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Cautions and Research Needs Identified at the Equol, Soy, and Menopause Research Leadership Conference[1–3]

Stephen Barnes* and Helen Kim

Department of Pharmacology and Toxicology, and Center for Nutrient-Gene Interaction, University of Alabama at Birmingham and Purdue University-University of Alabama at Birmingham Botanicals Research Center for Age-Related Disease, Birmingham, AL 35294

Abstract

This summary addresses the progress and limitations of existing research on the physiologic properties of the isoflavone daidzein metabolite equol. Previous research demonstrating that physiological equol is its S-enantiomer has led to the preparation of S-(-)equol–enriched products formed by the bacterial fermentation of soy germ. Although this product has interesting properties as described in this workshop, the following important issues must be addressed: *1*) the product should be evaluated against a preparation containing an equal amount of pure S-(-)equol to determine whether other components resulting from the fermentation are contributing to the physiological effects; *2*) evaluation of the cellular mechanisms of S-(-)equol using cell culture methods should be conducted at concentrations consistent with those encountered physiologically (in the nmol/L range) and in several cell lines representing a target tissue; and *3*) in follow-up studies in animal models and in human clinical trials, standardized preparations of S-(-)equol should be made available. Research opportunities now exist to determine whether equol's apparent effects on menopausal symptoms (hot flashes, sleep disturbances, bone health) in equol producers can be extended to equol nonproducers. It will be important to ensure that such research is not complicated by cultural differences, differences in lifetime exposure to soy products, experimental techniques, and other variables. Further areas of research that would benefit from the availability of S-(-) equol preparations include its use in skin care (either as an antioxidant or as an estrogen receptor agonist) and in the treatment of brain injury as well as postmenopausal cognitive decline. J. Nutr. 140: 1390S–1394S, 2010.

Introduction

The presentations at the Equol, Soy and Menopause Research Leadership Conference, held on June 16, 2009 in Washington DC, were organized into 3 general areas: soy and isoflavone research and metabolism; the relationship of equol to chronic diseases in humans; and the potential benefits of equol to women experiencing adverse symptoms during menopause. The following is a discussion of the cautions associated with conducting and interpreting research in the area and a summary of the research needs identified at the conference.

Cautions

Physiological equol vs. synthetic equol. There are 2 distinct areas of research on equol: studies on equol manufactured by fermentation using human intestinal bacteria from materials containing its precursor, daidzein, and studies on chemically synthesized equol. Equol can exist as the diastereoisomers S-(-) equol and R-(+)equol; however, S-(-)equol is the only form produced from daidzein metabolism in humans (1). Information most relevant to the health effects of equol in humans will necessarily derive from studies on human intestinal bacteria-produced S-(-)equol and not synthetic equol (2,3), which is a racemic mixture of S-(-)equol and R-(+)equol.

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* To whom correspondence should be addressed. E-mail: sbarnes@uab.edu.

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A majority of conference presentations concerned equol-enriched products containing S-(-)equol that are being used in preclinical and clinical studies. These studies required Institutional Review Board and Institutional Animal Use Committee approval as well as consideration of safety issues. Human studies may involve interactions with the FDA, because a specific manufactured product is involved.

Possible contribution of other substances to biological effects currently attributed to equol. S-(-)equol produced by bacterial fermentation of soy germ with human intestinal bacteria contains other isoflavone metabolites, some of which are possibly nonphysiologic. It remains to be determined whether the biological activities currently attributed to S-(-) equol in the fermented product are solely due to S-(-)equol alone. The research need can be addressed by conducting studies on pure S-(-)equol, which can now be chemically manufactured, and the bacterial products of daidzein metabolism. If the same amount of pure S-(-)equol and a bacterially produced S-(-)equol preparation lead to different outcomes, the other components of the product may contribute to the effects currently attributed to S-(-) equol alone.

Experimental design. Although convenient, preclinical studies in animals are not necessarily relevant to the human response to isoflavones because of differences in equol metabolism. In rodents and nonhuman primates (4–6) fed soy, the principal circulating metabolic form of the isoflavones is S-(-)equol, being 75% of the total isoflavones, and 4-5 times larger than the concentrations of daidzein and genistein. Experiments in rats suggest that a slower urinary clearance of equol compared with daidzein and genistein (7) accounts for its increased circulating amounts. Indeed, because very high circulating levels of equol result from fairly modest exposure to isoflavones in animal models, the relevance of animal studies to human experience is called into question.

In contrast to most other animals, humans do not make large amounts of equol and only ~30% of humans are capable of synthesizing equol (8–13), although in Japan and Korea, a larger group of individuals is able to synthesize equol (14). The recent work of Setchell et al. (15,16) on the pharmacokinetics of orally administered S-(-)equol and R-(+)equol in human participants raises interesting questions about equol's bioavailability. Using 2-^{13}C-labeled equol, an oral dose of 20 mg of S-(-)equol produced peak blood concentrations of all equol forms (conjugated and unconjugated) that were ~3 times higher than those for daidzein and genistein (after correcting for the different sizes of the doses) (17). It is possible that equol, unlike daidzein and genistein, is a terminal metabolite and is not dissipated into other chemical forms during transit through the intestine and intestinal wall into the blood stream.

Types of cell cultures and concentrations of isoflavones. Most studies using cell cultures to study mechanisms of action of isoflavones utilize levels ~2 orders of magnitude above the concentrations found in human exposure. Also, the use of unconjugated isoflavones does not match what is found in human blood (>95% conjugated metabolites). The cell lines typically used in these studies were developed because of their ability to be cultured rather than they are fully representative of the disease of interest. In addition, different types of cancer cells have a different pattern of metabolism for isoflavones (18,19). Attempts to determine the half maximal inhibitory concentration effects of genistein on human breast cancer cell proliferation revealed a correlation with the metabolites but not with genistein (18). Consideration of the cell line used in a preclinical study therefore is crucial to drawing conclusions.

Standardization of analytical methods. Analytical standardization represents another research need. Certified reference values do not exist for any soy isoflavone products. The Office of Dietary Supplements has contracted the National Institutes of Standards and Technology to manufacture of food matrix standards for several dietary supplements. A standard reference for soy is currently in development (20). In the past, concerns about the variability in the values being reported for estrogen receptors previously led the National Cancer Institute to develop a pooled standard reference product that was distributed to laboratories performing estrogen receptor analysis. The National Center for Complimentary and Alternative Medicine considers the use of standard products to be an extremely important point in its studies; products used in the center-funded studies have to go through extensive prereview by a Product Quality Working Group. In the absence of a certified standard, there should be a pooled sample that various laboratories can measure to check variability.

Research needs

What follows is a list of some of the most pressing research questions in the field.

Assuming there is a benefit from S-(-)-equol in equol producers, would nonproducers also benefit from equol? Some studies have reported a beneficial effect of S-(-)-equol. Equol producer status is determined by the presence of the bacteria and the conditions in the intraluminal space, i.e. the correct redox potential. Additional studies are needed to determine whether acute or chronic administration of S-(-)-equol to equol producers and nonproducers results in the same outcomes. Effects of equol on hot flashes, sensitivity to changes in temperature, sleep disturbance, and other symptoms reported as adverse effects by a majority of postmenopausal women are complicated by cultural differences, differences in lifetime exposure to soy products, study techniques, and other variables.

Improved sensitivity of questionnaire instruments. Questionnaires were developed for different purposes. Even scales designed to measure global quality of life should pair with symptom profiles appropriate for the topic of interest. There is a need for more sensitive questionnaire instruments to measure changes in the perception of quality of life to improve our objectives in outcome research. Further advancement will come from studying the underlying neurobiology, metabolic pathways, and measurable physiologic responses.

Is S-(-)equol a marker for other metabolic events in soy consumers? The factors that control bacterial equol production require further investigation. Additional information is needed about why there are fewer equol producers in Western populations (~30%) compared with Eastern populations (~50%), and the sensitivity of equol production to redox potential, both microscopically and to the gut in general. Whether S-(-)-equol is a marker for a different bioactive substance should also be investigated. Equol may be a surrogate marker for a minor metabolite, perhaps not even derived from the isoflavonoids. Examples of minor, but incredibly bioactive, compounds are the prostaglandins, thromboxanes, and leukotrienes, all metabolites of long-chain PUFA.

Are differences in response to equol related to time of exposure? In some Asian cultures, soy is a traditional part of the diet. In the West, the US, and Europe, individuals were not typically exposed to dietary soy in large amounts until the early 1990s. In contrast, Asian women who were in menopause between 2005 and 2009 have soy exposures dating back to the 1950s and 1960s. The differential effects of administration of soy or soy-derived materials to individuals with different exposure histories may be due to strong epigenetic phenomena, as is the case with exposure to soy and breast cancer. Adolescent exposure to soy is considered to be extremely important to determine future adult breast cancer risk (21,22). More recent studies suggest that childhood exposure to isoflavones may influence responses of adults to soy (23,24). Care should be taken to ensure that the right questions are being asked in nutritionally based epidemiological studies.

Because of differences in exposure history, outcomes of studies conducted in the US may not be comparable to outcomes in societies with large soy consumption from birth such as Japan or China. Furthermore, soy consumption patterns in the West have changed and a finding of undetectable blood concentrations of isoflavones today is rare. Controlling for different exposures in different eras is extremely important.

Reduction of variation on studies. Policy decisions are being made about the risk of specific chronic diseases based on studies on consumption of nutritional components. However, most studies for which answers to public health questions are sought are statistically inadequate. An obstacle that needs to be surmounted is having studies that are sufficiently statistically robust that loss of patients from the study or compliance problems do not corrupt the study conclusion. Clinical trials that are designed to provide answers for a whole population may not be affordable because of the large number of participants required. In addition, the frequent failure of a trial to execute the study as planned may yield insufficiently clear answers. However, as mechanisms become identified, it will be important to use this information to more carefully select the group to be studied. This can be achieved by narrowing the study group using the best available science about possible mechanisms. For instance, one could identify single-nucleotide polymorphisms in a receptor and then group individuals according to their responses. Another transforming approach is to use an advanced technology such as accelerator MS to be able to examine bone turnover with the use of calcium-41. The extreme sensitivity and specificity of accelerator MS for this rare isotope and the extended period of observation (5–10 y in the same participant) allows for multiple interventions to be tested in a pairwise manner, thereby substantially lowering the person-to-person variance in the experiment. Such an approach has been used to evaluate the effect of different soy supplements and standard therapies (residronate or estradiol and medroxyprogesterone acetate) on bone turnover in perimenopausal women (25).

Mechanisms of action of isoflavones beyond the estrogen receptor. Isoflavones may also elicit effects through mechanisms that do not involve the estrogen receptor (ER).[4] ER receptor activation is very complex and involves coactivators, repressors, and >30 other proteins (26). Isoflavones may affect other protein targets in the pathway. In contrast to peripheral targets (brain, breast, heart, etc.), cells lining the intestinal lumen may be exposed to very high concentrations of isoflavones (an estimated 50 or 60 μmol/L) and thereby other less sensitive mechanisms may come into play. Another region of the body where isoflavone concentrations are high (with the exception of genistein) is prostatic fluid, where isoflavone levels are 20–30 times higher than that in the blood (27–29). And because urine concentrates excreted compounds, the bladder is also exposed to high concentrations of isoflavones.

Other areas of work regarding the mechanisms of action of receptors are being pursued. Exosomes are 50- to 90-nm vesicles secreted by a wide range of mammalian cell types that carry proteins through the blood and thereby target other cells; this is an endocrine-like mechanism. Several polyphenols influence the way in which these exosomes are created, their content, and their fusion to other cells (30). Equol may regulate this process. Other isoflavone-dependent targets are the tumor necrosis factor-α pathway (31–33) and PPARα- and PPARγ-mediated pathways (34–37).

Importance of aglycones. The aglycones are present in fermented soy foods. They are more rapidly absorbed in the upper small intestine and thereby cause higher peak concentrations during the first enterohepatic cycle. Because they are products of fermentation with microorganisms, the aglycones undergo additional hydroxylation (e.g. to 6- or 8-hydroxyisoflavones) prior to the food being consumed (38). There are questions about whether this gives rise to increased bioactivity of the fermented soy food.

Role of intestinal microorganisms in human health. The microbiome is the most populous cell type in the human body. There are more cells in the gastrointestinal tract than in the rest of the body. Moreover, recent studies have shown that if microorganisms from the intestines of an obese mouse are transferred to a lean mouse, the lean mouse becomes obese, indicating that the metabolic state can affect either the composition or metabolic capacity of the microbiome in one animal's body, which can then directly affect another animal's health (39–41). Additional research is needed to determine whether isoflavones alter the microbiome and hence affect human health. The effect of high alcohol consumption and polyphenols on the microbiome and whether equol production plays a role in the physiological effects of alcohol should be determined.

The skin: another area where equol may have important bioactivity. Does equol have a beneficial effect in skin health? Many topical applications containing polyphenols that protect the skin have been developed. This area has 2 complementary areas, one where the added polyphenols have purported activity in sustaining the physical health and youthfulness of the skin and another where the added polyphenols may actually protect against skin cancer later in life. Both may involve antioxidant and antiinflammatory activities of the polyphenols.

Estrogen-like compounds in sustaining neuronal health and numbers. It is widely accepted that estrogen has benefits in brain health and function, including cognitive function. Given that at least some of the principal benefits of equol may be due to its similarity with estrogen, the effects of equol, especially taken as an additive, in the brain require further study. However, there has been controversy regarding the efficacy of estrogen or estrogen-like compounds in protecting cognitive function or

[4] Abbreviations used: AMS, accelerator mass spectrometry; ER, estrogen receptor; GSE, grape seed extract; NCCAM, National Center for Complimentary and Alternative Medicine; SNP, single-nucleotide polymorphism; TBI, traumatic brain injury.

preventing age- or postmenopause-linked cognitive decline. Nonetheless, cognitive dysfunction induced by ovariectomy clearly was attenuated by added estrogen or estrogen-like compounds, including the soy isoflavones (42). It will be important to determine whether equol alone can have the same benefit as the soy isoflavones in animal models of dementia including Alzheimer's disease. Estrogen and genistein independently prevented age-related mitochondrial dysfunction (43); in such models, it will be important to determine whether equol mimics or suppresses estrogen or genistein effects. An intriguing brain area where phytoestrogens and/or phytochemicals enriched in antioxidant activity may have activity is in modulating neurogenesis. In brain regions involved in cognition, neurogenesis is restricted to the dentate gyrus of the hippocampus [see (44)]. While there is complexity, recent studies showed that ablating neurogenesis prevented normal learning and memory, suggesting a requirement for neurogenesis in normal hippocampal functions in learning (45). If ablation of neurogenesis inhibits learning and memory, it is reasonable to propose that any compounds that enhance learning and memory have such actions by enhancing or protecting neurogenesis. Previous studies reported that grape seed extract enriched in the oligomeric proanthocyanidins enhanced cognitive function in young adult rats (46). Thus, it was not surprising that grape seed extract modulated neurogenesis in developmentally immature mouse brain (J. Cutts, L. Overstreet-Wadiche, and H. Kim, unpublished observations); however, it is also clear that not all increased neurogenesis is associated with "beneficial" effects in the brain, because increased neurogenesis was correlated with chemically induced seizures (47). Thus, neurogenesis will be a critically important area to explore for potential activity of the soy isoflavones, including equol. There is particular interest in identifying compounds that are safe in the brain whether or not they have the highest activity. The brain and the brain region are thus organs for much further investigation regarding the actions of soy phytoestrogens, including equol.

Recovery from traumatic brain injury. Screens of compounds from existing drugs with good safety profiles and from dietary supplements in a search for 'safe' agents have recently revealed that daidzein and biochanin A are strongly active in supporting recovery from traumatic brain injury (48). Genistein and daidzein are also being studied for their roles in wound recovery.

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