Evolution, Nutrition, Intestinal Microflora, and Prevention of Cancer: A Hypothesis

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Our diet and environment have changed rapidly since the start of industrialization. Our partially genetically determined behavior leads, in the affluent society, to the consumption of a diet rich in cholesterol, fat, energy, and salt and to a rapid increase in the incidence of so-called Western diseases. These diseases include all the major causes of death like various cancers and coronary heart disease. The industrially produced highly palatable diet contains too little essentially protective compounds, and our sedentary lifestyle, smoking, high alcohol consumption, and other behaviors further contribute to the development of disease. In fact cancer may be a relatively young disease going back to Hippocrates and ancient Greece about 2500 years ago, suggesting that evolution plays an important role.

In this presentation I will discuss the importance of the relations between phytoestrogens, mainly flavonoids and lignans, and steroids during evolution and the possible role of these evolutionary events and our intestinal microflora for adequate nutrition and availability of cancer protective compounds such as phytoestrogens.

Steroid Hormone-Dependent Cancer and the Structural Similarities Between Steroids and Phytoestrogens

Breast, endometrial, and prostate cancers are steroid hormone-dependent, and steroid hormone receptors have also been found in colon and other cancers. Estrogens play a definite role in the development of breast and endometrial cancer, but, in addition, they may have important roles with regard to prostate (1) and colon (2) cancer. After identification of the unknown phenolic compounds in urine (3–7), the structural similarities between the diphenolic lignans and isoflavonoids on one hand, and estrogens on the other hand and their very close molecular weights immediately suggested that they could interfere with steroid hormone metabolism or action at the cellular level or that they by themselves could have biological effects in the body. Then we found that postmenopausal breast cancer patients, living in Boston, Massachusetts, after one year's follow-up, had a very low excretion of lignans and somewhat lower equol in urine compared to vegetarians (8). These women consumed very low amounts of cereal fiber (about 3.5 g/day), one of the main sources of lignans. In addition, we observed very high values of both isoflavonoids and lignans in captive chimpanzees, highly resistant to the induction of breast cancer by various toxic compounds (9, 10) and in Japanese men and women with very low cancer incidence (11, 12). These observations supported our hypothesis of a protective role of these compounds in hormone-dependent cancer. Another possibility was that they are just markers of a protective diet.

Steroids, Flavonoids and Lignans During Evolution

Sterols as well as flavonoids (or at least their precursors) have been found in phylogenetically very early prokaryotic organisms like blue-green algae (cyanobacteria) (13–16). It is possible that the flavones in the green algae have been a result of some mutation of systems that introduce double bonds into the rings of steroids (16). According to Witzmann, there is probably no life without steroids (17) so the steroids must have been present very early. The basic steroid skeleton has not changed during about 1.5–2 billion years, and the complicated hormonal system in multicellular organisms is a much later development. Lignans, on the other hand, developed from flavonoid precursors (16) about 400 million years ago and isoflavones about 120 million years ago. At the time when plants came up from the water they required more structural support (vascular plants) and at that time lignins and lignans occurred. These compounds are typical plant compounds. Steroids are produced by bacteria, fungi, and plants but, according to present knowledge, only plants and fungi are able to produce flavonoids, and lignans are probably only produced by plants. Flavonoids

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have been found in a few animals, but they have all been of dietary origin.

Structural Similarities Between Key Steroid Metabolizing Enzymes and Flavonoid Metabolizing Enzymes and Proteins in Bacteria and Plants

Before we entered into studies on the role of nutrition and phytoestrogens in human health, we had recognized the important role of intestinal microflora and the composition of the diet in steroid hormone metabolism (18–20). When the mammalian lignans were discovered and found to be bacterial metabolites of plant precursors (3, 5, 21, 22), the importance of the intestinal bacteria not only for hormone metabolism but also for the production from plant precursors of important compounds with interesting biological activities became apparent. Michael Baker's presentation of his work dealing with the remarkable structural similarities between 17β-hydroxysteroid dehydrogenase, 11β-hydroxysteroid dehydrogenase, and 3α,20β-hydroxysteroid dehydrogenase, and some important proteins and enzymes in plants and soil bacteria (23–28), some of them living in symbiosis with alfalfa, soybeans, and other legumes (29–31), convinced me about the essential role that bacteria may have in mediating important signals from our environment. This symbiotic living is to the benefit of both the bacteria and the plant and why could it not be so also in the human body?

Gut Bacteria as Mediators of Plant Messages

It became obvious that our intestinal microflora may be even more important with regard to prevention of cancer than we previously had believed, despite that it was quite clear that gut bacteria mediate the effect of nutrition on hormone metabolism. It is interesting that similar functions in reproduction of plants are shown by both flavonoids and steroids, and they can act together (32–34). This implies that flavonoids, including isoflavonoids and similar compounds like lignans, may have actions in the human organism alone or together with steroids.

Unicellular organisms like bacteria are built to be able to take messages from the outside world in the form of lipid soluble compounds. The unicellular organisms use these compounds as signal substances, nutritional factors, or they can modify the compounds and send the signals to other organisms in the form of new molecules. We have not in the past understood the very important role played by our bacteria in the intestine because bacteria take up steroids (to a great extent deriving from bile), flavonoids, and lignans, use them themselves or modify them and secrete the modified products into the intestinal lumen so that the new compounds may be absorbed. Due to the fact that the bacteria have this property to take up and modify chemical structures, they are particularly useful in symbiotic living. Therefore bacteria live in symbiosis with plants, and our intestinal microflora are no exception. However, we must remember that antibiotics cause great disturbances in the life of these bacteria. A correct bacterial balance is probably very important, and diet is the way by which this balance must be achieved. Bacteria, having doubling times of very short duration (*E. coli* about 20 min), are probably able to adapt rather rapidly to dietary changes. A wrong diet yields an intestinal microflora with high activities of enzymes having negative effects on our health. Examples include high β-glucuronidase, azoreductase, and nitroreductase concentrations as a result of a high-meat diet as well as high concentrations of enzymes converting primary bile acids to more toxic secondary ones (35, 36).

Figure 1 shows a simplified scheme of the interaction between phytoestrogens, flavonoids, steroids, and soil or gut bacteria resulting in new messengers that then act on the plants and animals or man, respectively. The steroids, flavonoids, and lignans are consumed or reach the gut bacteria in animals and man by biliary secretion of their metabolites (18, 37, 38). Without the glycosidases in the gut, we would not be able to utilize the phytoestrogens and flavonoids because they cannot be absorbed without hydrolysis of their glycosidic bond (39–42). Germ-free rats excrete flavonoid glycosides unchanged in the feces (43). Similarly hydrolysis is needed for the biliary steroid conjugates (18) and also most likely for flavonoid and lignan conjugates before they can be reabsorbed. Figure 2 shows a more complicated scheme indicating that these new bacterial metabolites of phytoestrogens have been shown to inhibit very important steroid enzymes occurring in man, like the aromatase (44–47), the 17β-hydroxysteroid dehydrogenase type I (48), the β-hydroxysteroid dehydrogenase (49), and the 5α-reductase (50, 51), but they may act on many other enzymes. In this sense the phytoestrogens are very similar to steroids.

Figure 3 depicts how the flavonoids and phytoestrogens could affect estrogen metabolism in the gut, by inhibiting or stimulating the hydrolysis of biliary conjugates (52) or by inhibiting the formation of estradiol from estrone, the biologically most active estrogen. It has been shown that biliary estrone is reduced to estradiol in the gut (19), and this means a 10-fold biological activation of the estrogen. This highly active estrogen can then be reabsorbed (18). It is important to emphasize that we know practically nothing about the interactions between phytoestrogens and steroids in the gut, and this could be an important area of research in the future.

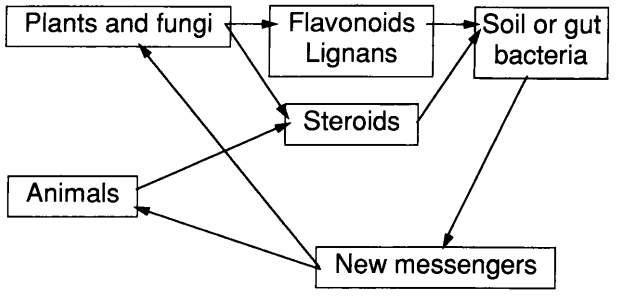

Figure 1. Simplified scheme showing the interaction between plants and fungi, soil or gut bacteria and animals.

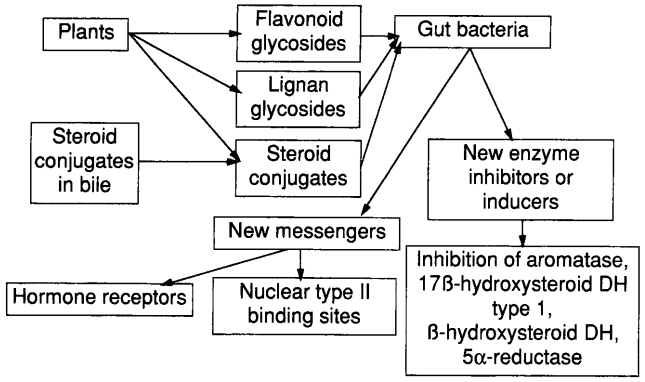

Figure 2. Scheme showing the interaction between plants, gut bacteria and man. Gut bacteria convert flavonoid, lignan, and steroid conjugates to new messengers interacting with hormone receptors or nuclear estrogen type II binding sites or inhibiting important steroid biosynthetic enzymes (aromatase. 17β-hydroxysteroid dehydrogenase type 1, β-hydroxysteroid dehydrogenase and 5α-reductase) or acting on liver enzymes (not shown).

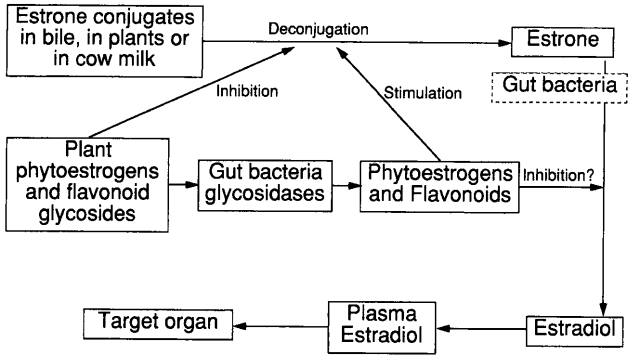

Figure 3. Hypothetical scheme showing interaction between human estrogens, plant phytoestrogens and flavonoids and gut microflora.

The intestinal bacteria cause the transformation of primary bile acids to more toxic secondary ones. They are also responsible for the formation of bile acid esters, (saponifiable bile acids) (53) that probably are less toxic than the original free bile acid as demonstrated for the aminoconjugates. Colon cancer patients and subjects who consume a low-fiber diet have a very low percentage of esterified bile acids (around 10%); omnivores on a normal diet have around 25%; and vegeterians 30%–80% (54).

The Hypothesis

The protection exerted against Western diseases by intake of plant food is to a significant extent mediated by our intestinal bacteria that 1–2 billion years ago obtained their flexible capabilities to handle and further refine phytoestrogens, including lignans, and flavonoids to cancer-protective compounds. The bisphenolic structure gives these compounds exceptional stability, and their structure and molecular weights make them particularly suitable for passing cell membranes and for interaction with enzymes and other proteins, like receptors.

In addition, if a proper diet is consumed, the intestinal bacteria have other beneficial effects preventing cancer. These factors will not be included in this discussion.

Phytoestrogens and Cancer Prevention

In plants the increasing size of the genome during evolution has been devoted to the synthesis of numerous very diverse compounds, like flavonoids and lignans with sensitive and exceedingly specific roles in the plant, but in animals it has resulted in a very complex regulatory hormonal system and behavioral pattern. The latter development has needed compounds with specific target organs. Evolution has caused only slight alterations of the steroid nucleus, but the development has occurred with regard to very specific receptors for steroids.

Plants are biochemically more sophisticated than animals, and they have developed an extremely complicated protective system, often highly specific for different types of plants and their predators (55). Because the animal organism does not have such a sophisticated biochemical protection system, it seems that animals, in order to protect themselves against diseases including infections, cancer, and coronary heart disease, may, in addition to their cellular and immunological defense system, need a supply of protective compounds from plants. In this process the intestinal microflora plays a fundamental role because these bacteria learned during the very early stages of evolution to take up signal substances like flavonoids and modify them for their own purpose and for the benefit of their plant hosts. Our intestinal microflora can in this way provide us with essential modified structures that we are not ourselves able to synthesize to keep us as a host in good health as long as possible. However, it must be kept in mind that the intestinal microflora may also produce compounds with negative effects on our body. These negative effects can many times be eliminated by a proper diet. The composition of the intestinal bacterial microflora depends on host physiology, microbial interaction, and environmental influence (mainly diet).

One of the main differences between flavonoids and lignans on one hand and steroids on the other is that the steroids act mainly *via* receptors, but phytoestrogens do not to our present knowledge have any specific receptors in animal or man. However, their action in animal and man may to some degree be mediated by steroid receptors. The anticancer effects seem to be mainly the results of interaction with other proteins than receptors (Table I). Flavonoids bind to many enzymes and affect enzyme activity. Root flavonoids of leguminosae act on the genome of symbiotic *Rhizobium* bacteria stimulating the nodulation gene (25). Nongenomic effects, as found also for steroids, occur (e.g., the inhibition of the aromatase enzyme (Table I)). Another possible mode of action is the inhibition of the conversion of estrone to estradiol by the 17β-hydroxysteroid dehydrogenase type 1 (48). Binding to the nuclear type II estrogen binding site abundant in many types of cancer cells and competing with estradiol may be one mode of action (56–

Table I. Anticancer Effects of Flavonoids and Lignans

Suggested mechanisms of anticancer effects	References
Inhibition of proliferation of solid tumor cells	(62)
Differentiation of leukemic cells	(62)
Inhibition of steroid enzymes	
Inhibition of aromatase (estrogen synthetase)	(44–47, 65)
Inhibition of 17β-hydroxysteroid dehydrogenase type 1	(48, 50)
Inhibition of 5α-reductase	(50)
Inhibition of β-hydroxysteroid dehydrogenases	(49)
Inhibition of tyrosine and other kinases	(62)
Decrease of sensitivity of breast cells to toxic compounds before puberty	(66, 67)
Stimulation of production of sex hormone binding globulin (SHBG)	(60, 68)
Binding to estrogen nuclear type-II binding sites	(57, 59, 68)
Prolongation of menstrual cycle	(69)
Inhibition of angiogenesis and tumor invasion	(70, 71)
Antioxidative effects	(72)

59) although this mechanism will remain very hypothetical until this binding site has been identified. Recently we found evidence suggesting that genistein stabilizes the sex hormone binding globulin (SHBG) mRNA in the same way as estradiol and in this way increases production of SHBG in HepG2 human liver cancer cells in culture (60). Other effects are seen in Table I. Thus, it seems that plant compounds other than vitamins may have essential roles in our body.

Flavonoids and lignans are phytoalexins, and they provide for the plants protection against viruses, fungi, bacteria, and insects. These effects may be important for the local protection of the gut against bacteria, viruses, and fungi. Antibiotics will decrease the hydrolysis of the glycosides by killing the glycosidase-producing bacteria and because the free aglycone usually is more active than the glycoside, the negative effects of antibiotics in the form of pathological growth of pathogenic bacteria, viruses, or fungi may be caused by elimination of these protective compounds. The effect of antibiotics on intestinal deconjugating enzymes has been well demonstrated for steroids (18, 19). Numerous studies show that the phytoestrogens inhibit cancer growth, stimulate differentiation, and have antioxidative properties (Table I) (61, 62).

Furthermore, epidemiologic evidence strongly supports the view that isoflavonoids and lignans are cancer protective. This will not be further discussed here as this topic was recently reviewed (61).

Because Japanese men have high levels of both isoflavonoids and lignans in the free + sulfate fraction in plasma (despite low values of lignans in urine) and very low incidence of hormone-dependent cancer (and colon cancer), we suggest that the sulfates comprising 5%–20% of total plasma phytoestrogen concentration (12, 63) may be biologically active, as is the case for estrone sulfate (64). Figure 4 shows some hypothetical mechanisms by which the phytoestrogens could reduce the intracellular production of estrogens first by inhibiting the conversion of androgens to estrogens (lignans inhibit the aromatase enzyme) and second by converting estrone to estradiol (inhibition by genistein of 17β-hydroxysteroid dehydrogenase type 1) (Table I). Phytoestrogens also reduce the activity of the 5α-reductase and may in this way prevent prostatic cancer (Table I). Furthermore, these compounds may form inactive or weakly active complexes with the estrogen receptor that competes with the estradiol-receptor complex for binding to the genome. All these mechanisms would theoretically lead to lower activity of biologically active sex hormones in target organs.

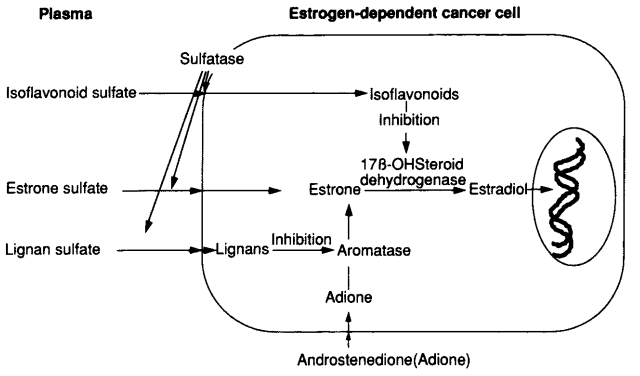

Figure 4. A hypothetical scheme showing interaction between isoflavonoids and lignans with intracellular biosynthesis of estradiol from estrone sulfate in an estrogen-dependent malignant cell.

Conclusion

It is concluded that for cancer prevention we need an intact well-functioning gastrointestinal microflora and a good supply of plant-derived protective compounds like vitamins, isoflavonoids, lignans, and maybe other types of flavonoids. This supply is ensured by consuming particularly soy (and rice; complex carbohydrates are important) and/or whole-grain products as well as various berries, seeds, vegetables, and fruits.

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