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SOY OF DIETARY SOURCE PLAYS A PREVENTIVE ROLE AGAINST THE PATHOGENESIS OF PROSTATITIS IN RATS

OM P. SHARMA,[1,2]* HERMAN ADLERCREUTZ,[4] JOHN D. STRANDBERG,[3] BARRY R. ZIRKIN,[1] DONALD S. COFFEY[2] and LARRY L. EWING[1]*

[1]Department of Population Dynamics, Division of Reproductive Biology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205, [2]The James Buchanan Brady Urological Institute, Department of Urology and [3]Division of Comparative Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, U.S.A. and [4]Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, Helsinki, Finland

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Summary—This study examined the effects of diet on the development of prostatitis in male rats. Adult male rats were placed on either of two specially formulated diets which differed from one another by the presence or absence of soy as the protein source. A third group of rats (control) was fed standard laboratory rat chow which also includes soy as a source of protein. After 11 weeks, it was found that rats maintained on soy-free diet developed prostatitis mainly in the lateral lobe of the prostate. Increased severity and incidence of prostatitis in rats maintained on the soy-free diet coincided with a significant decrease in urinary excretion of various phytoestrogens. There was no evidence of prostatitis in rats maintained on soy-containing diets. Urinary excretion of phytoestrogens in rats maintained on soy-containing diet was also not different from controls. These results suggest that soy as a dietary source plays a protective role against the development of prostatitis in rats, and indicate that the ventral, lateral and dorsal lobes of the rat prostate have different sensitivities to alterations in dietary factors.

INTRODUCTION

Prostatitis includes various inflammatory conditions affecting the prostate. Prostatitis is usually a focal process, histologically characterized by an accumulation of inflammatory cells, including neutrophils, lymphocytes, plasma cells and macrophages in the glandular lumina and stroma of the prostate [1]. In humans, such inflammatory changes are usually confined to one or more excretory ducts. These ducts are dilated and filled with inspissated secretion and macrophages with phagocytized foamy material. The intensity of the inflammatory reaction may vary, and both acute and chronic inflammatory changes may be present. Despite the fact that prostatitis is a widespread disease affecting 1 in 5 men, our knowledge of its etiology and pathogenesis remains unclear. For example, prostatitis may be caused by an infective agent, but it is also possible that there might be non-infectious causes of inflammation [2], including endogenous chemical, immunologic, obstructive, traumatic or autoimmune factors [3].

Previous studies have shown that genetic background, advancing age and hormonal imbalance contribute to the pathogenesis of prostatitis in the lateral prostate of Lewis, Copenhagen and Wistar rats [4–6]. A hormonal role in the enhancement of bacterial [7] and non-bacterial [6] prostatitis in rats has been suggested. Steroid hormones appear to play a significant role in the pathogenesis of prostatitis; specifically, estrogens have been shown to stimulate development of prostatitis [5, 6].

Diet appears to influence hormone production, metabolism, and excretion, and therefore may alter the incidence of prostate diseases [8–11]. Moore [2] indicated that prostatic inflammation might be associated with severe malnutrition. Dietary hormone-like substances each as certain diphenolic compounds, lignans and

*To whom correspondence should be addressed at: Department of Medicine, Box 202, University of Virginia Health Sciences Center, Charlottesville, VA 22908, U.S.A.

*Dr Ewing died during the preparation of this manuscript. He was an outstanding scientist, educator and a colleague, whose loss is felt deeply by all of us.

isoflavonoids, of soy origin, exhibit weak estrogenic activity both *in vitro* and *in vivo*, as well as antiestrogenic activity [12]. The possible effects of such factors on the prostate have not been considered. Soy is a rich source of isoflavonoid phytoestrogens (active hormone-like diphenolic components of plant origin structurally modified by intestinal microflora) [12, 13], and is the main protein source in commercial laboratory animal chow. In this paper, we assess the potential importance of diet, containing or devoid of soy, on the pathogenesis of prostatitis in rats.

MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats weighing between 375 and 450 g were purchased from Harlan Sprague–Dawley Inc. (Indianapolis, IN). Rats were housed two per cage on hardwood chip bedding (beta-chips; North Eastern Products, Warrensburg, NY) under controlled conditions of light (12 h light:12 h dark) and temperature (25°C). Water and food were provided *ad libitum*.

Diets

Control rats were fed commercial laboratory rat chow containing protein 14.0%, fat 6.0%, fiber 4.5%, carbohydrate 57.0%, ash 8.0% and moisture 11.0% (Agway Prolab RMH 1000, Waverly, NY). A second group of rats was fed specially formulated soy-free diet containing protein of casein source 17%, fat 5%, carbohydrate 66%, moisture 8.0%, dietary fiber 0.12% and ash 3.0%. A third group of rats was fed a diet with soy (plant sterols) which contained protein of soy source 17%, fat 5%, carbohydrate 71%, moisture 4%, dietary fiber 0.12% and ash 3.0% (Bio-Serv, Frenchtown, NJ). The specially formulated diets were identical in terms of ingredients except for the protein source (casein or soy). Previous studies [14] have shown that casein can be used as a source of protein in feed without major effects on general physiology.

Experimental design and treatments

Sixteen rats were divided into 3 treatment groups as follows: Group 1 ($N = 6$) fed on commercial laboratory rat chow; Group 2 ($N = 5$) fed on soy-free formulated diet containing protein of casein source; and Group 3 ($N = 5$) fed on formulated diet containing soy (plant sterols).

At the completion of the experiment (11 weeks), body weights were recorded and rats were killed by decapitation. The ventral, lateral and dorsal prostatic lobes were removed and briefly placed on ice. After weighing each lobe a portion was fixed for histology.

Urine collection

On the last day of treatment, rats were placed in metabolism cages and 24 h urine samples were collected for phytoestrogen analysis. Sodium metabisulfite (0.1% of the urine volume) was added to each sample as a preservative. Samples were stored at −20°C until analyses of various phytoestrogens were performed. Samples were shipped frozen on dry ice to Finland (Dr H. Adlercreutz) where urinary phytoestrogens were assayed.

Phytoestrogen determination

The phytoestrogens equol (Eq), daidzein (Da), *O*-desmethylangolensin (*O*-Dma), genistein (Gen), enterolactone (Enl) and enterodiol (End) were assayed in urine samples from each rat by combined capillary gas chromatography–mass spectrometry utilizing selected ion monitoring with an isotope dilution technique [15].

Histology

A portion of each lobe was fixed in 4% buffered formalin containing 2% glutaraldehyde (pH 7.4) and embedded in paraffin. Sections 5 μm in thickness were prepared from each lobe, mounted on glass slides and stained with hematoxylin and eosin. Selected sections from paraffin blocks were stained with azure eosin, Gram's stain, and Gomori's methanamine-silver nitrate [16] to identify possible microorganisms.

Pathology

All histopathologic examinations were performed by a veterinary pathologist (Dr John Strandberg). Lesions classified as prostatitis consisted of a spectrum of changes including focal intraluminal and interstitial accumulations of neutrophils, lymphocytes, plasma cells and macrophages. A specimen was considered positive for prostatitis if at least one area of active inflammation was seen on a microscopic section. The severity and extent of prostatitis was graded on a scale from 0 to 4+ where 0 reflected no prostatitis and 4+ very severe prostatitis affecting most of the acini. Several morphological features, including glandular

architecture, cell morphology, fibromuscular hyperplasia and degeneration were also evaluated histologically.

Statistics

Differences among treatment groups were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test [17]. Average severity of prostatitis in the different treatment groups was determined by averaging the grades given to each specimen in the group. All data are expressed as mean ± standard error of the mean (SEM).

RESULTS

Effect of diets on urinary excretion of phytoestrogens

The 24 h urinary excretion of several phytoestrogens (i.e. Da, Gen, Eq, *O*-Dma, Enl and End) in rats fed on commercial diet, soy-free or containing diet is depicted in Fig. 1. There was a significant reduction in the excretion of all of the measured lignan and isoflavonoid phytoestrogens in rats maintained on the soy-free diet. The urinary excretion of Da, Gen, Eq, and *O*-Dma in rats maintained on soy-containing diet was not significantly different from controls. Excretion of End and Enl, present in whole grain, was significantly lower in rats maintained on soy-containing diet than in controls.

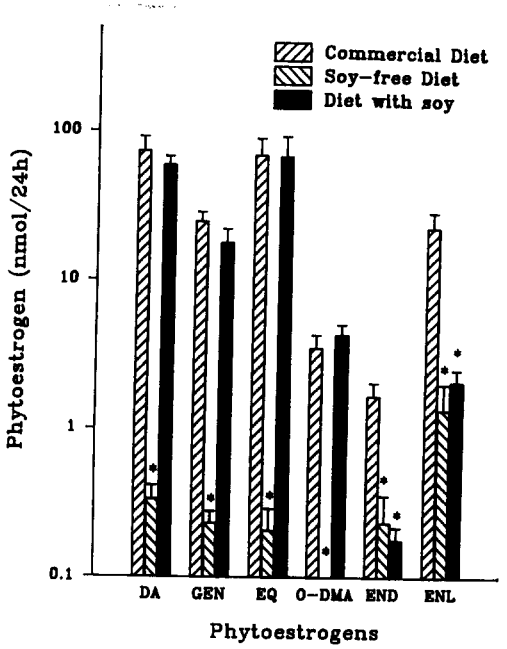

Fig. 1. Effect of regular and soy-free diet fed for 11 weeks on the urinary excretion of different phytoestrogens (nmol/24 h). Each bar represents mean ± SEM on the log scale. For abbreviations see text. * = Significantly different compared to controls ($P < 0.05$).

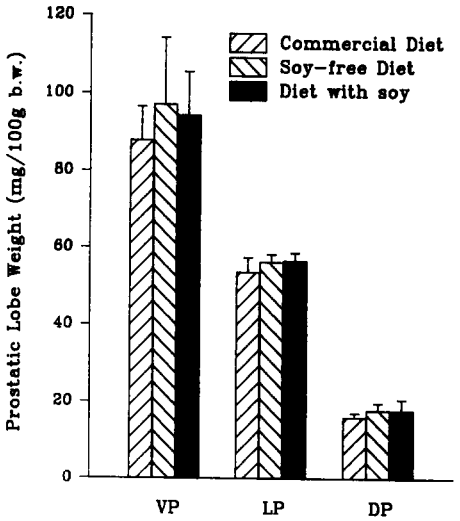

Fig. 2. Effect of different diets on relative weight of different lobes of the rat prostate. VP = ventral prostate, LP = lateral prostate, and DP = dorsal prostate. Each bar represents mean ± SEM.

Prostatic growth

To evaluate prostatic growth, prostatic weight was recorded. There was no statistically significant difference in the ventral, lateral and dorsal prostate weights of rats maintained on soy-free or containing diet when compared with control rats maintained on commercial diet (Fig. 2).

Prostatic histopathology

The ventral prostate in rats on the commercial diet had normal morphology typified by lobules of tubuloalveoli lined by a single layer of secretory epithelium surrounded by a thin basement membrane and an outer layer of smooth muscle cells. Intervening stromal fibrovascular matrix was scant. In addition to the smooth muscle cells investing each of the tubuloalveoli, the stromal elements located within and between lobules included nerve endings, fibroblasts, blood cells and collagen. In the control group, 1 out of 6 rats had mild inflammation in the ventral prostate. There was no evidence of inflammation or a significant change in the epithelial cell morphology or in the stromal compartment of the ventral prostate of rats maintained on soy-free or containing diet as compared to controls (Table 1).

The lateral prostate of control rats had, relative to the ventral prostate, smaller, more regular alveoli with epithelium infolding into the glandular lumens. Epithelial cells had

Table 1. Severity and percent incidence of prostatitis in the ventral, lateral and dorsal prostatic lobes (VP, LP and DP, respectively) of adult rats following feeding on different diets

		Prostatitis					
		Severity			Incidence (%)		
Treatments	*N*	VP	LP	DP	VP	LP	DP
Commercial diet	6	0.17 ± 0.17	0.17 ± 0.17	0	16	16	0
Soy-free diet	5	0	1.20 ± 0.37*	0	0	80	0
Diet with soy	5	0	0	0	0	0	0

Severity of prostatitis values are mean of grades (0 to 4+) given to each prostatic lobe ± SEM.
*The value is significantly different ($P < 0.05$) from the control group fed on the commercial diet.

prominent apical secretory vacuoles (Fig. 3A). The luminal secretion was more eosinophilic than that of the ventral prostate. There was a mild inflammation in the lateral prostate of one control rat.

In rats maintained on soy-free diet, the epithelial cells of some alveoli in the lateral prostate changed in character from columnar to stratified squamous cells. Some acini showed degeneration of the epithelium with accumulation of lymphocytes and plasma cells at the site of degeneration and glandular change of secretion. Inflammation was usually associated with these areas of dysplasia (Fig. 3B). The inflammation was composed of intraluminal accumulation of neutrophils, lymphocytes and macrophages as well as mononuclear cell infiltrations in the stroma. In some foci, inflammatory cells formed microabscesses within acini. Secretions were absent in these acini, which instead contained cellular debris. An extensive inflammatory response surrounding damaged alveoli included fibrosis (fibroblasts and collagen) with macrophages, lymphocytes and plasma cells in rats fed on soy-free diet. The acinar and stromal morphology of the lateral prostate in

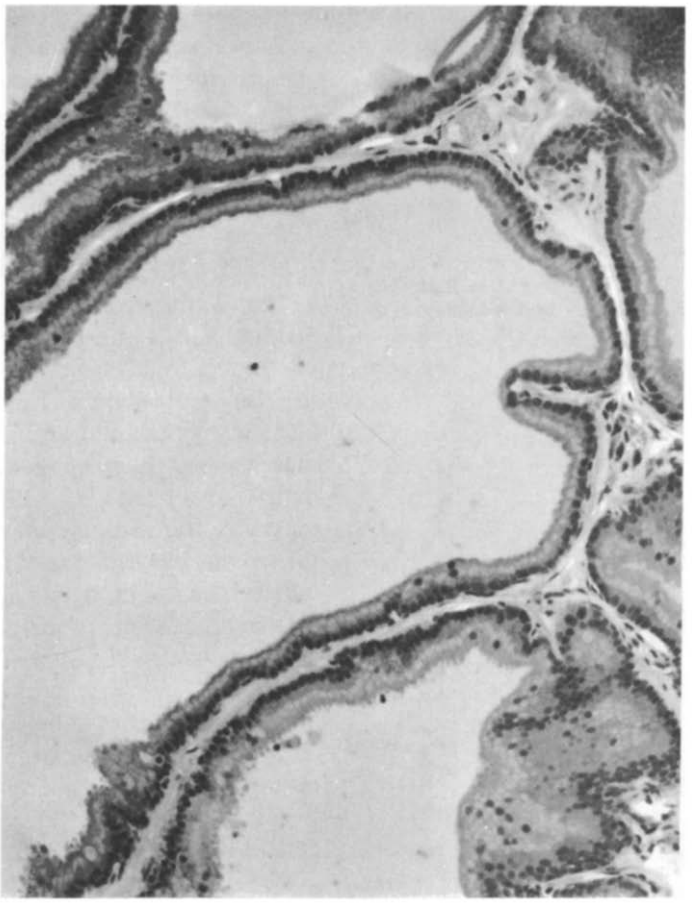

Fig. 3A. Photomicrograph showing lateral prostate in control rats maintained on commercial diet. Epithelium is consisted of low columnar or cuboidal cells with centrally located nuclei and clear supranuclear zone. Acini are filled with secretions. HE ×180.

rats maintained on soy-containing diet was normal. There was no evidence of inflammation in these rats.

The severity of the prostatitis was evaluated semi-quantitatively on a scale from 0 to 4+. The average severity of prostatitis in different treatment groups or different prostatic lobes was determined by averaging the grades (0 to 4+) given to each specimen in the group or lobe. The percent incidence and severity of prostatitis in the rat ventral, lateral and dorsal prostate are presented in Table 1. It is evident from this table that there was no sign of inflammation in the ventral or dorsal prostate following feeding the rats either soy-free or containing diets. Most interestingly, soy-free diet enhanced both the incidence and severity of prostatitis in the lateral prostate compared to controls. There was no evidence of prostatitis in the lateral prostate of rats fed on soy-containing diet. This clearly indicates that prostatitis occurred only when rats were fed soy-free diet. The inflammation was specific for the lateral prostate. There was no sign of inflammation in the dorsal prostate following any of the treatments. There was no evidence of the presence of micro-organisms in any of the prostate sections stained with azure eosin, Gram's stain and Gomori's methenamine-silver nitrate stains.

DISCUSSION

The high urinary excretion of lignan and isoflavonoid phytoestrogens in rats fed on commercial or soy-containing diet indicates that soy is a rich source of various phytoestrogens and their precursors [18]. The possibility that phytoestrogens and their precursors may play a protective role against prostatitis is supported by the correlation between the significant reduction in the urinary excretion of phytoestrogens (Eq, Da, *O*-Dma, Gen, Enl and End) and the marked increase in the occurrence of prostatitis in rats maintained on soy-free diet.

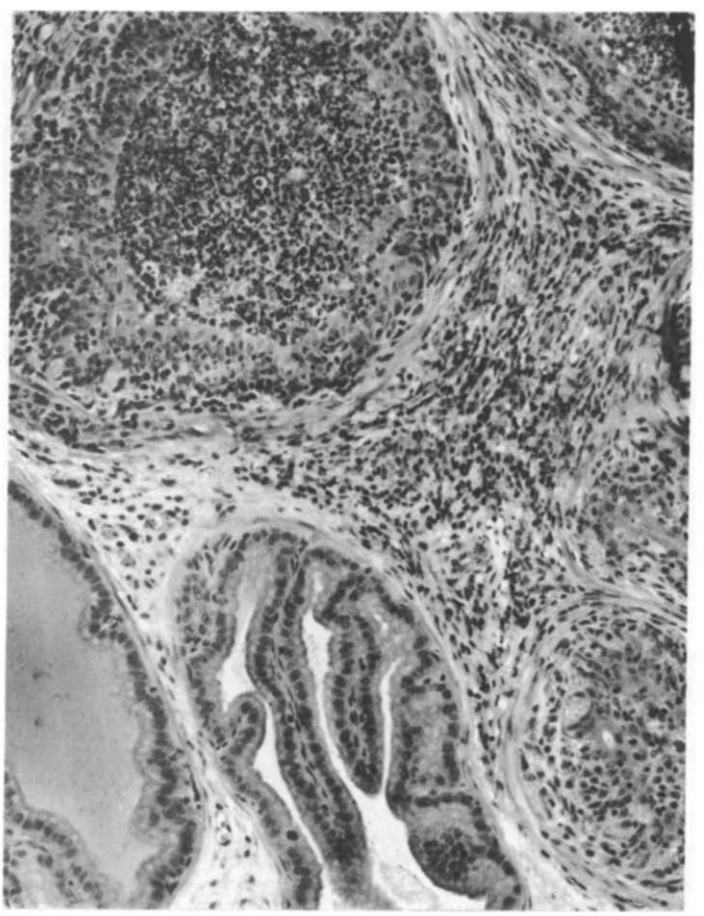

Fig. 3B. Representative acini and stroma in a lateral prostate affected by prostatitis following feeding rats on soy-free diet for 11 weeks. The lumen contains cellular debris, neutrophils, and lymphocytes. The epithelial cells lining the acinus show degenerative changes and have been partly shed. Macrophages and mononuclear cell infiltrations are seen in stroma. HE ×180.

In the present study, rats maintained on soy-free diet for 11 weeks developed severe inflammation in the lateral prostate, while rats maintained on soy-containing diet or commercial rat chow did not develop any sign of prostatitis. This finding suggests that soy of dietary source may play a protective role against the pathogenesis of prostatitis. The mechanism of this process is as yet unknown. One possible explanation could be that since soy-beans contain a number of phytoestrogens which are weak estrogens [12], the soy-free diet might disturb the androgen–estrogen ratio. As noted, lignans and isoflavonoid phytoestrogens are naturally occurring plant compounds having diphenolic structure, which may explain their estrogenic activity. These compounds stimulate sex hormone binding globulin (SHBG) synthesis by liver cells [13] of human. However, rats do not have SHBG, and thus the effects of dietary factors in rats could not be mediated via this mechanism. Rats have androgen-binding protein (ABP), a homologous protein to SHBG. Although ABP and SHBG are transcribed by the same gene [19], SHBG is synthesized in and secreted by the liver [20], whereas ABP is synthesized in and secreted by the Sertoli cells [21]. Further, their secretions are regulated in totally disparate ways [22]. Thus, it is possible that SHBG, which interacts with somatic cells, performs a different function than ABP, which presumably exerts its effects within the testis [23, 24]. The actions of the phytoestrogens are complex as they may exhibit both estrogenic as well as antiestrogenic properties, and bind to estrogen receptors [25, 26] and to the estrogen type II binding site [27]. We suggest that in these experiments the effect of soy diet was based on the estrogenic effect of the isoflavonoids present (please see below).

Of interest was the lobe-specificity in the response of the rat prostate to dietary factors. The data suggest that the rat ventral and dorsal prostates are protected against the development of prostatitis, but that the lateral prostate is not. Thus, the different prostatic lobes of the rat have different sensitivities to the inflammatory reactions induced by alteration in dietary factors. Basic biological differences exist between the different prostatic lobes of the rat. The histological structure differs in the various lobes of the rat prostate [28] as does the chemical composition of the lobes and their secretions [29]. The differential sensitivities of different prostatic lobes to prostatitis in rats is consistent with the heterogeneity in morphology and tissue chemical contents. The three lobes of the rat prostate differ in their protein composition [30], androgen receptor levels, regulation by androgens [31], and the rate of involution following androgen depletion [32]. Studies on androgen metabolism in different lobes of the rat prostate have revealed differences in the total activity of various enzymes such as 5α-reductase, 17β-hydroxysteroid oxidoreductase and 3α-hydroxysteroid oxidoreductase [33]. Wilson *et al.* [34] have demonstrated the lobular specific atrophy of the ventral prostate, but not of the dorsal prostate in aging (30-month-old) Fischer 344 rats. The presence of the prostatic type II estrogen binding sites has been shown exclusively in the dorsolateral prostate of the rat [35].

Lignan and phytoestrogens bind to the estrogen type II binding sites [26]. Such mechanisms may underlie the observed differences in susceptibility among the prostatic lobes to the dietary factors. We report the lateral prostate is the main site of prostatitis. This finding is in accordance with previous studies which have shown that prostatitis occurs in the lateral prostate of Lewis, Copenhagen and Wistar rats following different treatments [5, 6, 36]. The reason that the lateral lobe is the principal site of inflammatory reactions might be due in part to the differences in the drainage pattern of the lateral prostate compared to the other lobes [28]. The central and peripheral zones of the human prostate also differ widely in susceptibility to inflammatory processes [37], with the peripheral zone mainly involved in inflammation [37].

In conclusion, the present results indicate that the soy of dietary source plays a protective role against the development of prostatitis in Sprague–Dawley rats by as yet unknown mechanisms. These results also demonstrate that the ventral, lateral or dorsal prostatic lobes of the rat have differential sensitivities to the inflammatory reactions induced by alteration in dietary content. The role of diet should be carefully considered when studies concerning estrogen effects are carried out in humans and laboratory animals. Further studies are required to determine that which particular phytoestrogens of soy source are involved in this process.

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