Yonsei Medical Journal
Vol. 42, No. 4, pp. 395 - 404, 2001

Original Article

Development of Rat Prostatitis Model by Oral Administration of Isoflavone and Its Characteristics

Soo-Mee Kwon[1], Sun-Il Kim[1], Dong-Chan Chun[1], Nam-Hoon Cho[2], Bong-Chul Chung[3], Byung-Wha Park[4], and Sung-Joon Hong[1]

Departments of [1]Urology and [2]Pathology, Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul;
[3]Bioanalysis and Biotransformation Research Center, Korea Institute of Science and [4]Technology and Specialty Chemicals & Biotech Division, Pacific Corporation, Seoul, Korea.

Inflammation of the prostate can be induced experimentally in rats by the subcutaneous administration of estrogen. However, it is usually achieved at the price of some alteration in the sex steroid hormone balance and morphological changes in the prostate. In this study, a soy-extracted isoflavone mixture with weak estrogenic activity was administered orally in an attempt to induce prostatitis in a more physiologic way and to characterize the inflammation induced.

A total of 36 male Sprague-Dawley rats, 8 weeks old, were divided into 2 groups. The control group was fed with only an AIN-76A diet containing no detectable phytoestrogen and the experimental group was fed with AIN-76A and a soy-extracted isoflavone mixture (genistein 60.0% and daidzein 19.6%), 300mg/kg body weight for 9 weeks. The sequential body weight and prostate weight at necropsy were measured. A histologic examination and histomorphometry assessed the changes in the prostate. The serum concentrations of testosterone and dihydrotestosterone were measured to estimate the effects on the androgen level. Intraprostatic concentrations of genistein and daidzein were measured by gas chromatography/mass spectroscopy (GC/MS).

While no sign of prostate inflammation was apparent in the control group, severe inflammatory changes in the stroma, increased epithelial detachment and inflammatory exudates within the glandular lumen of the dorsolateral prostate were observed in more than 80%(15/18) of the experimental group. However, there was no significant difference in the ventral prostate between the two groups. The daidzein and genistein concentrations in both the lateral and ventral prostates were significantly higher in the experimental group than in the control group where no isoflavone was detectable. In addition, the concentrations were much higher in the dorsolateral than in the ventral prostate. Although the body weight gain was not consistent in the experimental group, there were no significant differences in the prostate weight and serum androgen level between groups.

In summary, when a soy-extracted genistein and daidzein-rich isoflavone mixture was administered orally into rats, prostatic inflammation with characteristic lobe specificity developed. The present method of inducing prostatitis seems to be a more physiologic than an estrogen-induced experimental model, and sequential pharmacokinetic studies might help in establishing this model as a more valuable tool in assisting future research in this field.

Key Words: Rat prostate, prostatitis, genistein, daidzein, estrogen

INTRODUCTION

Prostatitis is a very common disease affecting approximately 20 % of all adult males. However, its precise pathogenesis is still unclear. Apart from infective agents, non-infectious factors such as endogenous chemicals, immunologic factors, obstruction, trauma and autoimmune factors have been suggested as possible causes of prostatitis.[1] In rodent experiments, spontaneous nonbacterial prostatitis has been reported to occur and has been associated with a genetic background, advancing age and a hormonal imbalance. The latter has been demonstrated with various exogenous estrogen-induced prostatitis models.[2,3] However, the classic induction method, whereby estrogen is administered subcutaneously, is usually accom-

Received May 25, 2001
Accepted July 14, 2001

Reprint address: request to Dr. Sung Joon Hong, Department of Urology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea. Tel: 82-2-361-5806, Fax: 82-2-312-2538, E-mail: sjhong346@yumc.yonsei.ac.kr

panied by morphological and functional alterations such as prostatic atrophy and a reduction in secretory activity. Therefore, the estrogen-induced rat prostatitis model is not an appropriate experimental model.

Isoflavone, a diphenolic phytoestrogen, is an essential dietary component in many Asian populations, being ingested throughout life in the form of various soy-based foods. Many actions of isoflavone have been discovered which are related to the inhibition of cellular proliferation, but its principal action is related to its weak estrogenic activity.[4] therefore, it appears that chronic consumption of a diet with estrogenic activity beyond a certain amount, notably an isoflavone- rich diet, would exert an estrogenic effect on the human body. In male rats under the same feeding conditions, the prostate is the organ most concentrated with aglycone or total genistein,[5] and thus it appears to be the most important target organ in studying the effects of isoflavone in male. Overall, the high prevalence of prostatitis in men and evidence from animal studies suggests that the long-term consumption of estrogenic isoflavone might affect the normal prostate in one way or the other.

However, contrary to the consistent changes observed with estrogen administration, the results with isoflavone are often contradictory depending on routes of administration or dosage.[6-9] Furthermore, no study has been reported indicating that orally administered isoflavone can could lead to a prostatic inflammation.

In the present study, a high concentration of a soy-extracted genistein and daidzein mixture was administered orally to male Sprague-Dawley rats for 9 weeks. The body and prostate weight, histomorphometric changes of the prostate, the intraprostatic concentration of isoflavone and the serum concentration of androgen were assessed in order to determine whether orally administered isoflavone induces prostatitis and to define the characteristics of the inflammation induced.

MATERIALS AND METHODS

Extraction and analysis of isoflavone

The isoflavone extract was prepared as follows: Soybeans were placed into a tank with tap water at a ratio of 100g per liter and left at room temperature for 4 hours. They were then ground with a mixer and filtered. The resultant suspension of approximately 10% Brix was heated to 93-95℃ for 30 minutes and left to cool to 85℃. The protein was precipitated by adding $MgCl_2$ and 5% perlite was added to the supernatant and filtered. The filtered solution was applied to a column (5cm × 60 cm), packed with 500 ml of washed resin (XAD-7, Rohm and Haas Co., Frankfurt, Germany). The column was was subsequently eluted successively with 1000 ml of 1 N NaOH, 1000 ml of ionized water, 1000 ml of 30% ethanol and finally with 1000 ml of 70% ethanol. The recovered eluate was concentrated to 100 ml, then distilled water was added to obtain a 10% solid phase. It was sterilized at 121℃ for 15 minutes and cooled to 30℃. 5% of the *Aspergillus* species was added to the sample (vol:vol) and incubated at 30℃ for 72 hours. It was then centrifuged at 5,000 rpm and the sediment was washed 3 times with distilled water. After centrifugation, the sediment was mixed with 95% ethanol, heated to 80℃ then washed with ionized water to remove the ethanol. After filtration, the sample was concentrated and freeze-dried.

The isoflavone concentrate was analyzed with high performance liquid chromatography (HPLC) (Hitachi L-4200 UV/VIS detector, Tokyo, Japan). A mixture of 0.1% acetic acid in pure water and acetonitrile at a ratio of 70:30 was used as the solvent. The column packed with mightysil RP-18 GP 250 - 4.6 (Kanto Chemicals, Tokyo, Japan) was used with a flow rate of 1.0 ml/min and the detection wavelength was 254 nm.

The final components of the isoflavone concentrate were genistein 60.0%, daidzein 19.6%, glycitein 5.0%, water 2.5%, carbohydrate 0.41%, protein 1.48%, lipid 0.97% and other miscellaneous phytocomponents (lecithin and soy saponin) 10.04%.

Animals and diet

Thirty-six Sprague-Dawley male rats, 8 weeks old, were randomly grouped into experimental and control groups. The rats were fed with a

soy-free, casein-based AIN-76A(ICN Biomedicals, Aurora, Ohio, USA) diet in order to eliminate the potential influence of the phytoestrogens present in ordinary rodent diet. The rats were cared for in a specific pathogen-free environment. A controlled temperature (22℃) and humidity (55%) were used. The water and diet were supplied *ad libitum*. The care and use of laboratory animals in this experiment adhered to the Guidelines and Regulations for Use and Care of Animals in Yonsei University College of Medicine.

Treatment

In the experimental group, 300 mg/kg body weight of isoflavone concentrate was dissolved in 2 ml of distilled water containing 0.5% Tween-80 and 0.4% methylcellulose and administered through a stomach gavage in two divided doses daily for 9 weeks.[10] The rats in the control group were given only the vehicle. All animals tolerated the procedure well and did not show any notable adverse reaction throughout the period of administration.

At the end of the ninth week of treatment, the rats were anesthesized with an intramuscular ketamine/xylazine injection. After a lower abdominal incision, approximately 8 to 10 ml of blood was collected from the inferior vena cava and the serum was stored at -20℃ until analysis. All serum samples were analyzed within 1 month after collection. The ventral and dorsolateral prostates were carefully dissected and removed. One side of each prostate lobe was stored at -80℃ until analysis.

Measurement of body and prostate weight

The rats were weighed at the initiation of treatment and once each week thereafter. At necropsy, the prostate was separated from the adjacent tissues, The surrounding fat and capsule were removed by microdissection, and the ventral and dorsolateral prostates were weighed separately.

Histology and morphometric analysis

One side of each prostate lobe was fixed in 10% formalin and embedded in paraffin. Sections were cut at 5 ㎛ thickness, mounted on glass slides and stained with hematoxylin and eosin. The slides were examined under a light microscope and the histologic changes were compared.

Morphometric analysis of the same slides was performed by a simple-point counting method.[10] A computed digital imaging system consisting of a Sony CCD vision camera (Tokyo, Japan) mounted on an Olympus Vanox-S microscope (Tokyo, Japan), an IBM personal computer equipped with a frame-grabbing board (Matrox Electronics Systems, Dorval, Quebec, Canada) (with an auto image analyzing software (Optimas version 6.1, Media Cybernetics, Maryland, USA) and a flat computer monitor was used to facilitate the task. For each slide, images of 15 to 20 non-overlapping fields depending on the size of the specimen were captured separately at 100X magnitude, and each digitized image was saved in the computer hard disk. The prostate was divided into stromal and glandular compartments and the latter was subdivided into the epithelium and lumen. Each captured image was overlaid with a transparent 25/100-point double-square test grid attached to the monitor. If a point was superimposed over a certain tissue component, a point was scored for that particular component. 100 points were counted for each field and the area percentage, which is equivalent to volume percentage of each component, was calculated. Counting was done in triplicate.

Measurement of serum androgen level

Serum level of testosterone and dehydortestosterone (DHT) were measured by radioimmunoassay with a testosterone/dihydrotestosterone [^{3}H]assay system (Amersham pharmacia biotech, Bukinghamshire, UK). All measurements were made in triplicate.

Measurement of intraprostatic isoflavone level

Quantitative analysis for intraprostatic genistein and daidzein was done using gas chromatography/mass spectrometry (GC/MS). The prostatic tissues were homogenized by adding 0.5 ml of acetonitrile to 200 mg of the prostate. After

centrifugation, the supernatant was transferred to another tube by decantation. 0.5 ㎍ d2-estradiol (E2) was added to the specimen as the internal standard and was dried by evaporation in a rotary evaporator. To carry out enzyme hydrolysis, the residue was then dissolved in 1 ml of an acetate buffer (0.2N, pH 5.0) containing 50 ㎕ glucuronidase/arylsulfatase (from Helix Pomatia) and ascorbic acid (1 mg/ml). The sample was incubated overnight at 37℃. After hydrolysis, 100 mg 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 evaporated to dryness. The residue was dried in a vacuum desiccator over P_2O_5-KOH for 15 min, followed by derivatization with 50 ㎕ of the reagent mixture (MSTFA/TMCS, 100:1 volume ratio) at 60℃, for 30 min. After heating, 2 ㎕ aliquots were injected into a gas chromatography/ mass spectroscopy (GC/MS) (HP GC-MS system, Hewlett-Packard CO., Palo Alto, CA, USA) by an autosampler. All measurements were made in triplicate.

Statistics

Means and standard deviations were calculated for all data in both groups. An unpaired student T-test was used to compare the interval body weight change, the prostate weight, relative proportion of each component by morphometry, intraprostatic isoflavone concentrations and serum androgen levels between the two groups. A p value < 0.05 was considered significant.

RESULTS

Changes in body and prostate weight

The mean initial body weights in the control and experimental groups were 322.8 ± 21.1 g and 332.7 ± 21.5 g, respectively, which was not significantly different ($p > 0.05$). After 4 and 9 weeks of treatment with either isoflavone or the vehicle alone, the mean body weights in the control and experimental groups were 462.9 ± 23.1 g and 430.2 ± 21.5 g, 550.6 ± 37.1 g and 520.3 ± 50.6 g, respectively ($p < 0.05$). The mean body weight increases during the experiment in the control and experimental groups were 227.7 ± 35.3 g and 187.7± 39.2 g, respectively and was statistically significant ($p < 0.05$) (Table 1).

After 9 weeks of treatment with either isoflavone or the vehicle alone, the mean weights of the dorsolateral prostate in the control and experimental groups were 441.7 ± 93.7 mg and 443.3 ± 75.5 mg, respectively, which was not significantly different ($p > 0.05$). The mean ventral prostate weights were 651.7 ± 138.0 mg and 623.9 ± 134.2 mg, respectively. However, the difference was not statistically significant ($p > 0.05$) (Table 1).

Histologic and morphometric changes

Histologically, the ventral prostate of the experimental group did not show any clear difference from the control group. In the dorsolateral prostate of the experimental group, there was severe

Table 1. Changes in the Total Body and Prostate Weight in Adult Male Sprague-Dawley Rats with or without Isoflavone*

Groups[2]	No.	Body weight[1]				Prostate weight[1]	
		0 week	4 week	9 week	ΔE[3]	DLP	VP
control	18	322.8 ±21.1	462.9 ±23.1	550.6 ±37.1	227.7 ±35.3	441.7 ±93.7	651.7 ±138.0
experimental	18	332.7 ±21.5	430.2 ±21.5	520.3 ±50.6	187.7 ±39.2*	443.3 ±75.5	623.9 ±134.2

[1]Prostate weight were measured after 9 weeks administration of isoflavone mixture. The weights represent average standard deviation (S.D.).
[2]Eight weeks old rats were divided into control and experimental groups. In the experimental group, an AIN-76A diet and soy-extracted isoflavone mixture (genistein 60.0%, daidzein 19.6%) were given orally 300 mg/kg and in the control group, the AIN-76A diet and vehicle were given for 9 weeks.
[3] ΔE means the increased body weight between 0 week and 9 week.
DLP, dorsolateral prostate; VP, ventral prostate.
*$p<0.05$ by student t-test to the control side.

inflammation in the stroma and inflammatory cells and sloughed or degenerated epithelial cells were found packed in the glandular lumen (Fig. 1). The sloughed epithelial cells in the glandular lumen demonstrated various morphological features ranging from normal- appearing cells to cells with hyperchromatism and vacuolization. Most of the inflammatory cells were neutrophils and lymphocytes. However, eosinophils and macrophages were also present. These changes were observed in more than 80% (15/18) of the lateral prostates but not in the ventral prostate.

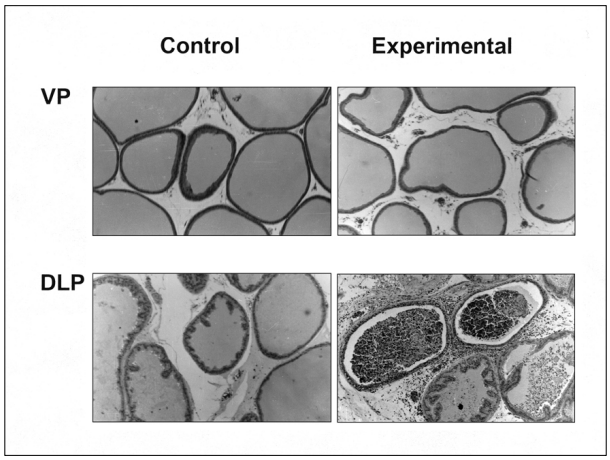

Fig. 1. Histological appearance of the ventral & dorsolateral prostate of normal adult male Sprague-Dawley rats after oral ingestion of isoflavone for 9 weeks. H & E stain. VP, ventral prostate. There is no significant difference in the histology of the experimental group compared to the control group. DLP, dorsolateral prostate. Compared with the control group, there is severe inflammation in the stroma and inflammatory cells and sloughed epithelial cells are packed in the grandular lumen of experimental group. ×60.

In the morphometric analysis of the prostate, the percentages of lumen in the dorsolateral prostate in the control and experimental groups were 37.3 ± 6.0% and 44.1 ± 7.8%, respectively, and in the ventral prostate, 41.8 ± 2.7% and 49.0 ± 8.3%, respectively, showing an increase in the experimental group, but not statistically significant ($p > 0.05$). The percentages of epithelium in the dorsolateral prostate in the control and experimental groups were 20.3 ± 2.6% and 17.7 ± 2.1%, respectively, and in the ventral prostate, 20.3 ± 2.5% and 17.8 ± 6.1%, respectively, showing decrease in the experimental group. The percentages of the stroma in the dorsolateral prostate in the control and experimental groups were 42.4 ± 4.4% and 38.2 ± 6.3%, respectively, and in the ventral prostate, 37.9 ± 1.2% and 33.2 ± 3.8%, respectively, showing decrease in the experimental group. However, the differences in the epithelium and stroma were not statistically significant ($p > 0.05$) (Table 2).

Changes in serum androgen concentration

The mean serum testosterone levels for the control and experimental groups were 2.78 ± 0.53 ng/ml and 2.53 ± 0.64 ng/ml, respectively showing a slightly higher level in the control group. However, the difference was not statistically significant ($p > 0.05$). The mean serum DHT levels showed no statistically significant difference either (0.09 ± 0.04 ng/ml in the control group, 0.07 ± 0.05 ng/ml in the experimental group) ($p > 0.05$) (Table 3).

Table 2. Changes in Percentage Area Density[1] of the Dorsolateral and Ventral Prostates after Oral Administration of Isoflavone*

Groups	No. animals	% Lumenal		% Epithelial		% Stromal	
		DLP	VP	DLP	VP	DLP	VP
control[2]	18	37.3 ± 6.0	41.8 ± 2.7	20.3 ± 2.6	20.3 ± 2.5	42.4 ± 4.4	37.9 ± 1.2
experimental[2]	18	44.1 ± 7.8	49.0 ± 8.3	17.7 ± 2.1	17.8 ± 6.1	38.2 ± 6.3	33.2 ± 3.8

[1]Percent Area Density measured after 9 weeks administration of the isoflavone mixture. The values are expressed as a mean±SD.
[2]Eight weeks old rats were divided into control and experimental groups. In the experimental group, an AIN-76A diet and soy-extracted isoflavone mixture(genistein 60.0%, daidzein 19.6%) were given orally 300 mg/kg and in the control group, a vehicle with the AIN-76A diet were given for 9 weeks.
DLP, dorsolateral prostate; VP, ventral prostate.
*$p<0.05$ by student t-test to the control side.

Table 3. Changes in the Serum Androgen Level[1] in Adult Male Sprague-Dawley Rats after Oral Administration of Isoflavone*

Groups	No.	Serum androgen (ng/ml)	
		T	DHT
control[2]	18	2.78 ± 0.53	0.09 ± 0.04
experimental[2]	18	2.53 ± 0.64	0.07 ± 0.05

[1]Serum androgen level measured after 9 weeks administration of the isoflavone mixture. The values are expressed as mean±SD.
[2]Eight weeks old rats were divided into control and experimental groups. In the experimental group, an AIN-76A diet and soy-extracted isoflavone mixture (genistein 60.0%, daidzein 19.6%) were given orally 300 mg/kg and in the control group, the AIN-76A diet and a vehicle were given for 9 weeks.
T, testosterone; DHT, dihydrotestosterone.
*$p<0.05$ by student t-test to the control side.

Intraprostatic isoflavone concentration

Genistein and daidzein were not detected in the prostate of the control group. The genistein and daidzein concentrations in the dorsolateral prostate of the experimental group were 373.4 ±157.3 pmol/g tissue and 271.0 ± 105.8 pmol/g tissue, respectively. However, in the ventral prostate, the genistein and daidzein concentration were only moderately elevated compared to the dorsolateral prostate with 87.7 ± 20.7 pmol/g tissue and 62.5 ± 40.7 pmol/g tissue, respectively. The differences between the groups were significant for both genistein and daidzein ($p < 0.05$) (Fig. 2).

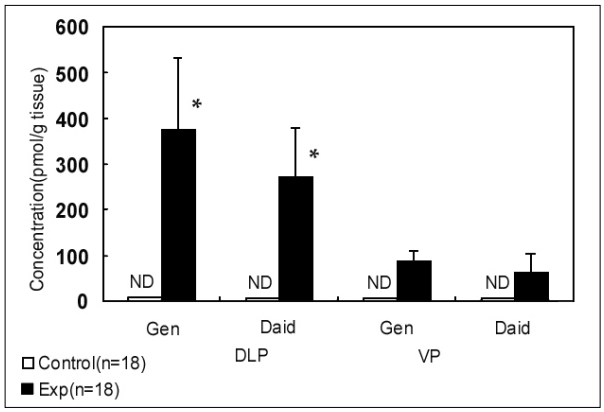

Fig. 2. Comparison of the intraprostatic concentration of genistein (Gen) and daidzein (Daid) between the control and experimental groups. The concentrations of both genistein and daidzein in the dorsolateral prostate (DLP) are significantly higher in the experimental group, whereas the concentrations in the ventral prostate (VP) are not different between the two groups. *$p < 0.05$. ND, not detected.

DISCUSSION

Isoflavone, which is a diphenolic phytoestrogen, has weak estrogenic activity and when injected subcutaneously into rats or mice, genistein can induce changes similar to rat prostatitis induced by estrogen.[8] However, it has not yet been proven whether the same changes can be induced by oral administration. These experiments showed that the oral administration of a soy-extracted genistein (200 mg/kg/day) and daidzein (66 mg/kg/day) mixture daily for 9 weeks induces prostatitis that is specifically localized to the dorsolateral prostate in more than 80% of rats.

Generally, subcutaneous administration of estrogen or synthetic genistein in rodents produces a negative feedback on the pituitary-gonadal axis with a decrease in the serum luteinizing hormone (LH) and androgen level with resultant atrophy of the prostate.[8,9] Changes in the urethroprostatic complex after subcutaneous injection of synthetic genistein are also similar to typical estrogenic effects, which are likewise dose-dependent and accompanied by endocrinologic changes and hepatic toxicity at high doses. According to Schleicher et al.[9] a subcutaneous injection of synthetic genistein at 50 mg/kg resulted in a tissue concentration of 1.89 nmol/g in the transplanted tissue, which is at least 5 times higher than the result in this study. At this dose, hepatic toxicity and a skin reaction at the injection site occurred in all animals.

In contrast to the subcutaneous injection, the results with oral administration show a com-

paratively lower estrogenic activity. Dalu et al.[7] reported that adding pure genistein in the rat diet for 3 weeks did not affect the body weight, the weight of the dorsolateral prostate and the serum testosterone level. This suggests that genistein might demonstrate estrogenic activity depending on the route and dosage. However, their results did not include any change in prostatic histology. Makela et al.[11] also reported that the short-term administration of soy did not show any estrogenic or antiestrogenic activity in the prostate.

In this study, the group given isoflavone demonstrated a significant reduction in body weight, along with an inflammation of the prostate. It is well recognized that rats treated with estrogen show reduced weight gain,[12,13] and this was shown to be associated with the inhibition of growth hormone release in the pituitary gland by estrogen,[14] or a lowering of the serum insulin-like growth factor (IGF)-I by estrogen.[15] Isoflavone also exerts a similar function by inhibiting tyrosine-specific protein kinase that which mediates the actions of the epidermal growth factor, the fibroblast growth factor and IGF-I.[16] Zhou et al.[17] also reported that isoflavone administration lowers the serum IGF-I level. Furthermore, a decreased concentration of plasma lipids and lipoproteins and an increased sensitivity to insulin was observed upon administration of soy protein, which is similar to that observed upon 17β-estradiol administration.[18] Overall, the observed decrease in body weight after ingesting isoflavone might be associated with estrogenic activity, a decrease in IGF-I concentration induced by the inhibition of tyrosine-specific protein kinase and a decrease in the plasma lipid concentration.

According to Chang et al.[5] prostate was the organ with highest tissue concentration of genistein when 15 mg of a genistein-fortified diet was given to male rats. In the present study, isoflavone administration did not result in a significant change in prostate weight, which is in concurrence with Weber et al.[19] However, the cumulative results are confusing with some reporting an increase[8] and others reporting a decrease in prostatic weight.[9] These seemingly contradictory results might be explained by the various forms of isoflavone used. Isoflavone is present in soy in the form of a β,D-glucoside conjugate. In the intestine, the aglycone form is liberated due to microbial β-glucosidase activity, which then undergoes glucuronidation in the intestinal cells and is transferred to the blood.[5] It is believed that the aglycone form is more readily absorbed and more bioavailable than the glycoside form. However, some observed only slight difference in the peak plasma concentration and no difference in the total absorption between glycoside and aglycone.[20]

The effect of isoflavone on prostatic growth is not clearly understood yet. Few of the experimental studies on of its effect on prostatic growth include inhibition of 5α-reductase activity in a benign prostatic hyperplasia tissue culture,[21] inhibition of prostatic cancer cell growth in a tissue culture,[22] and inhibition of the hypothalamus-pituitary-gonadal axis and the resultant decreased testosterone secretion leading prostatic growth inhibition in animal models.[9] The isoflavone used in the present study was extracted from soybeans, and although nearly 80% of its components are genistein and daidzein in the form of aglycone, possible effects of other minor components on the action of isoflavone could not be ruled out. The relative potency of the extracted isoflavone compared to the chemically synthesized one also remains questionable.

Concerning the changes in the circulating androgen level after isoflavone administration, Strauss et al.[8] reported a decrease in the serum and testicular testosterone level by isoflavone accompanied by a decrease in the serum and pituitary LH, suggesting that the estrogenic effect of isoflavone exerts inhibitory feedback on the hypothalamus-pituitary-gonadal axis. Other reports include no change in serum testosterone level,[7] or suggest that the decrease in the serum testosterone level reflects a secondary reaction to stress rather than the pure effect of isoflavone.[9] In the present study, no change in the serum androgen level was observed and histomorphometry revealed no significant difference in the volume percentage of glandular lumen, glandular epithelium and stroma between the two groups. Although no attempt was made to assess the status of the hypothalamus-pituitary-gonadal axis, it is presumed that these experimental condition does not place the prostate under the influence of

inhibitory feedback by isoflavone of a sufficient degree to exert a histologic change *in vivo*.

Nevertheless, previous data and this study clearly suggests that the route and dose of isoflavone are important determinants in producing prostatic inflammation and systemic estrogenic activity. Moreover, considering the reportedly protective effect of isoflavone against spontaneous prostatitis, more diverse effects could be expected to occur at various doses and administration routes.

Action limited to the prostate from our results also indicates that inducing inflammation by orally administered soy-extracted isoflavone is a more physiologic than estrogen induced method. It has an additional advantage of being from the same dietary source humans consume and may be of great value as a natural animal model in performing future research

The mechanism by which estrogen induces prostatitis is known to be first mediated by the inhibition of dopamine release at the hypothalamus, which in turn increases the production and secretion of prolactin from the pituitary gland.[23] Increased prolactin induces inflammation in the prostate in a dose dependent manner.[24] In addition, inflammation is the specifically more common in the dorsolateral prostate than in the ventral prostate. The inflammatory responses induced by an estrogen injection begin to show in the stroma 2 weeks later and further involve the lateral and the dorsal prostates in an orderly fashion, whereas only mild inflammation appears in the ventral prostate 10 weeks after the injection.[25] Recently, exposure of prostate to estrogen for short periods of time resulted in the up-regulation of proinflammatory transcripts, which continued until inflammatory cells appeared, implying that an endocrine-immune system interaction might be involved in the development of rat prostatitis.[26] It is not clear whether the inflammation induced by isoflavone is mediated by the same mechanism as with estrogen-induced inflammation. However, our results show that administering isoflavone orally demonstrates the same lobe specificity as in spontaneous prostatitis in Lewis rats as well as prostatitis induced by estrogen or subcutaneously injected genistein. Thus, it is evident that in rats, the dorsolateral prostate is more sensitive to inflammation inducers than the ventral prostate.

The more frequent inflammation in the dorsolateral prostate compared to the ventral prostate is explained by a higher sensitivity of the dorsolateral prostate to prolactin.[25] The observation that the proinflammatory effect of estradiol is blocked by bromocryptine, a dopamine agonist, implies that the effect of estradiol is mediated by prolactin.[2,24] Moreover, prolactin-like protein synthesis was demonstrated within the inflamed lateral prostate, which led us to believe that a locally produced prolactin-like protein is involved in the continuous facilitation of inflammation in the prostate.[27]

Pertaining to lobe specificity, it is recently being discovered that the estrogen receptors (ER) distribution varies in different lobes. ER beta is relatively more abundant in the prostatic epithelium, whereas ER alpha, which is abundant in the stroma, is the one thought to be linked to inflammation, and it is known to be most abundant in the lateral prostate and least in the ventral prostate.[28] In contrast, Sharma et al.[6] reported that the dietary administration of 17% soy protein inhibited spontaneous prostatitis in Louis rats. Moreover, the weak estrogen such as isoflavone can reportedly act as an antiestrogen by saturating the ER binding sites.[29] Therefore, the difference due to the dose and route of administration must be clarified in the future.

One interesting point is that the isoflavone concentrations were different in each lobe. The genistein and daidzein concentrations were significantly higher in the dorsolateral prostate of the group given isoflavone compared to the control whereas the difference in the ventral prostate was relatively low. This observation may be very important evidence that the difference in the isoflavone concentration in the different lobes may be an important factor for inducing prostatitis, but the exact mechanism is unknown. Although one may speculate the reason for the ower concentration of isoflavone in the ventral prostate compared to the dorsolateral prostate as being due to the different penetration rate, accumulation or excretion,[6] none is convincing. The matter needs further clarification using isoflavone as well as the estrogen induced prostatitis

model in rats. The rat prostate exhibits considerable heterogeneity in ductal morphogenesis and function between the lobes.[30] The dorsolateral prostate of rats is the embryological homologue of the human prostate.[31] the human prostate also exhibits some heterogenous features according to zones such as the different sensitivity and susceptibility to inflammation in the central and peripheral zones[32] and a 2.7 times higher concentration of prostate specific antigens in the transition zone than in the peripheral zone.[33] In this respect, future studies on the zonal difference in the isoflavone concentration may be clinically relevant.

Finally, in Asian countries, isoflavone is consumed for lifetime through soybean extracts that are a rich source of isoflavone. Moreover, the consequence is a much higher concentration of isoflavone in the prostatic fluid of men from this region than in Western men.[34] The incidence of prostatitis in western and eastern populations has not been compared in any report thus far. Interestingly, it has been reported that in age matched groups, Asian men have a smaller prostate than western men, but have higher incidence of prostatism.[35] As prostatitis induces prostatism, the inflammation induced by chronic ingestion of isoflavone in Asian men might be a possible factor that explains the high incidence of prostatism observed in these populations. Particularly, the different concentrations of genistein and daidzein in the different lobes suggest that the difference in the tissue isoflavone concentration may be another factor that proves lobe specificity in prostatitis induction. In this respect, further studies are needed.

The oral administration of an isoflavone mixture (300 mg/kg/day: genistein 60%, daidzein 19.6%) to normal adult male Sprague-Dawley rats resulted in lobe specific prostatitis, which was confined to the lateral prostate. This result is similar to the estrogen induced experimental prostatitis and is probably due to the weak estrogenic activity of isoflavone. Because the induction of prostatitis was produced by the oral administration of isoflavone and did not accompany any changes in the serum androgen levels and histomorphometry, the present study method seems to be physiologically more adequate than the estrogen induced model. Further studies on the biomolecular characteristics of the isoflavone-induced inflammation could furnish information for future research in this field.

REFERENCES

1. Meares EM. Nonbacterial prostatitis and prostatodynia. In: Lepor H, Lawson RK, editors. Prostate diseases. 1st ed. Philadelphia: W.B. Saunders Company; 1993. p.419-24.
2. Robinette CL. Sex hormone-induced inflammation and fibromuscular proliferation in the rat lateral prostate. Prostate 1988;12:271-86.
3. Naslund MJ, Strandberg JD, Coffey DS. The role of androgens and estrogens in the pathogenesis of experimental nonbacterial prostatitis. J Urol 1988;140: 1049-53.
4. Griffiths K, Morton MS, Denis L. Certain aspects of molecular endocrinology that relate to the influence of dietary factors on the pathogenesis of prostate cancer. Eur Urol 1999;35:443-55.
5. Chang HC, Churchwell MI, Delcios KB, Newbold RR, Doerge DR. Mass spectrometric determination of genistein tissue distribution in diet exposed Sprague-Dawley rats. J Nutr 2000;130:1963-70.
6. Sharma OP, Adlercreutz H, Strandberg JD, Zirkin BR, Coffey DS, Ewing LL. Soy of dietary source plays a preventive role against the pathogenesis of prostatitis in rats. J Steroid Biochem Mol Biol 1992;43:557-64.
7. Dalu A, Haskell JF, Coward L, Lamartiniere CA. Genistein, a component of soy, inhibits the expression of the EGF and ErbB2/Neu receptors in the rat dorsolateral prostate. Prostate 1998;37:36-43.
8. Strauss L, Mekella S, Joshi S, Huhtaniemi I, Santti R. Genistein exerts estrogen-like effects in male mouse reproductive tract. Mol Cell Endocrinol 1998;144:83-93.
9. Schleicher RL, Lamartiniere CA, Zheng M, Zhang M. The inhibitory effect of isoflavone on the growth and metastasis of a transplantable rat accessory sex gland carcinoma. Cancer Lett 1999;136:195-201.
10. Weibel ER, Gomez DM. A principle for counting tissue structures on random sections. J Appl Physiol 1962;17: 343-8.
11. Makela S, Santti R, Salo L, McLachlan JA. Phytoestrogens are partial estrogen agonists in the adult male mouse. Environ Health Perspect 1995;103:123-7.
12. Biegel LB, Flaws JA, Hirshfield AN, O'Connor JC, Elliott GS, Silbergeld EK, et al. 90-day feeding and one-generation reproduction study in Crl:CD BR rats with 17 beta-estradiol. Toxicol Sci 1998;44:116-42.
13. Wu-Peng S, Rosenbaum M, Nicolson M, Chua SC, Leibel RL. Effects of exogenous gonadal steroids on leptin homeostasis in rats. Obesity Res 1999;7:586-92.
14. Wiedemann E. Adrenal and gonadal steroids. In:

Daughaday WH, editor. Endocrine Control of Growth. New York: Elsevier; 1981. p.67-119.
15. Fisher JS, Kohrt WM, Brown M. Food restriction suppresses muscle growth and augments osteopenia in ovariectomized rats. J Appl Physiol 2000;88:265-71.
16. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987; 262:5592-5.
17. Zhou J-R, Gugger ET, Tanaka T, Gou Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. J Nutr 1999;129:1628-35.
18. Wagner JD, Cefalu WT, Anthony MS, Litwak KN, Zhang L, Clarkson TB. Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesterol ester content in ovariectomized cynomolgus monkeys. Metabolism 1997;46:698-705.
19. Weber KS, Jacobson NA, Setchell KD, Lephart ED. Brain aromatase and 5α-reductase, regulatory behaviors and testosterone levels in adult rats on phytoestrogen diets. Proc Soc Exp Biol Med 1999;221:131-5.
20. King RA, Broadbent JL, Head RJ. Absorption and excretion of the soy isoflavone genistein in rats. J Nutr 1996;126:176-82.
21. Evans BAJ, Griffiths K, Morton MS. Inhibition of 5α-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. J Endocrinol 1995;147:295-302.
22. Geller J, Sionit L, Partido C, Li L, Tan X, Youngkin T, et al. Genistein inhibits the growth of human-patient BPH and prostate cancer in histoculture. Prostate 1998;34:75-9.
23. Shull JD, Gorski J. Regulation of prolactin gene transcription *in vivo*: interactions between estrogen, pimozide, and alpha-ergocryptine. Mol Pharmacol 1990;37: 215-21.
24. Tangbanluekal L, Robinette CL. Prolactin mediates estradiol-induced inflammation in the lateral prostate of Wistar rats. Endocrinology 1993;132:2407-16.
25. Grayhack JT, Lebowitz JM. Effect of prolactin on citric acid of lateral lobe of prostate. Investig Urol (Berl) 1967;5:87-94.
26. Harris MT, Feldberg RS, Lau K-M, Lazarus NH, Cochrane DE. Expression of proinflammatory gene during estrogen-induced inflammation of the rat prostate. Prostate 2000;44:19-25.
27. Robinette CL. The role of prolactin in prostate inflammation(abstract). In: International Symposium on Biology of Prostate Growth Meeting Program; 1996 Mar 28-31; Washington D.C., 1996. p.57.
28. Lau KM, Leav I, Ho SM. Rat estrogen receptor α and β and progesterone receptor mRNA expression in various prostatic lobes and microdissected normal and dysplastic epithelial tissues of the Noble rats. Endocrinology 1998;139:424-7.
29. Holt S. Soya for health-The definitive medical guide. 1st ed. Larchmont(NY): Mary Ann Liebert, Inc.; 1996.
30. Hayashi N, Sugimura Y, Kawamura J, Donjacour AA, Cunha GR. Morphological and functional heterogeneity in the rat prostatic gland. Biol Reprod 1991;45:308-21.
31. Price D. Comparative aspects of development and structures in the prostate. NCI Monogr 1963;12:1-18.
32. McNeal JE. Regional morphology and pathology of the prostate. Am J Clin Pathol 1968;49:347-57.
33. Recker F, Kwiatkowski MK, Pettersson K, Piironen T, Lummen G, Huber A. Enhanced expression of prostate-specific antigen in the transition zone of the prostate. A characterization following prostatectomy for benign hyperplasia. Eur Urol 1998;33:549-55.
34. Morton MS, Matos-Ferreira A, Abranches-Monteiro L, Correia R, Blacklock N, Chan PS, et al. Measurement and metabolism of isoflavonoids and lignans in the human male. Cancer Lett 1997;114:145-51.
35. Tsukamoto T, Kumamoto Y, Masumori N, Miyake H, Rhodes T, Girman CJ, et al. Prevalence of prostatism in Japanese men in a community-based study with comparison to a similar American study. J Urol 1995; 154:391-5.