunitinib (dashed lines) per day. n = 9–12 mice per group.

Figure 2.

Echocardiographic analysis of 9–12 month old mice receiving Sunitinib and fed a phytoestrogen-supplemented (light gray bars) or phytoestrogen-free (dark gray bars) diet. Percent fractional shortening (**A**), left ventricular internal diameter during systole (LVID;s, **B**), and left ventricular volume during systole (LV Vol;s, **C**) in mice treated with vehicle (open bars) or Sunitinib (hatched bars) for 28 days. Error bars represent SEM. * $p < 0.05$, ** $p < 0.01$, compared to phytoestrogen-fed vehicle-treated control. $n = 4$ –5 mice in the phytoestrogen-supplemented diet groups, $n = 9$ mice in the phytoestrogen-free diet group.

Figure 3.

Crystal violet staining (**A**) or caspase activity (**B**) measured in NRVMs treated with Sunitinib alone (light gray bars), genistein alone (gray bars), or co-treated with Sunitinib and genistein (hatched bars). Staurosporine (dark gray bars, **B**) was used as a positive control for caspase activity. $n = 3$ NRVM preparations (60–90 pups per preparation). Error bars represent SEM. * $p < 0.05$, ** $p < 0.01$, relative to vehicle-treated.

Figure 4.

A. Quantification of inhibited RTKs by 10 μ M genistein in NRVMs. Horizontal line indicates no change in activation compared to vehicle-treated NRVMs. **B.** Venn diagram demonstrating shared inhibition of RTK activation among Sunitinib- (purple), genistein- (yellow), and Sunitinib and genistein- (green) treated NRVMs, relative to vehicle-treated. **C.** Inhibition of signaling molecules by Sunitinib alone (gray bars) or combined with genistein (hatched bars) in NRVMs. $n = 2$ NRVM preparations (60–90 pups per preparation), pooled.