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Rapid paper

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

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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 with Sunitinib alone. Thus, dietary soy supplementation should be avoided during administration of Sunitinib due to exacerbated cardiotoxicity, despite evidence for positive effects in cancer.

1. Introduction

Naturally occurring phytoestrogens such as genistein and daidzein that are present in soy have potent estrogenic and antioxidant cellular effects (Lissin and Cooke, 2000), and are key regulators of ion channel activity in the heart (Hool et al., 1998). Like other endocrine disruptors, genistein induces biphasic cellular responses. In cardiomyocytes, for example, concentrations of genistein present in the plasma of individuals taking soy supplements produce both cardioprotection as well as toxicity (Dang and Lowik, 2005; El Touny and Banerjee, 2009; Liew et al., 2003). At lower concentrations (<1 μM), genistein binds to estrogen receptors (Zava and Duwe, 1997), producing results that are thought to be largely beneficial though the cardioprotective effects of genistein and soy remain controversial (Sacks et al., 2006). By contrast, 1–10 μM genistein potently inhibits TKs, abrogating cardioprotective effects of preconditioning in ischemia/reperfusion models and inducing cardiomyocyte death via apoptosis (Fryer et al., 1998; Okubo et al., 2004). At these higher concentrations, the compound competitively binds the ATP-binding site of many membrane and cytosolic tyrosine kinases (Akiyama et al., 1987). Thus, although genistein has been shown to be cardioprotective in numerous animal models, higher plasma concentrations (1–10 μM) that can be achieved through soy supplementation can induce cardiotoxic effects (Si and Liu, 2008; Xu et al., 2009). Recently, our lab reported the direct molecular effect of genistein on phosphoproteins in adult cardiomyocytes and exacerbation of genetic cardiomyopathy by genistein (Haines et al., 2012). 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 (Jones and Kazlauskas, 2001; Salomon et al., 1995). Small molecule inhibitors of the ATP binding sites on RTKs successfully interrupt kinase activity and reduce uncontrolled cell growth in several forms of cancer (Zhang et al., 2009). 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), each of which have known roles in the growth and survival of tumor cells as well as in angiogenesis (Zhang et al., 2009). However, Sunitinib, like many other TKIs inhibit far more RTKs than originally thought (Hasinoff et al., 2008). Sunitinib was approved by the

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.

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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) (Joensuu, 2006; Raymond et al., 2011; Stadler and Szmulewitz, 2007). However, as with other TKIs used for cancer treatment, retrospective studies revealed that a significant number of patients developed cardiotoxicity during or immediately following administration of Sunitinib (Chu et al., 2007; Kerkela et al., 2006).

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 (Tsai et al., 2012). 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 (Moon et al., 2005). 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 (Sacks et al., 2006). 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.

2. Materials and methods

2.1. 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 227 mg genistein (LC Laboratories) and 205 mg 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) (Stauffer et al., 2006). Sunitinib (40 mg/kg/day) (Chu et al., 2007) 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.

2.2. Neonatal rat ventricular myocytes isolation (NRVMs)

NRVMs were isolated from 1-day-old Sprague-Dawley rat ventricles, as previously described (Maass and Buvoli, 2007).

2.3. Echocardiography

Digital images were obtained from mice in a prone position using 10 MHz-phased array transduced VingMed System Five (GE Medical Systems, Milwaukee, WI) echocardiography machine and analyzed using EchoPAC version 6 software (GE Medical Systems), as previously described (Stauffer et al., 2006).

2.4. Caspase activity measurements

NRVMs were plated at 100 cells/mL on 60 mm 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 (Stauffer et al., 2006).

2.5. 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. Seventy-five- to one hundred-microgram protein lysates from NRVMs treated for 36 hours with ethanol (vehicle), 150 ng/mL Sunitinib or 10 μM genistein were incubated individually with arrays overnight.

2.6. 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.

3. 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 40 mg/kg/day Sunitinib (Chu et al., 2007). 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 (Stauffer et al., 2006). Sixty percent of the phytoestrogen-fed animals died after administration of Sunitinib within approximately 1 week (Fig. 1). Three-fifths of the remaining mice treated with Sunitinib exhibited ocular

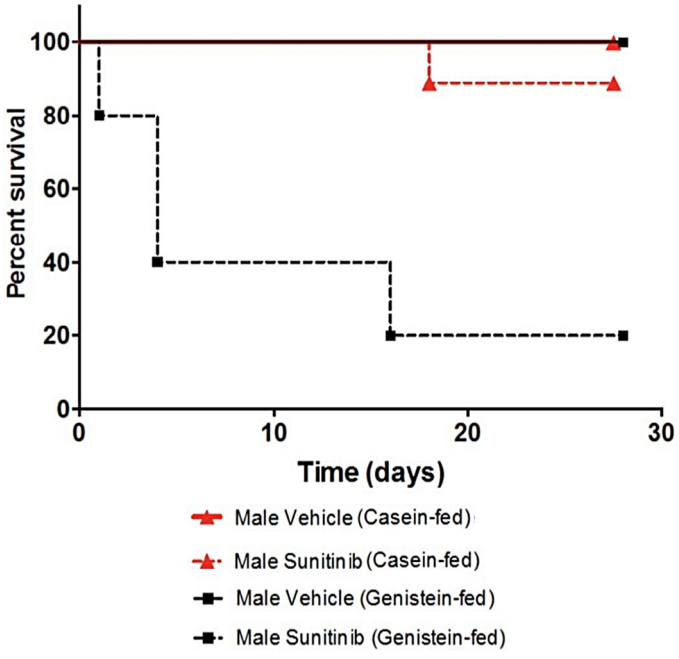

Fig. 1. Survival curves of male 9–12 month old mice fed a phytoestrogen-supplemented (black lines) or casein-based (red) diet. Mice received either vehicle (DMSO, solid lines) or 40 mg/kg/day Sunitinib (dashed lines) per day. $n = 9–12$ mice per group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)