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ANTIPROLIFERATIVE EFFECT OF LIGNIN - AND LIGNAN - RELATED PLANT PRODUCTS IN CANCER AND NORMAL CELLS. POSSIBILITY OF APPLICATION FOR ANTICANCER THERAPY?

ANTIPROLIFERATIVNI EFEKAT LIGNINSKIH I LIGNANSKIH BILJNIH PROIZVODA U ĆELIJAMA KANCERA I U NORMALNIM ĆELIJAMA. MOGUĆNOST PRIMENE ZA ANTIKANCER TERAPIJU?

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Apstrakt

Lignini i lignani su bitni proizvodi biljnog metabolizma. Lignin je polimer fenolnih alkohola i sastoji se od frakcije male i velike molekulske težine. Lignani, ne-strukturni biljni fenolni metaboliti, su dimeri i oligomeri fenolnih monomera. Izobilje lignina/lignana kao prirodnih proizvoda i indikacije njihove sposobnosti da inhibiraju rast malignih ćelija, su usmerili interes istraživačkih grupa ka istraživanjima ovih jedinjenja, u pogledu primene za antikancer terapiju. Neki od dosadašnjih rezultata su prikazani u ovom radu. Različite vrste lignana su izolovani iz biljaka koje su već ranije korišćene u tradicionalnoj medicini u lečenju različitih oboljenja. Oni su inhibirali proliferaciju malignin ćelija i imali kancerostatsku aktivnost. Mehanizam njihovog delovanja još nije razjašnjen, iako postoje indikacije da oni imaju fitoestrogensku aktivnost, ili da interaguju sa nekim enzimima važnim u razmnožavanju ćelija. Lignini su manje proučavani, ali retke studije pokazuju njihov potencijal u inhibiranju proliferacije malignih ćelija. U tom pogledu, uočena je razlika u delovanju sintetičkog lignina na proliferaciju ćelija između normalnih i malignih ćelija. Posebno potentna je bila ligninska frakcija male molekulske težine. Činjenica da je sintetisani lignin, ili njegove pojedine frakcije, čista supstanca, i da postoji mogućnost kontrolisanja i izmene uslova njegove sinteze, može biti interesantno za buduća proučavanja.

INTRODUCTION

Lignins and lignans are the major metabolic pruducts of phenylpropanoid metabolism in vascular plants. Together, they constitute some of the metabolically most expensive products generated by plants (1), and are derived from the aromatic amino acids, phenylalanine and tyrosine. Extension of the phenylpropanoid pathway from phenylalanine onwards, leads to both polymeric lignin and dimeric/oligomeric lignans (Fig. 1). Altogether, some 30-40 % of all organic carbon in vascular plants is stored in this manner. Lignin, the second abundant organic compound on the Earth's surface, provides mechanical support to the plant tissues and protection from physical, chemical stress and disease resistance. In the plant cell lignin is produced by oxidative coupling of the three phenolic alcohols/acids (coniferyl, p-coumaryl and synapyl), catalyzed by the enzyme peroxidase (2). Enzymatically synthesized lignin model compound, dehydrogenate polymer (DHP) from coniferyl alcohol, is the best lignin substitute, used in various experiments (3). Lignin consists of low (2000 - 8000) and high (60000 - 100000) molecular weight fraction (4). The lignans, non-structural phenolic metabolites, are dimmers or oligomers of phenolic monomers (5). There are plenty of structural variations among lignans from various plant sources (6).

Besides playing substantial roles in plant defense (5), many lignans are proposed to be good candidates for use in medicine and health maintenance (7, 8). Lignin was found to exert free-radical scavenging capacity (9, 10). Natural and synthetic lignin-like materials have shown antiviral, antitumor and immunopotentiating activities (11, 12).

Due to a lack of chemotherapeutics to efficiently control neoplastic processes, there is a need for discovering new, more efficient anticancer drugs that would distinguish malignant from normal cells. Abundance of lignins/lignans as natural products and indications of their potency in inhibition of malignant cells growth, made research groups increase their interest in studies of these compounds, concerning their application for anticancer therapy.

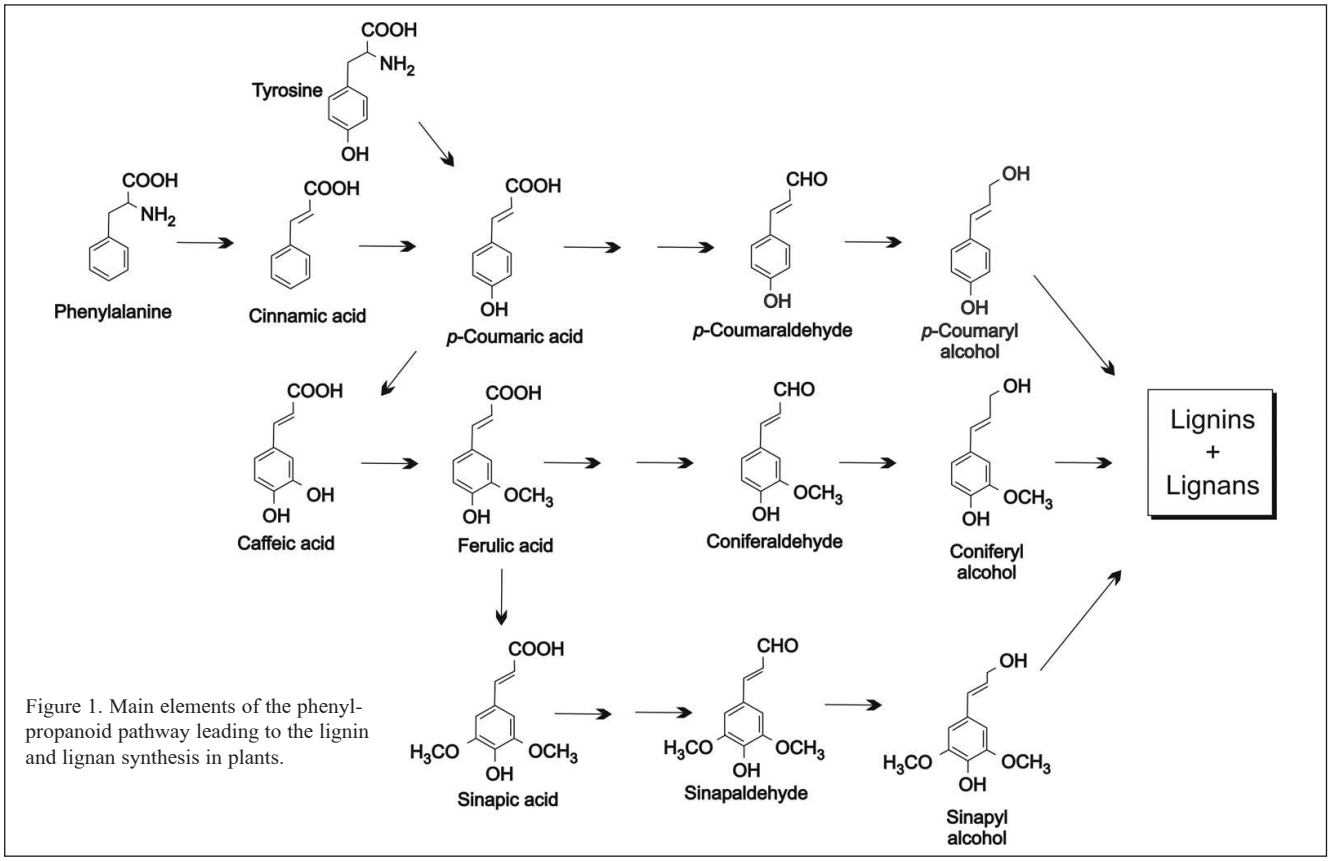

Figure 1. Main elements of the phenylpropanoid pathway leading to the lignin and lignan synthesis in plants.

ANTIPROLIFERATIVE EFFECT OF LIGNANS

A lot of plants have been traditionally used, for thousands of years, in curing various diseases. They have been employed as adaptogens or in alternative treatments against the microorganisms connected with certain body/organ disorders (13, 14). Such is for example *Schisandra chinensis*, widely distributed in northeast Asia, which can help in certain diseases, promoting oxygen-supply for the cells and potentiating the body's immune system (15). Various reports suggested that major bioactive constituents of *S. chinensis* were lignans which belong to the dibenzocyclooctadiene type (Fig. 2). It has been reported that dibenzocyclooctadiene lignans possess hepatoprotective, antiviral, antioxidant, cytotoxic, and cancer chemo-preventive activities (see for example 16). The recent studies demonstrated that dibenzocyclooctadiene lignans isolated from the fruit of *S. chinensis* inhibited cell proliferation in human lung cancer cells A549, and the antiproliferative mechanism of the active compound schisantherin C appears to be the induction of cell cycle arrest in G0/G1 phase through down-regulation of cyclin E and up-regulation of p27 in the cancer cells (17). In the course of screening for pharmacologically active substances from extracts of crude drugs used traditionally in Sino-Japanese herbal medicines, it was found that the 70 % ethanol extract from the fruits *of Arctium lappa* L. (native to the temperate regions of the old world, but also commonly cultivated in Japan) showed potent antiproliferative activity against B cell hybridoma cell, MH60. By bioassay-guided purification, a new lignan, (+)-7,8-didehydroarctigenin, together with the known lignans (-)-arctigenin and (-)-matairesinol were isolated as the active ingredients from an aqueous ethanolic extract of the fruits of *A. lappa* (18). On the basis of certain experimental results lignans have been proposed to have a phytoestrogenic activity towards mammals, but generally more experiments are needed to better assess their potential beneficial effects (7, 8, 19). The plant lignans and isoflavonoid glycosides are converted by intestinal bacteria to hormonelike compounds with weak estrogenic but also antioxidative activity; they have been shown to influence not only sex hormone metabolism and biological activity but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation, and angiogenesis in a way that makes them strong candidates for a role as natural cancer-protective compounds. Epidemiologic investigations strongly support this hypothesis because the highest levels of these compounds in the diet are found in countries or regions with low cancer incidence (7). Another study has shown that lignans may be moderate or weak inhibitors of human estrogen synthetase (aromatase) and that they bind to or near the substrate region of the active site of the enzyme. It is suggested that the high concentration of lignans in vegetarians, by inhibiting aromatase in peripheral and/or cancer cells and lowering estrogen levels, may play a protective role as antipromotional compounds during growth of estrogen-dependent cancers (20). Another group of authors proposed that enzyme topoisomerases may be one of the main cellular targets for anticancer drugs, among them lignans. According to the proposed mechanism, these drugs alter the catalytic functions of topoisomerases and convert these essential enzymes into lethal cellular weapons (21).

Some recent studies give encouraging results concerning potential use of lignans in anticancer therapy. Clinical intervention studies and experimental studies with lignan-rich diets suggest that lignans may have inhibitory effects on prostate cancer, but until recently no clinical or experimental studies with purified lignans have been published. Recently the effect of a plant lignan 7-hydroxymatairesinol (HMR) on LNCaP human prostate cancer xenografts in athymic mice was studied (22). The results suggest that dietary HMR started at the early phase of the tumor devel-

opment inhibit the growth of the LNCaP human prostate cancer xenografts in athymic male mice. Touillot et al. (23) examined associations between the risk of postmenopausal invasive breast cancer and dietary intakes of four plant lignans (pinoresinol, lariciresinol, secoisolariciresinol, and matairesinol) and their bioactive metabolites, the enterolignans. Analyses were further stratified by the combined estrogen and progesterone receptor (ER/PR) status of the tumors. The results of this large prospective study showed that higher dietary intakes of lignans were associated with a reduction in the risk of postmenopausal breast cancers, particularly those positive for both ER and PR. Yet individual differences in the metabolism of plant lignans into enterolignans and the mechanisms behind the potential biologic actions of enterolignans in carcinogenesis need to be better understood.

ANTIPROLIFERATIVE EFFECT OF LIGNINS

Inhibitory effect of lignins on proliferation of cancer cells has not been extensively studied, due to their high molecular weight which disables its transport through cell membrane into the cell interior. In one of the rare studies, it was found that artificial lignin, synthesized by polymerization of ferulic acid, inhibited growth of the tumor induced by a tumor promoter in mouse skin, after subcutaneous application. The polymer was dissolved in acetone and the effective concentration was 25 mg·ml-1 (24). In the pine cone extract, which showed inhibitory effect on the growth of ascites and solid tumor cells transplanted in mice, and also had antiviral and antimicrobial activities, lignin-related structures complexed with sugars and polysaccharides were found to be most effective components. Most active was found to be the low molecular weight fraction of lignin (11). In a recent study, the effect of different concentrations of synthetic lignin (DHP) on the growth of two human cell lines: human breast adenocarcinoma MCF7 and normal fetal lung fibroblast MRC5, was studied (25). The effects of short- (4 hours) and long- (72 hours) treatment duration were compared, in order to see whether there is any inhibitory effect of DHP on proliferation of malignant cells. The human transformed cell line was a control, to see if there is any difference in response to DHP in comparison with malignant cells. Besides, the aim was to compare the inhibitory potential of high and low molecular weight fraction of DHP on the cell proliferation. In the case of both normal cell line and human breast adenocarcinoma cell line, there was an effective inhibition by 1 mg·ml-1 DHP for only 4 hours, showing the similarity of the reaction of the two cell lines towards DHP. However, the growth of carcinoma cell line was found to be sensitive to the long-term treatment with the lower DHP dose (0.1 mg·*ml-1*) in comparison with the fetal cells. These data are important, depicting the difference in response between the normal fetal and malignant cells. These data might be a basis for further study of synthetic lignin as an agent that selectively influences the growth of malignant cells. Since polymers of high molecular weight such as DHP cannot pass the cell membrane, a possible mechanism of antiproliferative DHP action might be located at the surface of cell membane. In the second part of this study the cells were treated with low molecular weight DHP fraction, containing oligomer and low-molecular weight polymer, separated by filtration of the whole DHP solution. The results show the potency of this low molecular weight fraction to inhibit cell growth, even at lower DHP concentrations than in case when all fractions were present. The human breast adenocarcinoma cell line used in this study is estrogen receptor-positive; therefore the inhibitory effect of lignin on its proliferation may be possible as an antiestrogenic effect. It has been recently shown an inhibitory effect of lignans on E2 induced growth of tumors of the same type (26). The fact that synthesized lignin model compound, DHP, or its particular fractions, is a pure substance, and that there is a possibility of controlling and changing conditions of its synthesis, may be inspiring for its application in further studies of the effect on cell proliferation.

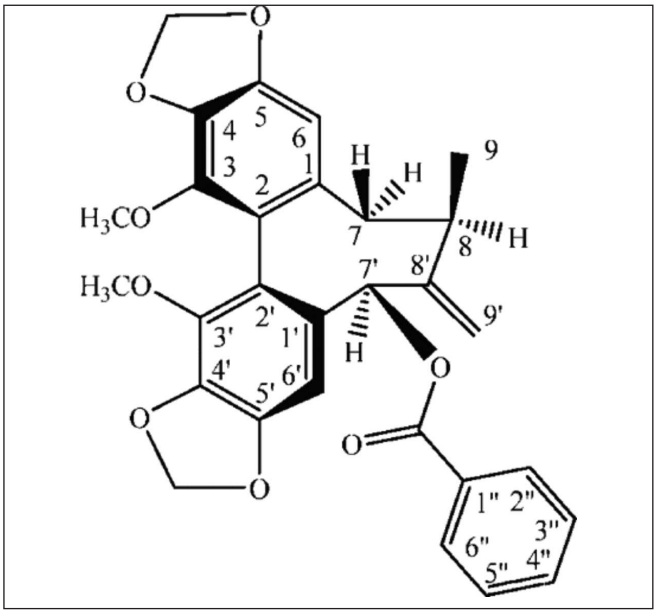

Figure 2. One of the 13 lignans isolated from the fruits of Schisandra chinensis. The structure-activity relationships of the dibenzocyclooctadiene lignans showed that the exocyclic methylene functionality was essential for antioxidant activity, while the benzoyloxy group probably enhances such activity (16).

There are different proposed mechanisms of inhibition of cell proliferation by lignins. Lignin-like substance suberin, extracted from *Querkus suber* cork, showed antimutagenic activity in *Euglena gracilis*, and the authors proposed that this effect may be due to the scavenging of reactive oxygen species (27). Some previous results indicated radical scavenging activities of lignin (9, 10). Sakagami et al. (28) showed that 125I-labeled natural and synthetic lignins administered orally to mice are absorbed through the digestive tract and excreted via urine, in addition to feces.

Although a lot of work is to be done in future in order to come to the clinical utilisation of the lignan- and lignin-related plant products in the anticancer therapy, the results of the up-to-date studies are encouraging. Generally, such studies contribute to the development of anticancer agents derived from natural products, which is one of the important research areas in the modern medicine.

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Abstract

Lignin and lignans are the major pruducts of plant metabolism. Lignin is polymer of phenolic alcohols and consists of low and high molecular weight fraction. The lignans, non-structural plant phenolic metabolites, are dimmers or oligomers of phenolic monomers. Abundance of lignins/lignans as natural products and indications of their potency in inhibition of malignant cells growth, made research groups increase their interest in studies of these compounds, concerning their application for anticancer therapy. Some of the up-to-date results are presented in this paper. Different kinds of lignans have been isolated from the plants that already have been used in traditional medicine as curing agents towards various diseases. They inhibited proliferation of malignant cells and had cancerostatic activity. Their mechanism of action is not yet clear, although there are indications that they have a phytoestrogenic activity, or that interact with some enzymes important in cell multiplication. Lignins are less studied, but rare studies show their potential in inhibition of malignant cell proliferation. In this respect, a difference in the effect of synthetic lignin on cell proliferation was observed between normal and malignant cells. Especially potent was low molecular weight lignin fraction. The fact that synthesized lignin, or its particular fractions, is a pure substance, and that there is a possibility of controlling and changing conditions of its synthesis, may be interesting for further related studies.

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