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Original Article

Comparative Study of Concentration of Isoflavones and Lignans in Plasma and Prostatic Tissues of Normal Control and Benign Prostatic Hyperplasia

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Objective: Isoflavones and lignans are phytoestrogens that have recently gained interest as dietary factors related to prostatic diseases. However, no data on the concentrations in prostate tissue in humans is available. Therefore, the concentrations of isoflavones and lignans in plasma and prostatic tissues according to the prostate volume were compared to determine their possible effect on the benign prostatic growth.

Methods: Fasting plasma and prostatic tissue specimens were acquired from 25 men over 50 years of age with similar normal dietary habits and no previous history of drug intake that could affect the isoflavones and lignans levels. The tissue was acquired either during a transurethral resection of the prostate in 15 patients with benign prostatic hyperplasia (BPH) with prostate volume over 40 ml or during a radical cystoprostatectomy in 10 patients with bladder cancer with a prostate volume <25 ml, who were used as the controls. Quantitative analysis of the isoflavones, specifically equol, daidzein and genistein and lignans, particularly enterodiol and enterolactone, was performed by gas chromatography-mass spectrometry.

Results: The mean prostatic concentrations of enterodiol, enterolactone, equol and daidzein in the BPH and the control groups were similar. However, the mean prostatic concentration of genistein was significantly lower in the BPH group than in the control group (65.43 ± 17.05 vs 86.96 ± 37.75 ng/ml, respectively, p=0.032). The plasma concentration of isoflavones and lignans in the two groups were comparable.

Conclusion: Isoflavones, but not lignans, have some influence the benign prostatic growth, and the prostatic concentration of genistein possibly has the closest association among them. More studies to further clarify the roles and mechanisms of isoflavone action on BPH including pharmacokinetic studies are recommended.

Key Words: Isoflavone, lignan, genistein, BPH

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a chronic disease affecting a significant portion of the aged male population in all parts of the world. It is generally accepted that BPH is less common in Asians than in Western countries. However, more recent reports have suggested that the prevalence of BPH or prostatism is increasing in Asian countries and may be equal to that of contemporary Western countries.[1] This trend may be more pronounced in urban inhabitants than in the rural inhabitants stressing the important role of diet in BPH pathogenesis.[2] The importance of diet in the disease process is not a new concept, as environmental factors, including the diet, have long been associated with a lower mortality rate by certain cancers in the Asian population than in Western countries as illustrated by Haenzel & Kurihara.[3] Key dietary compounds that are held to be responsible are isoflavones and lignans. They are diphenolic compounds classified as phytoestrogens due to their structural similarity to estrogen and their weak estrogenic action. Isoflavones are abundant in soy, the principal source of protein in the traditional Asian diet. Their concentrations in urine and blood have been shown to be high in Japanese men and women consuming a traditional Japanese diet.[4,5] Lignans are also abundant in cereals, grains, fruits and vegetables, with their

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principal source being linseed. The mechanisms of the actions of these phytoestrogens vary, such as the estrogenic effect,[6] the inhibition of enzyme activity such as 5α-reductase,[7] aromatase,[8] tyrosine-specific protein kinase,[9] the inhibition of angiogenesis,[10] and etc.. All of these actions result in an antineoplastic effect. However, some mechanisms, including the 5α-reductase inhibitory action and the inhibition of angiogenesis can also influence benign hyperplastic growth of the prostate.

The geographic difference clearly divides the East and West into the regions with high and low isoflavone consumption. However, the different isoflavone consumption levels cannot be directly translated into the difference in the prevalence of BPH. More important than the individual's dietary habit, is the actual bioavailability of isoflavones in the target organ, which must be reflected in the isoflavone concentrations in the prostatic tissue. This is because of a size variation between individuals even within a relatively homogenous population. In order to link a possible relationship between prostatic hyperplasia and the various phytoestrogens, including isoflavones, a direct measurement of the prostatic phytoestrogen concentration was made with concomitant plasma concentration. In addition, the possible relationship with the prostate volume was investigated.

MATERIALS AND METHODS

Patient selection

The candidates for this study were selected from male patients 50 years or older, who visited the outpatient department for prostatism, and who subsequently underwent a series of examinations to confirm their prostate related symptoms. In addition, people who had lived for more than 10 years in an urban area were enrolled. Similar dietary habits were assumed for all patients. The exclusion criteria included those with a previous history of drug intake that could affect the isoflavone and lignin concentration, those in poor general health, those who had a period of dietary restrictions within 3 months, those with any accompanying illness that could affect the diet such as diabetes mellitus or chronic renal failure, and those suspected of harboring prostate cancer. The prostate volume was measured with transrectal ultrasonography (B&K Medical, Panther 2002). Briefly, the maximum length, width and height of the prostate were measured and an integrated volumetric program automatically calculated the volume using the formula, volume = width $\times$ length $\times$ height $\times$ 0.5236. Men with a prostate volume between 25 ml and 40 ml were excluded from this study in order to emphasize the difference.

Caution was taken not to give any suggestion to the possible influence of dietary phytoestrogen on the prostatic disease, until the decision was made to treat the patient non-surgically.

Collection of samples

Fifteen patients among the candidates underwent a transurethral resection of the prostate (TURP). Five to ten chips of the inner prostatic tissue were collected immediately collected and stored at -153℃ in a liquid nitrogen tank until required. Afterwards, all 15 patients were pathologically diagnosed as having BPH.

As for the control transitional zone, the prostates from 10 radical cystectomy patients were used. Male patients undergoing a radical cystectomy for invasive bladder cancer had prostate volume measured with transrectal ultrasonography prior to the operation, and only those with a prostate volume $\leq$ 25 ml were selected. After the operation, a pathologist collected the specimen from the transition zone. A portion of the specimen was examined microscopically prior to the analysis to exclude the existence of any malignancy. After confirming that every patient had eaten, the non-fasting blood samples were collected in a heparinized tube and the plasma was stored at -20℃ until required.

Analytical method

Quantitative analysis for three isoflavones, equol, daidzein and genistein, and two lignans, enterodiol and enterolactone was done. The method used was a modification of a gas chromatography-mass spectrometry (GC-MS) method

published by Adlercreutz et al.[11] In brief, 0.5 mg *d2*-estradiol was added as an internal standard to the plasma and the mixture was dried in a rotary evaporator. To carry out the enzyme hydrolysis, the residue was then dissolved in 1 ml of an acetate buffer (0.2 N, pH 5.0) containing 50 μl of glucuronidase/arylsulfatase (from Helix Pomatia) and ascorbic acid (1 mg/ml). The sample was incubated overnight at 37℃. After hydrolysis, 100 mg of potassium carbonate was added to adjust the pH to 9.0. The mixture was extracted with 5 ml of ethylacetate and the organic layer was dried. The residue was dried in a vacuum desiccator over P_2O_5-KOH for 15 min, followed by derivatization with 50 μl of the reagent mixture (N-methyl-N-trimethylsilyltrifluoroacetamide/trimethylchlorosilane, 100 : 1 volume ratio) at 60℃, for 30 min. After heating, 2 μl aliquots were injected into the GC-MS (Hewlett-Packard 5989 Mass Engine) by an autosampler.

For the prostatic tissue, 200 mg of the specimen was homogenized with 0.5 ml acetonitrile. After centrifugation, the supernatant was transferred to another tube by decantation. 0.5 mg *d2*-estradiol was added to the supernatant as an internal standard and the same steps were repeated as for the plasma.

Statistical method

An unpaired student t-test was used to compare the mean isoflavone and lignan concentrations between the groups. A p value < 0.05 was considered statistically significant.

RESULTS

The mean prostate volumes of the BPH and the control group were 62.8 (43 - 97) ml and 21.7 (18 - 25) ml, respectively. The mean genistein concentration was highest in both the plasma and the prostate.

The enterodiol, enterolactone, equol, daidzein, and genistein concentrations (ng/ml) in plasma of the BPH and the control groups (mean ± SE) were 3.30 ± 0.52, 8.67 ± 1.38, 27.73 ± 7.68, 97.87 ± 16.92, 187.62 ± 20.01, and 2.26 ± 0.36, 8.17 ± 1.94, 13.68 ± 5.56, 80.35 ± 12.50, 155.08 ± 31.63, respectively. There was no significant difference between the two groups (p=0.053, 0.416, 0.075, 0.207, and 0.191 in order) (Fig. 1).

The enterodiol, enterolactone, equol, daidzein, and genistein concentrations (ng/ml) in the prostate of the BPH and the control group (mean ± SE) were 6.58 ± 1.44, 16.26 ± 3.54, 13.65 ± 3.28, 42.44 ± 5.70, 65.43 ± 4.40, and 4.97 ± 1.15, 27.78 ± 7.50, 11.81 ± 2.85, 49.46 ± 6.59, 86.96 ± 11.94, respectively (p=0.215, 0.068, 0.348, 0.217 and 0.032 in order) (Fig. 2).

The genistein concentration (ng/ml) in the BPH group was significantly lower compared to the control group.

DISCUSSION

The abundance of certain dietary ingredients, as a source of isoflavones such as soy, and grains, cereals, linseeds etc. as lignan sources has been considered as an important dietary characteristic that might be associated with the lower incidence of prostate cancer and BPH in Asian countries than their Western counterparts. In this respect, several studies have measured the bodily concentrations of phytoestrogens in different ethnic groups. According to Adlercreutz et al.,[4,5] the concentrations of isoflavones in the urine and the blood are high in Japanese men and women consuming a traditional Japanese diet, which is rich in soy products. Moreover, the concentrations

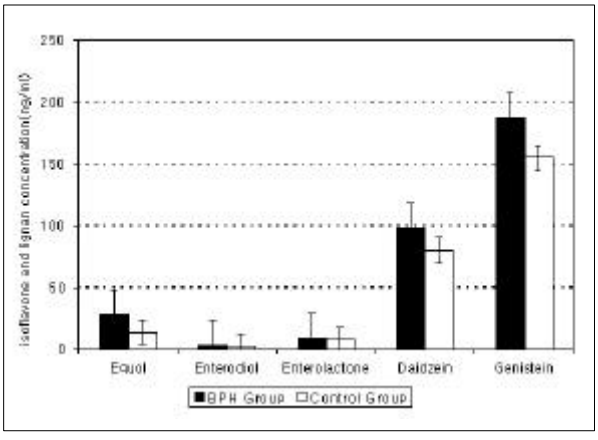

Fig. 1. Isoflavone and lignan concentration in plasma according to the prostate volume (ng/ml). No statistically significant differences compared to the BPH group.

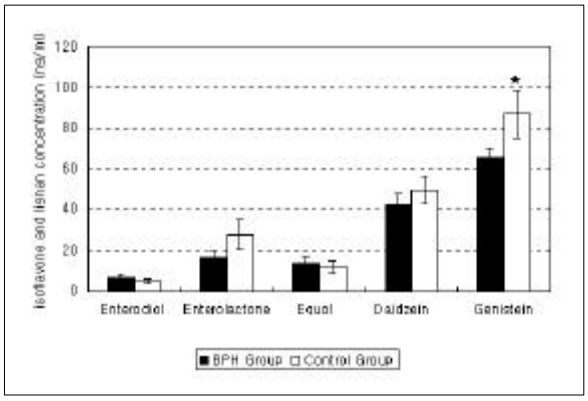

Fig 2. Isoflavone and lignan concentration in prostate tissues according to the prostate volume (ng/ml) *$p <$ 0.05 compared to BPH group.

of isoflavones were higher in Asian men than in Western men in a study comparing the phytoestrogen concentration in blood plasma and prostatic fluid from different ethnic groups.[12] Among the different isoflavones, genistein is the most studied, and mechanistic studies have revealed the inhibitory effects of genistein and other isoflavones on the abnormal proliferation of cells. These mechanisms include the inhibition of various enzymes such as 5α-reductase,[7] aromatase[8] and tyrosine-specific protein kinase activty[9] and angiogenesis inhibition.[10] Although some reports suggest that soy-containing foods reduce risk of the prostate cancer, there is a lack of clinical evidence that clearly demonstrates any difference in the development of prostate cancer.[13,14]

To our knowledge, this study is the first to show the presence of phytoestrogens in the human prostate by direct measurements of the tissue concentration. Among the different phytoestrogens, genistein and daidzein were the most concentrated, followed by enterolactone, equol and enterodiol. Equol is the final metabolite of daidzein and its concentration is probably not only a function of the daidzein concentration but is also dependent on the individual intestinal microflora. In an earlier study by Morton et al.,[12] the concentrations of various isoflavones and lignans were measured in the plasma and prostatic fluid by GC-MS, and the values between Hong Kong, Portuguese, and British men were compared. In this study, in which genistein was omitted, the mean isoflavones, daidzein and equol concentration, were highest in the prostatic fluid from Hong Kong men. The mean daidzein, equol, enterolactone, and enterodiol concentration in the prostatic fluid of Hong Kong men were 70, 8.5, 31, and 1.6 ng/ml, respectively, which were very similar to the results of this study. Furthermore, the values in the prostatic fluid were consistently higher than in the plasma, which suggests the ability of the prostate to concentrate these phytoestrogens. The plasma concentration of isoflavones also showed dose-dependent changes. Izumi et al.[15] measured the plasma isoflavone concentration after administering various forms of isoflavone at low (30 mg) and high doses (450 mg) to volunteers. In the case of the aglycone form, the highest plasma levels were reached by 2 hrs and 4 hrs after administering low and high doses, respectively. After 24 hrs, the level dropped to 1/7 of the peak level. In this study, the plasma concentrations of isoflavones and lignans in the two groups were similar. This may in fact be interpreted as indirect evidence that the two groups did not have overtly different affinity for isoflavones and lignans in their diet.

Although our study patients were all recruited from a homogenous population of the same ethnic origin and urban milieu, the phytoestrogens concentrations in the prostate showed considerable variation between individuals. These differences might simply reflect the subtle differences in diet, a less exaggerated manifestation than that shown between different populations or different rates of absorption and metabolism of the phytoestrogens. These issues were outside the boundary of this study due to lack of the individual data on individual dietary habits, and the pharmacokinetic profiles, However, considering the similarity of the plasma concentrations between the two groups, different rates of absorption and metabolism between each individual appears to be a more plausible explanation for the different prostatic concentrations. The pharmacokinetics of isoflavones in plasma and urine are well documented in humans through their oral challenge and subsequent detection in urine and plasma.[15-17] In a study investigating the tissue distribution of genistein in rats exposed at different doses in utero, through maternal milk, and as adults, significant dose-dependent increases in the total

genistein concentration in the prostate as well as other endocrine-responsive tissues were shown.[18] Unfortunately, due to the inaccessibility of the prostate to repeated collection as with urine or blood, the pharmacokinetics and direct evidence of a dose-dependency of isoflavone was not shown in the human prostate.

The results in this study revealed a significantly lower prostatic concentration of genistein in men with BPH than in men with a normal prostate. However, the lignan concentrations in the two groups were similar. However, the significance of this difference in the prostatic concentration of genistein according to the prostate volume is still unknown. In the field of BPH, studies examining the possible role played by the phytoestrogens are extremely limited. Geller et al.[19] were unique in suggesting a possible growth inhibitory function of genistein in BPH from a histoculture model. However, no clinical results, which indicate that the consumption of isoflavones reduces the development of BPH, have been reported. Recent experimental studies suggest that the expression of the vasculoendothelial growth factor (VEGF) positively correlates with the size of the prostate and that the decreased transforming growth factor-β (TGF-β) level leads to an abnormal proliferation of prostatic cells.[20,21] Moreover, there is experimental evidence showing that genistein inhibits angiogenesis and increases the TGF-β level.[22,23] Overall, these observations suggest that genistein might influence the growth regulatory mechanism in BPH. However, further studies to confirm this hypothesis are needed.

Using TURP chips to analyze the isoflavones and lignans levels raises the concern for the adequacy of the specimen when compared to radical prostatectomy specimens. This is because these chips are exposed to diathermy and are immersed for long periods of time in an irrigation fluid. However, TURP specimens have been used in various studies in conjunction with open prostatectomy specimens including PCR[24] and immunohistochemical studies,[25] without any important compromise in the results. In a study on the human growth hormone, a human placental lactogen and receptor assay using TURP specimens, Untergasser et al.[26] reported no difference between the fresh tissue and TURP specimen. Therefore, it is presumed that steroidal hormones, including phytoestrogens, are not influenced by the nature of the specimen used for their detection. Although there exist experimental limits imposed by technical difficulties in obtaining open resected prostate specimens in all patients, studies that compare the results according to the different methods of tissue acquisition will be needed. A recent animal study result demonstrated a lobe specificity of the inflammatory reaction of the prostate in response to isoflavone.[27] Similarly, a different response in humans between the BPH-prone transition zone and other zones could be anticipated, thus further studies should clarify this issue.

These results are not sufficient to conclude that isoflavone is involved in the hyperplastic growth of the prostate. However, as the diverse biologic actions of phytoestrogen are concentration dependent, the differences in the prostatic genistein concentration demonstrated in this study might suggest a relationship between the hyperplastic growth of the prostate and phytoestrogen. To clarify this possibility, further studies focusing on the influence of the active metabolites from the isoflavones and lignans in the human prostate, and the pharmacodynamics at the receptor level should be undertaken.

These results suggest that isoflavones, but not lignans, somehow influence the benign prostatic growth, and it is believed that the prostatic concentration of genistein has the closest association among them. More studies aimed at clarifying the roles and mechanisms of the actions of isoflavones and lignans on BPH including pharmacokinetic studies are needed.

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