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Review

Biological effects induced by estrogenic activity of lignans

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ABSTRACT

Background: Lignans are an important category of phytoestrogens and contribute to human health as food, supplements and medicines. Estrogenic lignans are classified by their various biological effects as revealed by assays used to evaluate estrogenicity, and cell signaling pathways and functions that are often associated with other signaling pathways and signal mediators. Although some assays give consistent results, others have shown contradictory results.
Scope and approach: The biological effects induced by estrogenic activity of lignans are summarized here by a comprehensive search of literature associated with their signaling pathways and cell functions.
Key findings and conclusions: Estrogenic lignans have structural characteristics, such as those mimicking the presence of and the distance between the hydroxyl groups at position 3 of the steroid A-ring and position 17 of the D-ring, and aromatic hydrophobicity. The signaling pathways induced by lignans include specific pathways, such as the estrogen receptor (ER) and mitogen-activated protein kinase (MAPK) pathways, and specific cell functions such as chromatin/epigenetic regulation, apoptosis, autophagy, cellular metabolism, translational control, cell cycle/DNA damage control, cytoskeletal/adhesion regulation, immunological/inflammatory response, neurodegenerative diseases and development/differentiation. In addition, crosstalk of receptor signaling was observed between estrogen signaling and other signaling pathways. Due partly to the fact that lignans do not cause adverse reactions or increase health risks, applications of estrogenic lignans in food, supplements, diagnostics, therapeutics and medicines have been explored. To further explore the applications of estrogenic lignans, it is essential to understand their mechanism of action, especially at the cell signaling level.

1. Introduction

Lignans consist of a large group of natural products characterized by the phenylpropane (C_6–C_3) unit. The group of lignans is divided into lignans (containing two C_6–C_3 units linked by a 3β-(8-8′) bond), neolignans (containing two C_6–C_3 units but not linked by 3β bonds) and hybrid lignans (containing a C_6–C_3 unit bound to another structure, such as flavonoids in flavonolignans) (Kumar, Silakari, & Kaur, 2012), which are further divided into 8 major types, furofuran (pinoresinol, sesamin, etc.), furan (lariciresinol, olivil, etc.), dibenzylbutane (secoisolariciresinol, dihydrocubebin, etc.), dibenzylbutyrolactone (matairesinol, hinokinin, etc.), aryltetralin (podophyllotoxin, β-peltatin, etc.), arylnaphthalene (taiwanin, justicidin A, etc.), dibenzocyclooctadiene (steganacin, etc.) and dibenzylbutyrolactol (cubebin, etc.), according to their structural characteristics, such as the carbon skeleton, oxygen presence and cyclization pattern (Satake, Ono, & Murata, 2013; Suzuki & Umezawa, 2007). There are structural variations of lignans, such as (8-*O*-4′) neolignans (or 8,4′-oxyneolignanes) where two C_6–C_3 units are linked by an ether oxygen, cyclolignans which contain an additional carbocyclic ring, “seco”-prefixed lignans which have ring cleavage, and “nor”-prefixed lignans in which one or more carbon atoms are lost from the skeletal atoms (Moss, 2000).

Lignans are biosynthesized from phenylpropanoid monomers through coniferyl alcohol with the aid of dirigent proteins and other proteins such as those with reductase/dehydrogenase/hydroxylase/methyltransferase/oxygenese activities. Norlignans, such as those with 7-8′, 8-8′ and 9-8′ linkages, are considered to be synthesized through lignin biosynthesis followed by the loss of a carbon, or through appropriate modifications of phenylpropanoid monomers. After biosynthesis, lignans and norlignans are deposited in the heartwood region of trees to protect against rotting (Suzuki & Umezawa, 2007).

1.1. Biological effects induced by lignans

Lignans are known to induce various biological activities

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including antitumor activity, antimitotic activity, antiviral activity, inhibition of the activity of enzymes such as cytochrome oxidase and cAMP phosphodiesterase, and various physiological activities such as cathartic activity, cardiovascular effects, allergenicity and toxic activities towards insects, microbes, fungi, fish, plants and mammals (MacRae & Towers, 1984). Their pharmacological properties include stress-reducing activity, anti-inflammatory activity, relaxation effects, analgesic effects, hypertensive effects, antioxidant activity, liver protective effects, hypolipidemic activity and phytoestrogenic activity (Deyama & Nishibe, 2010).

1.2. Lignans as phytoestrogens

Phytoestrogens are a group of plant-derived compounds having estrogenic activity, and therefore, potentially having various effects on human health, such as effects on breast and prostate cancers, cardiovascular disease, menopausal symptoms and osteoporosis (Adlercreutz & Mazur, 1997; Mazur, Duke, Wähälä, Rasku, & Adlercreutz, 1998; Ososki & Kennelly, 2003; Price & Fenwick, 1985; Usui, 2006). The well-characterized phytoestrogens are isoflavones, coumestans and lignans, while other compounds, such as anthraquinones, chalcones, prenylflavonoids and saponins, have also been investigated (Ososki & Kennelly, 2003). Meanwhile, steroidal compounds with estrogenic activity, such as β-sitosterol, are found in almost all plants, although they are excluded from phytoestrogens according to a strict definition (Dixon, 2004). In addition, plant lignans have been said to not have inherent estrogenic activity (Adlercreutz, 2002), because only their metabolites actually reveal their activity when they are taken as a food.

Lignans and their glycosides are metabolized by intestinal flora into enterodiol and enterolactone, collectively called the enterolignans, which may act by binding to ERs, whereas ER-independent activities have been observed in tumor growth suppression, angiogenesis inhibition, apoptosis inhibition and others (Satake et al., 2013). Biologically active metabolites of phytoestrogens or estrogenic compounds derived from plant lignans, such as enterolactone, enterodiol, matairesinol and secoisolariciresinol and their monohydroxylation/glucuronidation/sulfation products, could be used as dietary biomarkers, based on the association of lignin levels in body fluids with dietary fiber intake (Lampe, 2003).

Phytoestrogen content in foods, such as secoisolariciresinol and matairesinol, was examined by gas chromatography-mass spectrometry (GC-MS), and the results revealed that flaxseed (*Cuscuta epilinum*) contained the highest level of lignans among the foods tested, while other sources, such as grains, seeds, vegetables, fruits and beverages, were also sources (Mazur, 1998). Later, the content of enterolignan precursors including lariciresinol and pinoresinol was examined by liquid chromatography-tandem MS (Milder, Arts, van de Putte, Venema, & Hollman, 2005).

Lóránd et al. tentatively proposed groups of phytoestrogens (beneficial estrogens) and xenoestrogens (harmful estrogens) from the viewpoint of risk assessment, although there are exceptions in these categories and the definition of estrogenicity itself has been controversial (Lóránd, Vigh, & Garai, 2010). Pharmaceutical or any beneficial use of chemicals requires proper control of their efficacy, and thus, when phytoestrogens are used as a drug or a supplement, or as materials for any beneficial use, it is quite important to understand their cell-based molecular mechanisms. The same is true for accurate and reliable risk assessment of chemicals. In this review, the molecular aspecst of estrogenicity of lignans is focused.

2. Estrogenic activity of lignans

Estrogen is a female hormone and exerts its effect first by binding to ERs and then through mechanisms at multiple levels, such as receptor/ligand interaction, transcription, translation and cell functions (Fig. 1). In the genomic pathway, nuclear ERs act as a transcription factor, and, upon binding with estrogen, the complex, usually in the form of ER dimers, up- or down-regulates the expression of target genes by binding to DNA at the transcriptional regulatory sequence, or the estrogen-responsive element (ERE). In contrast, membrane ERs are involved in the non-genomic pathway, where the binding of estrogen to ERs initiates transcription-independent signaling pathways, resulting in rapid regulation of cell functions or slow regulation of the expressions of estrogen-responsive genes. The estrogen signal may be transduced into other signaling pathways, such as the epidermal growth factor receptor (EGFR) pathway, by receptor crosstalk, which can affect the function of the same cell, or other cells in the vicinity or at distant locations, through autocrine, paracrine or endocrine signaling, respectively, which may contribute to homeostatic networks (see Section 3.3).

2.1. Assays for estrogenic activity

A variety of assays to evaluate the estrogenic activity of chemicals has been developed based on the molecular mechanism of estrogen action (summarized in Fig. 1), including the ligand-binding assay, reporter-gene assay, yeast two-hybrid assay, transcription assay, protein assay, cell assay, animal test and signaling pathway analysis (for details, see Kiyama & Wada-Kiyama, 2015).

The ligand-binding assay is based on the ability of a test chemical to compete with ^{3}H-labeled 17β-estradiol (E_2) or non-radioactive compounds for binding to ERs, and is often expressed as the concentration showing 50% inhibition. In a reporter-gene assay, such as the chemically activated luciferase expression, or CALUX assay (Sonneveld, Jansen, Riteco, Brouwer, & van der Burg, 2005), and yeast estrogen screen, or YES assay (Arnold, Robinson, Notides, Guillette, McLachlan, 1996), various reporter-gene constructs have been used in combination with reporter genes, such as the luciferase and β-galactosidase (lacZ) genes, and transcriptional regulatory sequences, such as the ERE, in the promoter region of the constructs, to detect the ability of the ER to transactivate the reporter gene upon its binding to ligands. The yeast two-hybrid assay (Nishikawa et al., 1999) is based on the interaction between two transcription-regulatory proteins. It uses two expression vectors expressing different fusion proteins, such as those containing the GAL4 DNA-binding domain/human ER ligand-binding domain and those containing the ER-binding domain of a co-regulator/GAL4 transactivation domain/lacZ. In the presence of an estrogenic chemical, the interaction between GAL4/ER and co-regulator/lacZ occurs and lacZ is subsequently detected by colorimetric assay. Transcription assays, such as the reverse transcription-polymerase chain reaction (RT-PCR) and DNA microarray assay, detect the transcripts of genes to quantify the expression of ER target genes, such as *TFF1* (*pS2*) gene, or sets of estrogen-responsive genes to evaluate the effect of estrogenic chemicals on the gene expression. Protein assays, such as Western blotting, mass-spectrometry (MS) and the enzyme-linked immunosorbent assay (ELISA), qualitatively and quantitatively evaluate a protein or sets of proteins induced by estrogenic chemicals. In cell assays, cell growth and proliferation are detected by a variety of methods including counting live cell numbers by the dye exclusion method or by fluorescence-activated cell sorting (FACS). Specific cell lines are often used, such as MCF-7 cells in the E-screen assay (Soto et al., 1995) and Ishikawa cells for the detection of estrogen-inducible alkaline phosphatase activity (Pisha & Pezzuto, 1997). Finally, animal tests, such as the uterotrophic assay, have been used from the early days to detect estrogenicity, where the genetic, developmental and behavioral effects of chemicals can be examined. However, a number of technical,