REVIEW PAPER

ISOFLAVONOIDS − DUAL ACTION ON ESTROGEN RECEPTORS

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Abstract: Isoflavonoids are phytoestrogenic compounds found mainly in plants from the Fabaceae family and also in soy-based foods. Isoflavonoids exhibit (anti)estrogenic effects, acting on estrogen receptors due to the structural similarities with estrogenic hormones (17β-estradiol). The aim of our minireview is to highlight the pharmacokinetic and pharmacodynamic properties of isoflavonoids, in order to sustain the beneficial effects in different pathologies (osteoporosis associated with menopause, breast cancer, prostate cancer, protective cognitive functions) but, at the same time, to aware about the possible adverse effects on long-term administration.

Keywords: isoflavonoids, estrogen, receptors, menopause, SERM.

1. Introduction

Nowadays, there is an increased research interest in phytoestrogen compounds as they are widely used by vegetarians (through the consumption of soy, soy-based foods and vegetable products) and also by women at menopause (as dietary supplements) due to the beneficial effects produced in this physiological state, improving both somatic and psychological symptoms (Dong et al., 2011; Ahsan et al. 2017; Setchell, 2017).

Phytoestrogens are compounds of vegetal origin, classified according to their chemical structure in four classes: isoflavonoids, flavonoids, coumestans and lignans. Isoflavonoids and flavonoids represent the active substances with an important role in phytotherapy based on pharmacological studies. Isoflavonoids are found mainly in plants from the Fabaceae family, the most important source being represented by soybean (*Glycine max*). Actually, the food sources of isoflavonoids are soy milk, tofu, roasted soybeans etc. (Rietjens et al., 2017).

Isoflavonoids are benefic in pathologies like breast cancer, prostate cancer, cardiovascular diseases, metabolic syndrome associated with type II diabetes, but also in hypothyroidism and in cognitive dysfunctions (Cano and Garcia-Peres, 2010; Colacurci et al., 2013; Delmanto et al., 2013; Sathyapalan et al., 2018).

2. Pharmacokinetic aspects

Regarding the absorption and metabolism of isoflavonoids, after oral administration, glycosides are enzymatic hydrolysed to aglycone and carbohydrates by *β*-glucosidase and other enzymes in the gastrointestinal tract, but also by intestinal bacteria, then are absorbed through enterocytes into blood stream.

After absorption, isoflavonoids are -glucurono or sulfo-conjugated. Bacterial intestinal flora play a vital role in the isoflavonoids metabolism, producing both hydrolysis and transformation into the most important metabolites (from genistein and daidzein: dehydrodaidzein, dehydrogenistein, equol or secondary metabolites: *p*-ethylphenol from genistein and final transformation into CO_2) (Franke et al., 2014).

The scientific literature indicates that isoflavonoids have different effects depending on individual microbial flora. The isoflavonoid metabolites have grater estrogenic effects than the `parent` molecules (i.e. S-equol, the intestinal flora metabolite of daidzein, presents higher estrogenic activity than daidzein). Microbial flora produces enzymes capable of metabolizing food, xenobiotics and isoflavonoids, and differs from one person to another. There are notable interindividual differences, only 30-50% of individuals are reported to have the ability to turn isoflavones into equol, depending on ethnicity and lifestyle (Reverri et al., 2017; Kolátorová, et al., 2018).

Regarding the pharmacokinetic parameters, daidzein and genistein reach the maximum concentration range (C_{max}) 2-8 hours after administration, the half-life being about 6-8 hours. An increased intake of isoflavonoid-containing products, for example the consumption of soybean 2-3 times a day, can lead to clinically relevant concentrations (Kano et al., 2006).

3. Mechanism of action related with the chemical structure

The interaction of isoflavonoids with the estrogen receptors (ER) is due to their structural similarity with 17β-estradiol (endogenous agonist), although they do not have a steroidal structure like C_{18}-hormones.

The chemical structures of 17β-estradiol and daidzein, genistein, glicitein (the most important isoflavonoids) are presented in **Fig. 1**. Each structure presents two hydroxyl (-OH) groups on both sides. The key point differentiating 17β-estradiol from isoflavonoids, is the distance between the two hydroxyl groups, that influences the affinities to the ER (Simons et al., 2012).

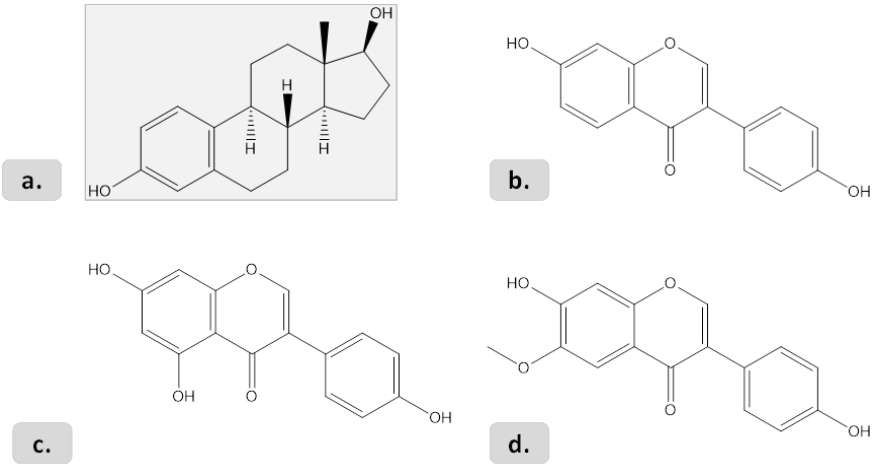

Fig. 1. Chemical structures of estradiol(a), daidzein(b), genistein(c), glicitein(d)

Because of this structure, phytoestrogens may act as agonists, but also as antagonists (in the presence of endogenous agonist), having, at the same time, similar structure with tamoxifen (an antiestrogenic molecule). In fact, these structures act as selective estrogen receptor modulators (SERMs) with comparable affinity for the ER, but a lower intrinsic activity than the endogenous agonist (Sathyapalan et al., 2018).

The ER are part of a superfamiliy of nuclear receptors. There are two subtypes of ER: alpha (ER-α) and beta (ER-β). The ER-α are predominantly found in the urogenital system (endometrium, uterus, kidneys), mammary gland, pituitary gland, while ER-β are found in bone, prostate, brain, cardiovascular system and reproductive organs. The main activity of these receptors, as nuclear receptors, is to regulate the expression of different genes (through the process of transcription and translation) or repressing other genes (Pang et al., 2018).

The beneficial effects of isoflavonoids are due to their interaction with the ER-β subtype. On the other hand, by activating the ER-α receptors, isoflavonoids are responsible for inflammatory and malignant processes. However, it seems that some isoflavonoids such as genistein and daidzein present a selective action on ER-β, producing predominantly the beneficial effects of isoflavonoids.

Regarding the detailed molecular mechanism of action, isoflavonoids cross the cell membrane (being sufficiently lipophilic) and form a complex with the ER. The isoflavonoid-receptor complex traverses the nuclear membrane, attaches itself to DNA, to the estrogen responsive element `ERE`, and initiates transcription processes of the genes with mRNA generation. The mRNA will undergo the ribosomal translation process, finally producing the different estrogen-specific effects. At the same time, during this process, other genes are repressed and the transcription process is inhibited.

The action of isoflavonoids on these receptors is dual, because they act as agonists or partial agonists (antagonists in the presence of endogenous agonist). On the other hand, genistein binds to both estrogen receptors (α and β) but with a 5-6-fold greater affinity for the ER-β. The position and number of hydroxyl groups influences the receptor selectivity. Thus, genistein has increased affinity for ER-β whereas the removal of a hydroxyl group at daidzein decreases the binding affinity (Dixon and Ferreira, 2002). There are studies which demonstrate the affinity of genistein for ER subtypes (Paterni et al., 2014; Jiang et al., 2018). In **Table 1** it can be observed that genistein has a high relative binding affinity (RBA) for the ER-β subtype, influencing minimally the ER-α subtype, compared to 17β-estradiol.

Table 1. The RBA of 17ß-estradiol and genistein for estrogen receptor (ER) subtypes

Compound	**RBA* for ER-α (%)**	**RBA for ER-β (%)**
17ß-estradiol	100	100
Genistein	5	36

Note: *The relative binding affinity (RBA) was calculated as the ratio of the concentrations of agonist and competitor required to reduce the specific ligand-binding by 50% (the ratio of IC_{50} values) (Kuiper et al., 1997; Paterni et al., 2014).

Thus, these isoflavonoids have beneficial effects in menopausal women (with low levels of estrogen), but may have less beneficial or even harmful effects in young women (acting as antagonists). In this regard, the effect of isoflavonoids in pathologies of estrogen-rich tissues, for example the effect on the mammary glandular breast cancer remains to be investigated (Nagat et al., 2014; Senthilkumar et al., 2018).

An additional mechanism of action recently proposed for genistein is the tyrosine-kinase receptor binding and therefore the modulation of the activities of hormones that use tyrosine phosphorylation for intracellular signal transfer (eg. insulin, insulin-like growth factor etc.) (Amanat et al., 2018).

This mechanism of action was also investigated in the study of Glisic et al. (2018), which demonstrated that isoflavonoids would have a beneficial effect on glucose metabolism, reducing the risk of developing type II diabetes in non-diabetic women, lowering insulin resistance. It interferes directly with lipid metabolism (lipogenesis and lipolysis), but indirectly at the level of the intestinal, hepatocyte and skeletal cells. It seems that isoflavonoids suppress the synthesis of genes involved in gluconeogenesis, but also have antioxidant effects (Glisic et al., 2018).

4. Effects on bone tissue

Isoflavonoids produce stimulation of osteoblast activity and decrease osteoclast activity. The presence of a free hydroxyl group in position 7 (for genistein) is very important to exert the antiosteoporotic effect. Synthesis of osteoclasts is inhibited by binding to ER-β in the bone cells. Isoflavonoids increase alkaline phosphatase levels, a marker for bone cell differentiation and proliferation, highlighting the idea of an inhibitory effect on the bone resorption process (Gupta, 2014; Muhammad et al., 2018). Regarding the action on bone resorption, a recent study compared the preventive osteoporotic effect in menopausal women treated with low concentrations of estrogen hormones (replacement therapy) and standardized soy isoflavonoid extract. The conclusion was that there are no major differences between the preventive effects of hormonal therapy and isoflavonoid therapy, both decreasing the bone resorption process (Tit et al., 2018).

5. Effects on adipose tissue

The adipose tissue is influenced by isoflavonoids by decreasing insulin-induced lipogenesis in both adipocytes and preadipocytes and increasing adrenaline-induced lipolysis. It was observed a decrease in the circumference of adipocytes, therefore the decrease in fat deposits occurs by decreasing the size of the cells. At different stages of development, isoflavonoids may also influence the number of adipocyte cells (Harmon et al., 2001).

Thus, isoflavonoids produce qualitative and quantitative decreases in fat deposits. Genistein may cause weight loss by inhibiting mRNA responsible for LPL synthesis, rather than producing lipolysis (Grossini et al., 2018).

6. Other recently discovered benefic effects

Considering that isoflavonoids are acting like SERMs, in a recent study on experimental animals, Fáber et al. (2018) show the protective effect of genistein on the skin flap viability. This effect could be potentially applied in plastic surgery to women undergoing a reconstructive intervention, but the mechanism responsible for these effects is not clearly defined, so further investigations should be conducted (Fáber et al., 2018). There are also studies offered by the scientific literature that suggest the protective cognitive functions of

isoflavonoids, but these effects appear to depend on age, comorbidities, plant type and the type of isoflavonoids administered (Roozbeh et al., 2018).

Conclusions

Regarding the major interest among women in phytoestrogen supplementation (especially isoflavonoids) for preventing or treating different pathological conditions and regarding the new trend of population to become vegetarian, more studies following the effects of isoflavonoids should be done in order to evaluate the balance benefit-risk of intake of high amounts of isoflavonoids, because in long-term administration they can act like endocrine disruptors.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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