REVIEW ARTICLE

Isoflavones in gynecology

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http://dx.doi.org/10.1590/1806-9282.64.06.560

SUMMARY

KEYWORDS: *Isoflavones. Menopause. Postmenopause. Hormone therapy.*

INTRODUCTION

Isoflavones are the most common forms of phytoestrogens and they are found in soy, soy products (soy milk, tofu, soy beverages, and soy flours), lentils, green peas, and alfalfa and bean sprouts. The main isoflavones are genistein, daidzein, and glycytine. They may be found in nonconjugate form (aglycone) and in conjugated form (glycosylated).[1] Isoflavones are nonsteroidal compounds structurally similar to natural estrogen, as they exhibit a phenolic ring with a hydroxyl radical attached to carbon three. This structure gives them a capacity for high-affinity selective binding to estrogen receptors, thereby enabling them to engage in estrogenic activity in human tissues. Isoflavones have an estrogenic or anti-estrogenic effect depending on their concentration, on endogenous sex steroids, and on the specific target organ in the interaction with the estrogen receptors. The fact that there are two types of estrogen receptors, alpha and beta, endows the different target organs with specificity to phytoestrogens.

ISOFLAVONE METABOLISM

Isoflavones are generally found in food in their main forms, as genistein, daidzein, and glycytine, i.e., bound to beta-glycosides and sugars. However, the human body does not absorb these forms. Thus, they change into smaller molecules through the action of specific enzymes for absorption without the sugar molecule.[2] Once ingested, the biologically inert glycosylated isoflavones undergo acid and enzymatic hydrolysis by gastric acids and intestinal glycosi-

DATE OF SUBMISSION: 30/6/18
DATE OF ACCEPTANCE: 30/6/18
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dases. However, it is the intestinal bacteria, through hydrolytic enzymes, that cause the changes in the glycosylated forms of isoflavones. The enzymes, which are called β-glucosidases, hydrolyze conjugated daidzein and genistein, converting them into their nonconjugate forms – namely aglycones– which can then be absorbed by the intestinal epithelium.[2] The bacteria break the heterocyclic bonds in the chemical structure of the isoflavone molecules changing these into phenolic acids, which can be absorbed, conjugated, excreted, or metabolized. The aglycone forms of isoflavone may undergo further transformation into other types of specific metabolites, such as equol or O-desmethylangolensin from daidzein, and p-ethynylphenol from genistein.[2]

Isoflavone absorption occurs by varied means and its metabolic pathways are not yet fully understood. After absorption, the molecules are believed to incorporate into chylomicrons, which in turn move into the lymphatic system and subsequently, into the circulatory system. By way of the latter, isoflavones reach all tissues, where they exert their biological effects, influencing the activity of all cells with estrogen receptors. After producing their estrogenic or anti-estrogenic effects, isoflavones enter the hepatic circulation, where they are secreted into bile, then partly reabsorbed into the enterohepatic circulation and partly excreted through feces and urine. The study of bacterial flora and of isoflavone metabolism is extremely important, for they have a bearing on blood concentration and on intestinal bioavailability and absorption.

ACTION MECHANISMS OF ISOFLAVONES

Isoflavone effects on the body vary from tissue to tissue given the affinity of isoflavones for specific receptors. The fact that isoflavones have a chemical structure similar to that of endogenous estrogens and that they bind to the same receptors as the latter, this enables isoflavones to regulate the gene expression of estrogen-regulated products.[3] There are two known types of estrogen receptors: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Isoflavones show greater affinity for ERβ, and estrogens for ERα.[3] The estrogenic potential of isoflavones is low compared to that of 17-β-estradiol, i.e., approximately 1/1000.[3] Genistein has a 4% binding affinity for ERα and 87% for ERβ, whereas daidzein, which is much less potent, exhibits a 1% and 5% affinity for ERα and ERβ, respectively. The high affinity of isoflavones for ERβ in comparison with ERα and the different distribution of such receptors in the tissues suggest a tissue-selective activity of the compounds. Hence, isoflavones would exert estrogenic action in some tissues, such as coronary vessels, but not in other tissues, such as the endometrium.[3,4] Some authors have classified isoflavones as selective modulators of estrogen receptors (SERMs). Isoflavones would act in similar ways to SERMs on estrogen receptors.[4] SERMs are nonsteroidal synthetic agents that bind to estrogen receptors inducing changes in the receptor's biological activity according to the type of tissue. Isoflavones may also exert their biological effects by means other than estrogen receptors. For example, isoflavones would act through tyrosine kinase receptors and other peptide receptors on the plasma membrane of certain cells. Other potential action mechanisms of isoflavones include cell-cycle regulation and antioxidant effects. Furthermore, isoflavones play an important role in preventing menopause-related disorders and chronic diseases, such as heart diseases, cancer, and diabetes.[5]

HORMONE THERAPY

Throughout a woman's life, ovarian changes take place leading to decreasing estrogen levels (hypoestrogenism) mainly associated with atrophy of the genital tract. Vulvovaginal symptoms develop and may include dryness, itching, burning, and pain, especially during sexual intercourse. Although the natural process of aging is a determinant of estrogen deficiency, it has been established that the chief etiological factors are vaginal epithelial deterioration and atrophic vaginitis.

To relieve hypoestrogenic symptoms, hormone therapy (HT) is currently the treatment of choice.[6] Vasomotor instability (hot flashes) frequently occurs in hypoestrogenism, and it manifests as a sudden sensation of intense heat along with flushing and sweating.[6] It usually lasts for less than 10 minutes. Vasomotor instability may also underlie anxiety symptoms, palpitations, and sleep disorders. Although the prevalence of vasomotor symptoms varies in accordance with race and ethnicity, over 50% of women report vasomotor symptoms at some point during menopause.

However, studies have attributed to estroprogestative or estrogen therapy the onset of side effects,

such as increased risk of breast and endometrial cancers and of thromboembolism. Therefore, its use has been questioned.[7] For this reason and given the increasing demand for alternative natural therapies, a significant number of women have been using phytoestrogens, particularly isoflavones, to relieve the symptoms of hypoestrogenism.[2]

ISOFLAVONES AS HORMONE THERAPY

Questions still linger about the beneficial effects of isoflavones on the female reproductive system and its appendages, particularly the breasts. Therefore, there is a demand for evidence-based benefits of isoflavone consumption in preventing and treating the undesirable effects of hypoestrogenism. The requirement is being met through experimental and epidemiological studies.

A prospective cohort study showed that the higher the isoflavone ingestion, the higher the protection against breast cancer in Latin, African, Japanese, and American women.[8] However, a study analyzing the effects of isoflavones on breast cancer-related genetic pathways demonstrated that a high concentration of plasma genistein induces the overexpression of genes that stimulate the cell cycle proliferation pathway. This raises the concern that the consumption of high soy concentrations could make women more vulnerable to breast cancer.[9]

In vitro studies report that high concentrations of isoflavones reduce cell proliferation, whereas low concentrations exert stimulating effects.[1] Low isoflavone concentrations are also capable of modifying the expression of some vital genes for cell survival, cell cycle control, and apoptosis.[10]

Laboratory studies have shown that genistein and daidzein can inhibit tyrosine kinase (PTK) by blocking the signaling pathway between the growth factor and its receptor and the DNA, thus thwarting activation of both cell proliferation and angiogenesis.[11] It is well known that kinase-dependent cyclins (KDCs) and cyclin-dependent kinase inhibitors regulate different phases of the cell cycle. Hence, these regulators are important targets for cancer therapy and prevention. In a study evaluating the combination of genistein and daidzein, the authors detected an increase in p53 and a reduction in cyclin B1 protein expression.[12]

Experimental work carried out with female rats receiving different isoflavone doses showed that isoflavones do not stimulate breast proliferation. Instead, they have a protective effect due to the reduced capacity of isoflavones to bind to ERα.[13] In a later study of the effect of soybean isoflavones on the expression of genes which control cell growth, the authors observed cell cycle blockage and potential cancer prevention.[14] Notwithstanding the numerous studies for or against the use of isoflavones in postmenopausal women with breast cancer, we believe they should not be indicated, for the studies are much too controversial.

In a case-control study of estrogen-dependent ovarian cancer conducted in Southern China, Lee et al. [15] reported that daily consumption of at least 120g of isoflavones, when compared to less than 61g, had a protective effect on women. They assumed the outcome was related to apoptosis induction and to growth and proliferation inhibition of the tumor cells. They added that isoflavones could boost the production of sex hormone-binding globulin (SHBG) in the liver, leading to a reduction in bioavailable estrogens in the plasma. Another hypothesized mechanism was the inhibition of aromatase activity in the ovary. This enzyme converts androgens into estrogens, and such is the case *in vitro*.

The examination of meta-analyses of randomized controlled trials to evaluate the effectiveness of phytoestrogens in vasomotor symptoms and their side effects in postmenopausal women revealed considerable divergence among authors. Nevertheless, most reported mitigation of the symptoms, as well as improvement in the quality of life; none reported any side effects.[16] On the other hand, Del Giorno et al.[17] showed there was no significant improvement in menopausal symptoms and sexual satisfaction after the use of isoflavones derived from *Trifolium pratenses*. There are many published meta-analysis studies of soy isoflavones and vasomotor symptoms. The most recent comprehensive meta-analysis examined the results of 19 clinical trials of soy isoflavones for treating hot flashes and concluded that isoflavone supplements derived from the chemical synthesis of plant extracts were significantly more effective than placebo in reducing the severity of the heat waves et al.[18]

Atrophy of the genitals in menopause ranges from 10% to 50%. The lack of circulating estrogens favors a reduction in collagen and elasticity, resulting in vulvovaginal atrophy and dryness. These in turn give rise to diminished lubrication, which causes discomfort and dyspareunia (pain) during sexual inter-

course.[19] Studies assessing isoflavone action on the vagina after menopause are scarce. [20] Epidemiological studies of postmenopausal women using gel isoflavone reported improvement in vaginal trophism with attendant improvement in vaginal symptoms, pH, and increase in estrogen receptor expression, indicating that isoflavones are possibly a good therapy option for vulvovaginal atrophy relief.[21] Experimental studies with female rats under long-term isoflavone treatment showed vaginal epithelium trophism, confirming an isoflavone-induced trophic effect.[13, 22]

A randomized double-blind study of postmenopausal women who consumed soy isoflavones showed that long-term use neither affects endometrial thickness nor increases hyperplasia or endometrial cancer.[23]

Studies conducted with female rats showed that genistein and daidzein induced several genomic responses in the uterus. However, dosages deemed normal did not stimulate cell proliferation and thus these isoflavones may be considered agonists and/or SERMs.[22] High dosages prompted isoflavones to have a trophic effect on the endometrium, but when combined with estrogens, they did not present an additive effect.[14] A prospective clinical study that assessed the endometrium of 32 menopausal women for six months stated that three women exhibited endometrial changes suggestive of endometrial stimulation.[24] In still another study involving high doses of isoflavones, the uterus of oophorectomized female rats presented endometrial squamous metaplasia.[13]

Asian countries have a lower fracture rate than Western countries, such as the United States. This difference may be related to the fact that soybean food products are rich in isoflavones and are consumed daily by Asian women. Thus, many studies report the beneficial effects of isoflavones as inhibitors of the effects of bone resorption. In this respect, they are similar to estrogen, which is known to suppress bone resorption activity. According to in vitro models, isoflavones suppressed osteoclast formation. A meta-analysis study reported that isoflavones significantly attenuated bone loss in postmenopausal women. [18]

The beneficial effects of isoflavones include not only a reduction in bone loss, but also the stimulus for bone formation and for increased bone mineral density.

Antioxidant properties of isoflavones in the female reproductive system

Isoflavones are also known for their antioxidant properties, among which the capacity for regulating the enzyme expression and activity of the antioxidant system and for inhibiting oxidation of cell components through direct sequestration of free radicals by its phenolic rings or its ability to chelate the metallic ions involved in the oxidative process.[25]

Excessive production of free radicals may create an inadequate environment for normal physiological reactions, giving rise to a number of diseases of the female reproductive system, including endometriosis, polycystic ovary syndrome (PCOS), and infertility, without any apparent cause.

In the female reproductive system, free radicals play a key role in the regulation of several signaling pathways in folliculogenesis and oocyte maturation, in the cyclic changes in the endometrium, and in embryo implantation. Hence, oxidative stress exerts its influence throughout a woman's reproductive lifespan and modulates the decline of fertility as a woman ages.[26]

A study conducted with infertile women showed that dietary soybean consumption while they underwent treatment with an assisted reproduction technique seemed positive for the likelihood of pregnancy.[27] Likewise, Unfer et al.[28] noted that the pregnancy to delivery rate among the women who underwent in vitro fertilization and received isoflavone supplementation was almost double the number of women who did not ingest any isoflavones. Another study with infertile Japanese women revealed that diets rich in genistein and daidzein could reduce the risk of deep endometriosis. [29]

Reduction in oxidative stress by isoflavones has been demonstrated in several in vivo models. Genistein and daidzein have also been associated with a decrease in the risk of chronic pathologies, such as neurodegenerative, cardiovascular, and metabolic diseases, as well as cancers, partly due to their antioxidant activities.[30]

An experimental study with sexually mature female rats revealed that treatment with genistein diminished follicular atresia and raised the number of surviving ovarian follicles, suggesting genistein contributes towards lengthening the reproductive lifespan.[31]

Finding that isoflavones play a role as antioxidants has widened its potential uses not only in treating hypoestrogenism-derived disorders, but also in preventing and treating conditions associated with an increase in oxidative stress.

CONCLUSIONS

Isoflavones play many roles in offsetting diverse menopausal symptoms. Nonetheless, further studies are required to ensure outcome reliability.

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation with this paper.

PALAVRAS-CHAVE: *Isoflavonas. Menopausa. Pós-menopausa. Terapia hormonal.*

REFERENCES

1. Bedani R, Rossi EA. Isoflavonas: bioquímica, fisiologia e implicações para a saúde. Bol. CEPPA. 2005;23(2):231-64.
2. Setchell KDR. The history and basic science development of soy isoflavones. Menopause. 2017;24(12):1338-50.
3. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA. 1996;93(12):5925-30.
4. Cassidy A, de Pascual TS, Rimbach G. Molecular mechanisms by which dietary isoflavones potentially prevent atherosclerosis. Expert Rev Mol Med. 2003;5(24):1-15.
5. Esteves EA, Monteiro JBR. Efeitos benéficos das isoflavonas de soja em doenças crônicas. Rev Nutr. 2001;14(1):43-52.
6. NAMS - North American Menopause Society. The 2012 hormone therapy position statement of the North American Menopause Society. Menopause 2012;19(3):257–71.
7. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-33.
8. Morimoto Y, Maskarinec G, Park SY, Ettienne R, Matsuno RK, Long C, et al. Dietary isoflavone intake is not statistically significantly associated with breast cancer risk in the Multiethnic Cohort. Br J Nutr. 2014;112(6):976-83.
9. Shike M, Doane AS, Russo L, Cabal R, Reis-Filo J, Gerald W, et al. The effects of soy supplementation on gene expression inbreast cancer: a randomized placebo-controlled study. J Natl Cancer Inst. 2014;106(9):189.
10. Li Y, Anand-Srivastava MB. Implication of multiple signaling pathways in the regulation of angiotensin II induced enhanced expression of Giα proteins in vascular smooth muscle cells. Can J Physiol Pharmacol. 2012;90(8):1105-16.
11. Li H, Yu YY, Hu X, Cao SW. Research on the interactions between genistein and its glucosides with DNA. Guang Pu Xue Yu Guang Pu Fen Xi. 2008;28(8):1905-9.
12. Choi YH, Lee WH, Park KY, Zhang L. p53-independent induction of p21 (WAF1/CIP1), reduction of cyclin B1 and G2/M arrest by the isoflavone genistein in human prostate carcinoma cells. Jpn J Cancer Res. 2000;91(2):164-7.
13. Carbonel AA, Simões RS, Santos RH, Baracat MC, Simões Mde J, Baracat EC, et al. Effects of high-dose isoflavones on rat uterus. Rev Assoc Med Bras. 2011;57(5):534-9.
14. Carbonel AA, Calió ML, Santos MA, Bertoncini CR, Sasso GD, Simões RS, et al. Soybean isoflavones attenuate the expression of genes related to endometrial cancer risk. Climacteric. 2015;18(3):389-98.
15. Lee AH, Su D, Pasalich M, Tang L, Binns CW, Qiu L. Soy and isoflavone intake associated with reduced risk of ovarian cancer in southern Chinese women. Nutr Res. 2014;34(4):302-7.
16. Thomas AJ, Ismail R, Taylor-Swanson L, Cray L, Schnall JG, Mitchell ES, et al. Effects of isoflavones and amino acid therapies for hot flashes and co-occurring symptoms during the menopausal transition and early postmenopause: a systematic review. Maturitas. 2014;78(4):263-76.
17. del Giorno C, Fonseca AM, Bagnoli VR, Assis JS, Soares JM Jr, Baracat EC. Effects of Trifolium pratense on the climacteric and sexual symptoms in postmenopause women. Rev Assoc Med Bras. 2010;56(5):558-62.
18. Taku K, Melby MK, Kronenberg F, Kurzer MS, Messina M. Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials. Menopause. 2012;19(7):776-90.
19. Levine KB, Williams RE, Hartmann KE. Vulvovaginal atrophy is strongly associated with female sexual dysfunction among sexually active postmenopausal women. Menopause. 2008;15(4 Pt 1):661-6
20. Tedeschi C, Benvenuti C, Research Group EG. Comparison of vaginal gel isoflavones versus no topical treatment in vaginal dystrophy: results of a preliminary prospective study. Gynecol Endocrinol. 2012;28(8):652-4.
21. Lima SMRR, Campaner AB, Auge APF. Isoflavones derived from Glycine max (L.) Merr. in the treatment of vaginal atrophy: A new frontier. Rev Assoc Med Bras. 2017;63(9):727-8.
22. Carbonel AAF, Lima PDA, Lim JJ, Fuchs LFP, Paiotti APR, Sasso GRDS, et al. The effects of soybean isoflavones and 17β-estradiol in uterus and mammary glands of diabetic rat models. Gynecol Endocrinol. 2018;34(4):314-9.
23. Quaas AM, Kono N, Mack WJ, Hodis, HN, Felix JC, Paulson RJ, et al. Effect of isoflavone soy protein supplementation on endometrial thickness, hyperplasia, and endometrial cancer risk in postmenopausal women: a randomized controlled trial. Menopause. 2013;20(8):840-4.
24. Wolff LP, Martins MR, Bedone AJ, Monteiro IM. Endometrial evaluation in menopausal women after six months of isoflavones. Rev Assoc Med Bras. 2006;52(6):419-23.
25. Erba D, Casiraghi MC, Martinez-Conesa C, Goi G, Massaccesi L. Isoflavone supplementation reduces DNA oxidative damage and increases O-β-N-acetyl-D-glucosaminidase activity in healthy women. Nutr Res. 2012;32(4):233-40.
26. De Bruin JP, Dorland M, Spek ER, Posthuma G, van Haaften M, Looman CW, et al. Ultrastructure of the resting ovarian follicle pool in healthy young women. Biol Reprod. 2002;66(4):1151-60.
27. Vanegas JC, Afeiche MC, Gaskins AJ, Mínguez-Alarcón L, Williams PL, Wright DL, et al. Soy food intake and treatment outcomes of women undergoing assisted reproductive technology. Fertil Steril. 2015;pii :S0015-0282(14)02529-1.
28. Unfer V, Casini ML, Gerli S, Costabile L, Mignosa M, diRenzo GC. Phytoestrogens may improve the pregnancy rate in in vitro fertilization–embryo transfer cycles: a prospective, controlled, randomized trial. Fertil Steril. 2004;82(6):1509-13.
29. Tsuchiya M, Miura T, Hanaoka T, Iwasaki M, Sasaki H, Tanaka T, et al. Effect of soy isoflavones on endometriosis: interaction with estrogen receptor 2 gene polymorphism. Epidemiology. 2007;18(3):402-8.
30. Yoon GA, Park S. Antioxidant action of soy isoflavones on oxidative stress and antioxidant enzyme activities in exercised rats. Nutr Res Pract. 2014;8(6):618-24.
31. Zhuang XL, Fu YC, Xu JJ, Kong XX, Chen ZG, Luo LL. Effects of genistein on ovarian follicular development and ovarian life span in rats. Fitoterapia. 2010;81(8):998-1102.