ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 812

MEDICA - ODONTOLOGICA

AN EXPERIMENTAL MODEL OF PROSTATIC INFLAMMATION FOR DRUG DISCOVERY

by

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TURUN YLIOPISTO
Turku 2008

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ISBN 978-951-29-3661-8 (PRINT)
ISBN 978-951-29-3662-5 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2008

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ABSTRACT

There is increasing evidence to support a significant role for chronic non-bacterial prostatic inflammation in the development of human voiding dysfunction and prostate cancer. Their increased prevalence with age suggests that the decrease of testosterone concentration and/or the ratio of testosterone-to-estradiol in serum may have a role in their development. The main objective of this study was to explore prostatic inflammation and its relationship with voiding dysfunction and prostate carcinogenesis by developing an experimental model. A novel selective estrogen receptor modulator (SERM), fispemifene, was tested for the prevention and treatment of prostatic inflammation in this model.

Combined treatment of adult Noble rats with testosterone and estradiol for 3 to 6 weeks induced gradually developing prostatic inflammation in the dorsolateral prostatic lobes. Inflammatory cells, mainly T-lymphocytes, were first seen around capillaries. Thereafter, the lymphocytes migrated into the stroma and into periglandular space. When the treatment time was extended to 13 weeks, the number of inflamed acini increased. Urodynamical recordings indicated voiding dysfunction. When the animals had an above normal testosterone and estradiol concentrations but still had a decreased testosterone-to-estradiol ratio in serum, they developed obstructive voiding. Furthermore, they developed precancerous lesions and prostate cancers in the ducts of the dorsolateral prostatic lobes. Interestingly, inflammatory infiltrates were observed adjacent to precancerous lesions but not in the adjacency of adenocarcinomas suggesting that inflammation has a role in the early stages of prostate carcinogenesis.

Fispemifene, a novel SERM tested in this experimental model, showed anti-inflammatory action by attenuating the number of inflamed acini in the dorsolateral prostate. Fispemifene exhibited also antiestrogenic properties by decreasing expression of estrogen-induced biomarkers in the acinar epithelium. These findings suggest that SERMs could be considered as a new therapeutic possibility in the prevention and in the treatment of chronic prostatic inflammation.

Keywords: Prostatic inflammation, voiding dysfunction, prostate cancer, estrogen, androgen, experimental model, SERM

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KOKEELLINEN MALLI ETURAUHASTULEHDUKSEN LÄÄKEHOIDON KEHITTÄMISEKSI

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TIIVISTELMÄ

Viimeaikaiset tutkimustulokset ovat antaneet viitteitä, että kroonisella eturauhastulehduksella ilman bakteerilöydöstä on merkitystä eturauhassyövän ja toiminnallisen virtsaamishäiriön synnyssä. Eturauhastulehduksen syntymekanismia ei tunneta, mutta miehen ikääntymiseen liittyvällä alentununeella androgeeni-estrogeenisuhteella saattaa olla merkitystä eturauhassairauksien ja virtsaamishäiriöiden synnyssä. Tämän väitöskirjatyön lähtökohtana oli selvittää muuntuneen hormonitasapainon merkitystä eturauhastulehduksen synnyssä sekä tutkia tulehduksen mahdollista yhteyttä toiminnalliseen virtsaamishäiriöön ja eturauhassyöpään kehittämällä kokeellinen malli. Kehitetyssä mallissa tutkittiin uuden selektiivisen estrogeenireseptorin muuntelijan (SERMin), fispemifeenin, vaikutusta eturauhastulehduksessa.

Aikuisten Noble-rottien yhdistetty testosteroni- ja estradiolikäsittely sai 3-6 viikossa aikaan vaiheittaisesti etenevän tulehduksen eturauhasen dorsolateraalisissa lohkoissa. Tulehdus eteni perivaskulaarisista, T-lymfosyyteistä koostuvista pesäkkeistä stroomaan ja edelleen rauhasten ympärille. Pidennettäessä hormonikäsittelyaikaa 13 viikkoon, rauhasten sisälle kertyi tulehdussoluja. Tulehduksen lisäksi eläimille kehittyi virtsaamishäiriö. Eläimille, joiden testosteronitaso oli normaalia korkeammalla, ja joiden androgeeni-estrogeenisuhde oli alentunut, kehittyi tyhjennysoireita (obstruktiivisia oireita). Pidemmillä käsittelyajoilla eläimille kehittyi dorsolateraalisten eturauhaslohkojen laskutiehyeisiin syövän esiasteita, jotka etenivät eturauhassyöviksi. Syövän esiasteiden läheisyydessä todettiin tulehdussolukertymiä, mutta syöpien ympärillä tulehdusta ei todettu. Tämä viittaa siihen, että tulehduksella on merkitystä eturauhassyövän kehityksen alkuvaiheissa.

Uudella SERM-yhdisteellä, fispemifeenillä, todettiin anti-inflammatorinen vaikutus kehitetyssä kokeellisessa eturauhastulehdusmallissa. Fispemifeeni vähensi tulehtuneiden rauhasten lukumäärää. Lisäksi fispemifeeni toimi antiestrogeenisesti vähentämällä estrogeenin lisäämien proteiinien ilmentymistä eturauhasen epiteelissä. Tässä tutkimuksessa saadut tulokset tukevat ajatusta, että SERM-yhdisteitä voidaan hyödyntää kehitettäessä uusia lääkeainehoitoja kroonisen eturauhastulehduksen estossa ja hoidossa.

Avainsanat: Eturauhastulehdus, virtsaamisoireet, eturauhassyöpä, estrogeeni, androgeeni, kokeellinen malli, SERM

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ABBREVIATIONS

AR	Androgen receptor
BPH	Benign prostatic hyperplasia
CD3	T-lymphocyte
CD4	Helper T-lymphocyte
CD8	Cytotoxic T-lymphocyte
CP/CPPS	Chronic prostatitis / chronic pelvic pain syndrome
CPSI	Chronic Prostatitis Symptom Index
DHT	5α-dihydrotestosterone
DLP	Dorsolateral prostate
E_2	17β- estradiol
EPS	Expressed prostatic secretion
ERα/β	Estrogen receptor α/β
H&E	Hematoxylin & eosin staining
HGPIN	High-grade prostatic intraepithelial neoplasia
LGPIN	Low-grade prostatic intraepithelial neoplasia
LP	Lateral prostate
LUT	Lower urinary tract
LUTS	Lower urinary tract symptoms
NIH	National Institutes of Health
PIN	Prostatic intraepithelial neoplasia
PR	Progesterone receptor
PSA	Prostate-specific antigen
PRL	Prolactin
RB	Rhabdosphincter
SERM	Selective estrogen receptor modulator
T	Testosterone
$T+E_2$	Testosterone and estradiol treatment
VP	Ventral prostate

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals I- IV:

I Bernoulli J, Yatkin E, Talvitie EM, Santti R, and Streng T. Urodynamic changes in a noble rat model for nonbacterial prostatic inflammation. Prostate 2007, 67(8):888-899.

II Bernoulli J, Yatkin E, Konkol Y, Talvitie EM, Santti R, and Streng T. Prostatic inflammation and obstructive voiding in the adult Noble rat: impact of the testosterone to estradiol ratio in serum. Prostate 2008, 68(12): 1296-1306.

III Bernoulli J, Yatkin E, Laakso A, Anttinen M, Bosland MC, Vega K, Kallajoki M, Santti R, and Pylkkänen L. Histopathological evidence for an association of inflammation with ductal PIN-like lesion but not with ductal adenocarcinoma in the prostate of the Noble rat. Prostate 2008, 68(7): 728-739.

IV Yatkin E*, Bernoulli J*, Lammintausta R, and Santti R. Fispemifene (Z-2-{2-[4-(4-Chloro-1,2-diphenylbut-1-enyl)-phenoxy]ethoxy}-ethanol), a novel selective estrogen receptor modulator, attenuates glandular inflammation in an animal model of chronic nonbacterial prostatitis (J Pharmacol Exp Ther. 2008, in press).
* These authors contributed equally to the work.

1 INTRODUCTION

The National Institutes of Health (NIH) classification divides prostatitis into four categories: I) acute bacterial prostatitis, II) chronic bacterial prostatitis, III) chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) subdivided into inflammatory (IIIA) or non-inflammatory (IIIB) classes and IV) asymptomatic inflammatory prostatitis (Nickel et al., 1999). CP/CPPS accounts for 90% of all chronic prostatitis cases and only 5 to 10 % of men with symptoms of prostatitis are characterized to have bacterial origin prostatitis. CP/CPPS has been estimated to affect 10-14% of men of all ages (Mehik et al., 2000b; Schaeffer, 2003). Asymptomatic inflammatory prostatitis has been found in 44% of biopsy samples and even in >95% of prostatectomies (Blumenfeld et al., 1992). This means that practically all men have histopathological signs of inflammation in the prostate.

The symptoms of CP/CPPS are heterogeneous, principally pain in the pelvic region as well as irritative or obstructive symptoms on voiding (Schaeffer, 2006). The etiology of CP/CPPS is unclear. It may develop from an autoimmune basis (reviewed by Batstone et al., 2002; Motrich et al., 2007; Rivero et al., 2007). The current consensus suggests that CP/CPPS arises from interaction between immune, endocrine and neuronal causes (Pontari and Ruggieri, 2004). The initiator of the inflammatory process may be a local infection, chemical irritation, an immunological response to systemic infection or hormonal imbalance.

A statistically significant positive correlation was recently found between histological grade of chronic prostatic inflammation and lower urinary tract symptoms (LUTS) (Nickel et al., 2007c). The correlation was weak but did not preclude the possibility that patients with chronic prostatitis may be more likely to develop voiding dysfunction with age. Numerous mechanistic explanations have been proposed for the associations of human voiding dysfunctions and CP/CPPS. Among them, high pressure voiding causing reflux of urine or semen into prostatic ducts leading to an increased intraprostatic pressure is often mentioned (Mehik et al., 2003). The increased luminal pressure may cause leakage of pro-inflammatory factors through the prostatic epithelium into stroma and trigger inflammatory reaction.

Inflammation in the prostate gland has recently been related also to benign prostatic hyperplasia (BPH) (reviewed by Kramer et al., 2007; Mishra et al., 2007; Nickel, 2008a). Histological inflammation can be found in the majority of BPH pathological samples. Aged men experiencing LUTS are likely to have BPH and they are often labelled to have LUTS caused by benign prostatic enlargement secondary to BPH. However, prostate size correlates poorly with the extent of the LUTS, particularly with storage symptoms. No significant relationship has been found between prostate size and changes in the symptom index over time. Other causes than prostate size should be considered (Koskimäki et al., 1998). A possibility remains that cytokines secreted by inflammatory cells have systematic influence or when released into secretion by glandular inflammation they would influence neurogenic mechanism or muscular

functions of the lower urinary tract and cause functional changes such as rhabdosphincter dyssynergia and/or abnormal detrusor contractility.

In addition, there is increasing evidence for the association of chronic prostate inflammation with human prostate cancer (reviewed by Nelson et al., 2004; MacLennan et al., 2006; De Marzo et al., 2007). Both epidemiological data (Dennis et al., 2002; Barqawi et al., 2004) and molecular studies have provided evidence suggesting that inflammation-derived growth factors and cytokines contribute to the development of prostate cancer (DeMarzo et al., 2003; Nelson et al., 2003; Palapattu et al., 2005). Also histopathological studies suggest that proliferative inflammatory atrophy (PIA) lesion might be a precursor to prostatic intraepithelial neoplasia (PIN) and prostate cancer (DeMarzo et al., 1999b). Despite that high-grade PIN is well-established as precursor of prostatic carcinoma (Bostwick and Qian, 2001; Montironi et al., 2006) its association with inflammation is not well-understood.

Taken together, the understanding of chronic non-bacterial prostatitis may be the key to the understanding of voiding dysfunctions/LUTS, BPH and prostate cancer, and successful treatment of chronic prostatitis could decrease the risk of these diseases. Unfortunately, as presented, the cause of non-bacterial inflammation is not known. One possible initiator of the inflammation is a hormonal cause. The prevalence rate of CP/CPPS increases with age (Roberts et al., 1998). There is also age-related decrease in the serum testosterone concentration (Kaufman & Vermeulen 2005) causing imbalance to testosterone to estradiol ratio (T-to-E_2 ratio). The association might be causal and explained by the weakening anti-inflammatory action of testosterone and the intensifying pro-inflammatory influence of estradiol (Straub, 2007). Another reason for abnormal hormonal milieu is obesity. Decreased androgen levels and increased estrogen levels have been observed in obese men (Schneider et al., 1979; Kley et al., 1980; Vermeulen 1996). Unfortunately, no data has been given whether obesity is related to chronic prostatitis. However, suggested hormonal conditions promoting prostatic inflammation would be remarkably similar to those known to cause gynecomastia (Braunstein, 1999).

At present, there is no evidence that testosterone replacement therapy would have preventive or therapeutic significance in CP/CPPS patients. Testosterone is peripherally converted to estradiol, leading to supraphysiological serum estradiol levels (Gooren and Bunck, 2004). Thus testosterone replacement therapy would not necessarily increase the ratio of the T-to-E_2 and have the desirable anti-inflammatory effect. Sex hormone metabolizing enzymes and sex steroid receptors offer more potential targets for drug discovery. Indeed, the selective estrogen receptor modulator (SERM), toremifene, has been shown to decrease the incidence of prostate cancer in an animal model (Raghow et al., 2002) and in patients with high-grade prostatic intraepithelial neoplasia (Price et al., 2006). Recent finding of TMPRSS2-ERG gene fusion (regulated by estrogen receptor dependent mechanism) even in 30-70 % of prostate cancer cases make estrogen action even more attractive target (Tomlins et al., 2005; Saramäki et al., 2008; Setlur et al., 2008). However, while considering the

numerous potential drug candidates, a feasible experimental model to screen the effects and safety of drug candidates in the prostate should be available.

The aim of the present work was to study the possible causal relationship of obstructive voiding, precancerous lesions and prostate cancer with prostatic inflammation by developing an experimental model. Adult male Noble rats were treated with androgen and estrogen for different periods. Careful serum hormone concentration monitoring was used to observe hormonal changes; histological and immunohistochemical methods were established to improve histopathological assessments, and urodynamical measurements were used to detect alterations in voiding. Fispemifene, a novel SERM was tested as a new therapeutic option for non-bacterial prostatic inflammation.

2 REVIEW OF LITERATURE

2.1 Structure and function of the prostate gland

2.1.1 Development, blood supply and innervation of the prostate

The prostate develops from the embryonic urogenital sinus, composed of urogenital sinus epithelium and urogenital sinus mesenchyme. Both these as well as androgens are needed for prostate development. The result of this interaction is the differentiation of mature prostatic tissue in which the epithelium has a secretory phenotype and the mesenchyme develops into mature prostatic stroma composed of mainly smooth muscle cells (Cunha et al., 2003)

The prostate lies between the urinary bladder and the pelvic floor surrounding the prostatic urethra (Villers et al., 1991). The main arterial blood supply to the prostate is from the inferior vesical artery and less from the middle rectal arteries. The veins of the prostate form the prostatic venous plexus around the sides and base of the prostate; between the true capsule of the gland and the outer fibrous sheath. The plexus drains into the internal iliac veins. The lymph vessels of the prostate drain into the internal iliac lymph nodes.

The human prostate receives a dual autonomic innervation from both parasympathetic (cholinergic) and sympathetic (noradrenergic) nerves via the prostatic plexus; parasympathetic nerve fibers from the sacral segments of the spinal cord and sympathetic fibers from the hypogastric nerves. Both cholinergic and noradrenergic nerves innervate the smooth muscle bundles of the prostatic stroma and cholinergic nerves also innervate the smooth muscle of the capsule (Dixon et al., 1999). In addition, the prostatic stroma smooth muscle cells and its blood vessels express various adrenergic receptor subtypes, which respond by contraction to α_1 adrenergic stimulation (Rosenzweig et al., 2004). The nerves supplying the prostate contain, in addition acetylcholine and noradrenaline, a variety of neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) (Dixon et al., 1999; Zermann et al., 2000). Stimulation of the parasympathetic nerves increases the rate of prostatic secretion while activation of muscarinic receptors in the prostatic glandular epithelium causes secretion (Dixon et al., 1999; Nadelhaft et al., 2003).

2.1.2 Structure of the prostate

The prostate has a base and apex, and four surfaces: posterior, anterior, and two inferolateral surfaces. The base of the prostate is the upper surface adjacent to the preprostatic uretha and bladder neck; the apex of the prostate is the lowest part. The

histology and anatomy of the prostate has been studied by different methods and described by different models (Dixon et al., 1999). The most detailed studies of the anatomy, histology and pathology of the prostate has been described by McNeal (McNeal, 1968; McNeal, 1988). He has described four different regions of the prostate, the largest region being the anterior fibromuscular and non-glandular region. The remaining glandular prostate is subdivided into three zones: peripheral (PZ), central (CZ) and transitional zones (TZ) in relation to the urethra. The peripheral zone is located posterolaterally, the central zone is located at the base, and the 2 lobes of the transition zone locate along the proximal urethra (Villers et al., 1991).

The prostate gland consists of glandular tissue (glands) and fibrous and muscular tissues around the glands. The ducts from the glands converge and open into the prostatic urethra. Individual acini have epithelium that varies from inactive, low cuboidal to active, pseudostratified columnar depending on the degree of stimulation by androgens. The epithelium in acini, but also in the ducts (excluding the main ducts near the urethra) is composed of secretory cells that are separated from the basement membrane and prostatic stroma by a layer of basal cells (De Marzo et al., 1999a). Basal cells are generally flattened, having small nuclei and only a little cytoplasm. They can be distinguished from the secretory cells, because they express specific cytokeratins and lack secretory markers (De Marzo et al., 1999a). The proliferation activity of the epithelium occurs in this basal cell layer. The transitional epithelium, which lines the prostatic urethra and main ducts, has single layer of columnar secretory cells in the luminal surface, and only a relatively sparse cytoplasm (Dixon et al., 1999).

There are histological differences between the zones e.g. in the central and peripheral glandular zones that might lead to differences in the function and presence of prostatic diseases (Dixon et al., 1999). The ducts and acini of the peripheral and transitional zone are often smaller and not circular compared to the larger ducts and acini in the central zone. There are also differences in the ratio of stromal to epithelial tissue between the zones, and this probably gives one reason why prostate diseases often arise in specific zones (Villers et al., 1991).

2.1.3 Function of the prostate

At ejaculation, the human prostate releases approximately 1 ml of slightly acidic fluid that makes up 20 % of the total ejaculate volume (Dixon et al., 1999). Fluid that is secreted by epithelial cells lining the acini and prostate ducts is rich in proteins, enzymes and metal ions. It includes proteases such as prostate-specific antigen (PSA), prostatic acid phosphatases (PAPs), immunoglobulins and zinc (De Marzo et al., 1999a).

The physiological role of PSA is believed to be liquefying the seminal fluid. Transcription of PSA is influenced by androgens, restricting production to the prostate epithelium. It is released into seminal fluid in which PSA concentrations range from 0.3 to 3 mg/ml (10-100 μmol/l) (reviewed by Lilja et al., 2008). Normally, the PSA is

tightly held in the prostate and only a minor proportion leaks into the circulatory system. In the blood, median PSA level is approximately 0.6 ng/ml (reviewed by Lilja et al., 2008). PSA exists in the blood in multiple forms as free or complexes with the various protease inhibitors or as proprotein or mature protein. In the detection and monitoring of prostate cancer different forms are used such as total PSA, free PSA, and PSA density (reviewed by Lilja et al., 2008). The influence of prostatic diseases on PSA levels are discussed in sections 2.2.3.1 and 2.3.3.1.

The prostate fluid nurtures and protects sperm during transport to potential ovum fertilization (De Marzo et al., 1999a). Other functions that the prostate exhibits are enzymatic activities. Prostatic proteases are involved in the degradation of other accessory sex gland products, and prostatic secretion takes part in enzymatic conversion of seminal vesicular fructose into glucose. Further, the prostate seems to have autocrine/paracrine regulatory activity. The prostate locally produces growth factors, that play a role in normal development of the function, but which may also participate in the development of prostatic diseases. Growth factors that have been found to be produced in the prostate include e.g. fibroblast growth factors (FGF) and insulin-like growth factors (IGF) (Dixon et al., 1999).

2.2 Prostatic inflammation

2.2.1 The National Institutes of Health (NIH) classification of prostatitis

A classification system for the prostatitis has been developed by the National Institutes of Health (NIH) in 1995 (Nickel et al., 1999). The NIH prostatitis classification system involves categories I to IV (Table 1) based on symptoms and microscopic and culture evaluation of prostate-specific specimens [expressed prostatic secretion (EPS), ejaculate, post-prostate massage urine, prostate biopsy] (Nyberg et al., 1999).

Table 1. The NIH prostatitis classification (Summarized from Nickel et al., 1999 and Habermacher et al., 2006)

NIH classification	Characteristics	Prevalence
Category I	Acute bacterial prostatitis	2-5% of cases
Category II	Chronic bacterial prostatitis	2-5% of cases
Category III	Chronic prostatitis/ Chronic pelvic pain syndrome A. Inflammatory B. Non-inflammatory	90-95% of cases
Category IV	Asymptomatic inflammatory prostatitis	unknown

2.2.2 Pathology of acute and chronic non-bacterial prostate inflammation

The normal prostate contains some inflammatory cells consisting of scattered stromal and intraepithelial T- and B-lymphocytes, macrophages and mast cells (Lucia and Torkko, 2004; De Marzo et al., 2007).

In the mildest form of acute prostatitis in asymptomatic patients, small numbers of neutrophils are found in the secretory cell layer and within the gland and duct lumen. The secretory cell layer is broken with increasing severity and glands become filled with inflammatory exudate containing intact and fragmented neutrophils, degenerating epithelial cells and macrophages engulfing necrotic debris. Concomitantly, prostatic glands often go atrophic, shrunken and irregular. It has been suggested that when the epithelial layer is destroyed, the inflammatory process spills into the adjacent stroma but currently there is no data to support this progression from glandular to stromal form (Boag and Young, 1999).

In the chronic form of prostatitis, the pathology is best described in asymptomatic patients but qualitatively symptomatic non-bacterial prostatitis is similar. The most common pattern of inflammation is lymphocyte infiltration adjacent to the prostatic acini; periglandular inflammation localized mainly in the peripheral zone. In periglandular inflammation, scattered lymphocytes are often seen, but even lymphoid nodules or follicles are present. Subtyping of lymphocytes has shown that the majority are T-lymphocytes with a smaller number of B-lymphocytes (John et al., 2001). In cases with large numbers of periglandular inflammation, the degree of epithelial infiltration is also greater and the effects on epithelial cells are more prominent. In these cases, CD8 lymphocytes (cytotoxic/suppressor T-lymphocytes) predominate in the intraepithelial component while CD4 lymphocytes (helper T-lymphocytes) locate in the stroma. The proportion of CD8 lymphocytes has been shown to increase with the increased degree of the inflammation (Boag and Young, 1999; Doble, 1999).

In addition to periglandular inflammation in the chronic form of prostatitis, stromal and glandular inflammation also commonly coexists. There are sheets and clusters of lymphocytes in the stroma without contact to glands. Infiltrates may also be present around small veins (Kohnen and Drach, 1979). In the glandular form of the inflammation, neutrophils and macrophages are typically found in the lumina, and lymphocytes in the epithelium. Mild acute glandular inflammation (neutrophil-rich) in combination with chronic periglandular and stromal inflammation (lymphocyte-rich) is common finding (Boag and Young, 1999).

2.2.3 NIH category III prostatitis (chronic prostatitis/chronic pelvic pain syndrome)

2.2.3.1 Symptoms and diagnosis

At present, there is no standard diagnostic method for chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and it should be emphasized that category III prostatitis is often a diagnosis of exclusion (Habermacher et al., 2006). Patients are clinically characterized by symptoms of pain or discomfort in the pelvic region and possibly obstructive (hesitancy, weak stream) or irritative (frequency, urgency) voiding symptoms (Habermacher et al., 2006; Schaeffer, 2006). Pelvic pain is a prerequisite symptom for diagnosis of CPPS since studies have shown that it is the most consistent symptom differentiating category III prostatitis from other mimicking conditions (Habermacher et al., 2006). Symptoms tend to worsen and ameliorate with time in a cyclical fashion (Bergman and Zeitlin, 2007).

Chronic prostatitis-like symptoms are evaluated by using the National Institutes of Health Chronic Prostatitis Symptom Index, NIH-CPSI which has also been translated into other languages, including a valid Finnish version (Leskinen et al., 2003). Laboratory tests to differentiate NIH categories IIIA and IIIB includes Meares-Stamey 4-glass test (or 2-glass test), that rests on finding an excessive amount of leucocytes in expressed prostatic secretion (EPS) after prostate massage or in urine samples obtained before and after prostate massage (refers to specimens from urethra and bladder) (Doble, 1999; Richard et al., 2003). The differentiation is still problematical. Firstly, leucocytes may normally exist in EPS and the voided bladder specimen. Secondly, the finding of leucocytes in EPS of patients with non-bacterial prostatitis is episodic and thirdly, the technique to demonstrate leucocytes in EPS has an effect on the results (Richard et al., 2003).

In addition, several studies have indicated that there does not seem to be a correlation between the finding of the leucocytes in EPS or inflammation on needle biopsy and severity of clinical symptoms of prostatitis (reviewed by Pontari, 2003; Schaeffer, 2003; Pontari and Ruggieri, 2004; Wiygul, 2005; Nickel, 2008a). A recent study examined the relationship between symptoms of prostatitis and histological inflammation (Nickel et al., 2007b). No differences were observed between the distribution of inflammation status in men with or without chronic prostatitis-like symptoms. Significant correlations were found between average chronic inflammation and total CPSI score and subscores for urinary symptoms and quality of life. Still, the authors emphasized the magnitude of correlations being small and more studies are needed to determine whether symptoms and inflammation are truly associated.

The significance of the alterations in the PSA levels concerning prostatitis is still undetermined. Increased PSA levels have been found in relation to asymptomatic prostatic inflammation (Kobayashi et al., 2008). Increased levels have been considered to be caused by leakage of PSA from the acini and ductal lumina to the interstitium,

because prostate duct integrity is disturbed (Kandirali et al., 2007). In the study by Gumus et al. (2004) inflammation, especially in the perivascular field, was found to increase serum PSA levels. This was considered to be a result of the destroyed barrier between glandular tissue and blood flow. However, the relationship between PSA and men with symptomatic prostatitis has been less studied. Nadler et al. (2006) studied men with CP/CPPS (NIH category IIIA and IIIB). The authors concluded that although small elevations in PSA and percent free PSA were present in patients with CP/CPPS, these differences were not clinically significant nor they could thus be used as an effective biomarker for the condition.

2.2.3.2 Epidemiology

Prostatitis affects 10-14% of men of all ages and ethnic origins. Even 50% of men at some point in their lives suffer from this condition. The overall prevalence of physicians' diagnoses of prostatitis has been around 10% (Schaeffer, 2003). A population-based study in Finland showed overall lifetime prevalence 14.2 % (Mehik et al., 2000b). Roberts et al. (1998) showed that incidence of chronic prostatitis increases with age and at 30-40 years of age there is a peak in incidence. In addition Nickel et al. (2001a) showed that the young men indeed often suffer from chronic prostatitis; prevalence being 11.5% in men younger than 50 years compared to men over 50 years of age with prevalence 8.5%. Race has not been considered to impact on the prostatitis diagnosis. However, the prevalence rate of prostatitis-like symptoms was lower in Japanese men (4.9 %) (Kunishima et al., 2006) when compared to 9.7 % in Canadian men (Nickel et al., 2001a).

2.2.3.3 Etiology

The etiology of CP/CPPS remains unclear despite many studies of possible causes. It is possible that patients in this category have different etiologies (Rivero et al., 2007). Some studies have suggested CP/CPPS to be a cluster of related syndromes without a single unifying cause and some even suggest that the prostate is not responsible for the symptoms (Habermacher et al., 2006).

The current consensus suggests that CP/CPPS arises from interaction between immune, endocrine and neuronal causes as well as the psychological status of the individual (Pontari and Ruggieri, 2004; Wiygul, 2005; Habermacher et al., 2006). The pain of chronic prostatitis may indeed be a result of neurogenic inflammation in the peripheral and central nervous systems (Pontari & Ruggieri, 2004). Neurogenic inflammation is triggered by the release of substances from nerves, such as substance P and calcitonin gene-related peptide which causes edema, neutrophil accumulation, are implicated in the formation of radical oxygen species, and promote contraction of the ureter, bladder and urethra (Geppetti et al., 2008). Interestingly, it has been observed that men with CP/CPPS are much more likely to suffer from chronic sinuitis pointing to systemic

inflammation mechanisms (Pontari, 2003). The possible initiators may thus include infectious, inflammatory, mechanical, hormonal or neuronal causes (Pontari and Ruggieri, 2004; Habermacher et al., 2006). The reflux and influx of sterile urine is for example considered to cause inflammation in the prostate. Interestingly, in CP/CPPS patients, intraprostatic pressure has been shown to be increased (Mehik et al., 1999; Mehik et al., 2000a). Despite the triggering factor, the inflammatory process may cause tissue edema and increased intraprostatic pressure leading eventually to chronic pain (Mehik et al., 2003).

Prostatitis may also develop from an autoimmune basis (Alexander et al., 1997; Batstone et al., 2002; Motrich et al., 2007; Rivero et al., 2007). Normally the immune system acquires self-tolerance by deletion of autoreactive T cells in the thymus in the perinatal period and by functional suppression of autoreactive T and B cells at later stages of development. However, there may occure a failure in the maintenance of self-tolerance and failure to discriminate between self and non-self antigens (Wiygul, 2005). Based on immunology studies and seminal plasma cytokine levels it has been suggested that prostatic inflammation is mediated by an adaptive immune response directed against self genital tract antigens, with an autoimmune process (Richard et al., 2003; Motrich et al., 2007). Interestingly, autoimmune diseases are more common in women than men. This suggests that hormones, especially estrogens, may be involved the development of autoimmune diseases. As estrogens are also considered as one important possible cause also for prostatitis, the role of estrogens in prostatic inflammation is discussed more thoroughly in sections 2.5.3 and 2.6.3.1.1.

2.2.3.4 Medical treatment options for CP/CPPS

As discussed above, the pathogenesis and the diagnosis of CP/CCPS are largely unknown and unspecified. For these reasons, effective treatment for CP/CPPS remains uncertain and current treatments focus on symptomatic relief (Dimitrakov et al., 2006; Schaeffer, 2006). The therapies used include antibiotics, α-blockers, anti-inflammatories and 5α-reductase inhibitor.

The role of antibiotics especially in category IIIB is still unclear, and it has been suggested that antibiotics should not been used for long-periods or repeated courses. Anti-inflammatory therapy such as the use of non-steroidal anti-inflammatory drugs (NSAID) has been shown to provide at least some symptomatic improvement in CP/CPPS patients. However, considering the potential side-effects, their use is not recommended for long-periods (Schaeffer, 2003).

Recent studies suggests that the α_1-adrenoceptor antagonists, α-blockers, have a role in the management of prostatitis syndromes (Mehik et al., 2003; Nickel, 2006a; Lee et al., 2007; Nickel 2008b). α-blockers have already been used in the treatment of lower urinary tract symptoms (LUTS) but they have gained attention in the treatment of chronic prostatitis for several reasons: they relax smooth muscle in the prostate, urethra

and bladder; α_1-receptors located in the central nervous system (CNS) may be implicated in the pain and in addition, α-blockers may reduce neurogenic inflammation in the lower urinary tract (Geppetti et al., 2008; Nickel 2008b).

5α-reductase type 2-inhibitor, finasteride, decreases conversion of testosterone to dihydrotestosterone and it has been used in the treatment of benign prostatic hyperplasia (BPH). Recently, its efficacy in CP/CPPS has also been studied. Leskinen et al. (1999) showed finasteride to reduce pain and voiding symptoms and Kaplan et al. (2004) showed quality of life and pain to be improved. The mechanisms are still unclear and suggested explanations include, for example, reduction of edema and pressure sensations in the prostate, reduction in the glandular part of the prostate and inflamed area and shrinkage of the prostate during finasteride treatment (Leskinen et al., 1999). Finasteride may thus be effective especially in men with CPPS and BPH (Nickel et al., 2004).

There are also several novel suggested medical approaches to treat pain and muscle spasticity such as tricyclic antidepressant medications and anticonvulsants (Nickel et al., 2007a). Phosphodiesterase 5 (PDE5) inhibitors such as sildenafil (Viagra) have been used for erectile dysfunction. In addition, they have also been hypothesized to be effective in the treatment of CP/CPPS due to their capability to relax prostatic duct smooth muscle (Grimsley et al., 2007). Interestingly, the use of mepartricin has been shown to provide significant symptomatic improvement in men with CPPS (De Rose et al., 2004). Mepartricin is a compound which belongs to the polyene macrolide class of antifungal agents, and it has been developed as a drug for the treatment of BPH (Shakutou et al., 1999). Mepartricin studied both in humans and rats, is able to increase fecal estrogen excretion resulting in a decrease in estradiol levels in serum (Tavanti et al., 1989; Del Vecchio et al., 1990; Shakutou et al., 1999). This raises the question of the significance of antiestrogenic therapy in CP/CPPS in the future and warrants efficacy and safety clinical trials.

2.3 BPH, PIN-lesion and prostate cancer

2.3.1 Benign prostatic hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) is common age-related proliferative abnormality of the human prostate, especially noted within the transition zone and periurethal areas (Lee and Peehl, 2004; Wang et al., 2008). BPH is correctly defined as enlargement of the prostate gland from the progressive hyperplasia of stromal and glandular prostatic tissue. Clinical BPH refers to the lower urinary tract symptoms (LUTS) associated with benign prostatic enlargement causing bladder outlet obstruction (BOO) (Nickel, 2008a).

Despite the high prevalence of BPH, its detailed pathogenesis remains unknown (Wang et al., 2008). The cause is considered to be multifactorial including for example, the effects of androgens and estrogens in the growth of stromal and epithelial cells (Shibata et al., 2000) and interestingly, impairment of blood supply to the lower urinary tract causing chronic ischemia (Berger et al., 2005; Berger et al., 2006a; Berger et al., 2006b). In addition, inflammation seems to play an important role in the initiation, development as well as evolution of BPH, suggesting that BPH is an inflammatory disease (Kramer et al., 2007; Mishra et al., 2007; Nickel, 2008a; Wang et al., 2008). Histologically, nodules of BPH patients contain infiltrates of T-lymphocytes, macrophages and B-lymphocytes that are chronically activated. These infiltrating cells are responsible for the production of cytokines which may support fibromuscular growth in BPH (Wang et al., 2008). Another explanation of stromal overgrowth may come from the fact that estrogens increase important growth factors such as TGFβ1 and 2, basic fibroblast growth factors, and insulin-like growth factors (Eaton 2003). Although there is currently no candidate for a foreign antigen that would trigger immune response, evidence has been obtained that inflammation in BPH would have an autoimmune component (Kramer et al., 2007).

Based on the observations between inflammation and BPH, new treatment strategies based on anti-inflammatory action should be considered. At present no evidence has been obtained e.g. from the use of anti-inflammatory therapy or use of non-steroidal anti-inflammatory drugs in BPH patients (Wang et al., 2008). At present the lack of representative BPH *in vivo* models complicates drug development. In addition to man, spontaneous BPH is only observed in dogs and chimpanzees (Mahapokai et al., 2000) and in the rat and mouse prostate spontaneous BPH is totally absent.

2.3.2 Prostatic intraepithelial neoplasia (PIN-lesion)

Prostatic intraepithelial neoplasia (PIN) is well-established as precursor of prostatic carcinoma (Bostwick and Qian, 2001; Brawer, 2005; Montironi et al., 2006) though all studies have not found PIN as predicative for prostate cancer (Postma et al., 2004). PIN is abnormal proliferation within the prostatic ducts, ductules, and acini (Bostwick and Qian, 2001). Currently, PIN is divided into two grades (low and high; LGPIN and HGPIN, respectively) (Montironi et al., 2006). The continuum from LGPIN to HGPIN and early invasive cancer is characterized by basal cell layer disruption; progressive loss of markers of secretory differentiation; increasing nuclear and nucleolar abnormalities and proliferative activity (Montironi et al., 2006). Interestingly as proliferation normally occurs in the basal cell layer, in PIN lesions the greatest proliferation occurs on the luminal surface (Bostwick and Qian, 2004). HGPIN, that can be identified based on prominent nucleoli within an existing duct structure, is divided into four main patterns: tufting, micropapillary, cribriform and flat (Brawer, 2005). The clinical importance of recognizing HGPIN is based on its strong association with prostatic carcinoma though there are no clinical differences between these four patterns (Bostwick and Qian, 2004).

The incidence, extent and grade of PIN increase with age. The average incidence has been reported ranging from 4 to 16 % (Bostwick and Qian, 2004). In addition to age, race and a spectrum of molecular and genetic abnormalities similar to prostate cancer have also been observed to effect on PIN incidence (Sakr and Partin, 2001). PIN seems to represent an intermediate stage between benign epithelium and invasive malignant carcinoma by preceding prostate cancer by several years (Brawer, 2005). As with prostate cancer, PIN is multicentric and is also often located in the peripheral prostate zone (Brawer, 2005). Because PIN is found as a strong marker of risk for prostate cancer, it represents an appropriate target for prostatic chemoprevention (Sakr and Partin, 2001; Brawer, 2005). Indeed, androgen deprivation therapy often used in prostate cancer treatment, also decreases the prevalence and extent of HGPIN (Bostwick and Qian, 2004). In general, basal cells in the normal prostate have no androgen receptor but luminal secretory cells express a high level of androgen receptor and show sensitivity to androgens (van Leenders et al., 2003; Bostwick and Qian, 2004). Thus the luminal cells of HGPIN probably share this same androgen sensitivity (Bostwick and Qian, 2004).

2.3.3 Prostate cancer

2.3.3.1 Etiology

Prostate cancer is a leading cause of illness and death among men in the United States and Western Europe (Crawford, 2003b; Nelson et al., 2003; De Marzo et al., 2007). Incidence of prostate cancer increases with age, e.g. autopsy series have showed small prostatic carcinomas in 29 % of men 30-40 years of age and in 64 % of men 60-70 years of age (Nelson et al., 2003). To evaluate whether population-based screening could reduce mortality from prostate cancer, multicentre trial, European Randomised Screening Program of Prostate Cancer (ERSPC), has been started but yet no conclusive data is available (De Koning et al., 2002; Finne et al., 2003; Van der Kwast et al., 2003). At present, screening of prostate cancer is based on PSA measurement, digital rectal examination (DRE) and definitive diagnosis also requires transrectal ultrasound (TRUS)-guided needle biopsy. Prostate cancer causes PSA to be released into the circulatory system and to increase serum PSA levels. However, PSA is not specific to prostate cancer: benign prostate diseases often cause increase in serum PSA and on the other hand, most men with increased PSA do not have prostate cancer. Thus PSA testing may be best considered as an indicator of risk to be considered in combination with other factors. In addition, it has been suggested that the release of PSA into blood might begin early in the process of carcinogenesis, and could even be a causative factor (Lilja et al., 2008).

Dietary and lifestyle-related factors as well as androgens have long been considered as contributors to the risk of prostate cancer (Nelson et al., 2003). The association between obesity and prostate cancer has also been considered but the precise

association is still unclear (Goldstraw et al., 2007). Normally, prostate cancer incidence rates have been low in Asia, but the rate e.g. in Japan has been continually increasing. The major contributing factor has been considered to be change in the diet to more Western style with higher fat and meat intake (Crawford, 2003b). The risk of prostate cancer among Asians also increases when they immigrate to North America (Nelson et al., 2003). These both support the significance of environmental and lifestyle-related factors in causing prostate cancer. It should be noted that improved diagnostic methods such as the use of PSA testing and more biopsies guided TRUS to the detection of prostate cancer may also be factors contributing to the increased incident rate of prostate cancer in Japan (Crawford et al., 2001).

Whereas the majority of prostate cancers are a result of environmental and cultural habits, approximately 5 to 10 % of all prostate cancer cases are hereditary (De Marzo et al., 1999a; Crawford, 2003b; Crawford, 2003a). Studies of inherited prostate cancer-susceptibility genes have revealed an increased risk of prostate cancer with the mutant RNASEL-gene (encodes endoribonuclease that degrades viral and cellular RNA) and MSR1-gene (macrophage-scavenger receptor 1 expressed in the macrophages in the inflamed prostate) (Nelson et al., 2003). Recently, interesting finding was done when prostate-specific, strongly androgen-regulated gene TMPRSS2 (transmembrane protease, serine 2), was identified to have fusion with Ets family gene (oncogenic transcription factor; ERG) even in 50-70 % of prostate cancer cases (Tomlins et al., 2005; Rubin 2008). In Finnish study by Saramäki et al. (2008) the frequency of TMPRSS2-ERG fusion was ~30%. TMPRSS2-ERG fusion is regulated by estrogen receptor (ER) dependent mechanism yielding to prostate cancers that are molecularly distinct from other prostate cancers (Setlur et al., 2008). TMPRSS2-ERG fusion has been frequently but not unequivocally associated with more aggressive prostate cancers and poorer prognosis (Demichelis et al., 2007; Kumar-Sinha et al., 2008). Indeed, some studies have concluded fusion to have favorable prognosis (Saramäki et al., 2008). Concerning precancerous lesions, approximately 16% of HGPIN lesions have TMPRSS2-ERG fusion associated with concurrent TMPRSS2-ERG prostate cancer suggesting fusion to be an early event (Mosquera et al., 2008).

2.3.3.2 Prostate inflammation as a contributing factor for prostate cancer

About 20% of all human cancers are caused by chronic infection or chronic inflammatory states, including those affecting the liver, stomach and large intestine (De Marzo et al., 2007; Mantovani et al., 2008). The molecular mechanisms that underlie the pathogenesis of inflammation-associated cancer are complex. Activated, phagocytic inflammatory cells may release highly reactive chemical compounds, that cause oxidative or nitrosative damage to DNA in the epithelial cells. This in turn results in damaged cells that must be replaced by cell division from resident stem cells. Inflammatory cells also secrete cytokines that promote epithelial cell proliferation and stimulate angiogenesis. Furthermore, inflammatory cells might facilitate epithelial cell invasion into the stromal and vascular compartments leading to metastasis (Lucia and Torkko, 2004; De Marzo et al., 2007; Sciarra et al., 2008).

There is also emerging evidence that inflammation has a role in the etiology of prostate cancer (reviewed by Ho E et al., 2004; De Marzo et al., 2007; Sciarra et al., 2008; Haverkamp et al., 2008). This evidence is obtained mainly from epidemiological and molecular pathological studies (De Marzo et al., 2007). The anatomical relationship between inflammation and cancer studied by histopathological methods is inconclusive (Lucia and Torkko, 2004). There are many reasons for this e.g. difficulties in acquiring prostate tissue from men without indication for biopsy (Sutcliffe and Platz, 2007); the lack of systematic assessment and quantification of inflammation in biopsy samples (Karakiewicz et al., 2007), and the view that inflammation may contribute earlier events in carcinogenesis rather than being present at the tumor site (Lucia and Torkko, 2004).

The proliferative inflammatory atrophy (PIA) may offer a possible link between prostatic inflammation and cancer (De Marzo et al., 1999b). It was designated to discrete foci of proliferative glandular epithelium with the morphological appearance of simple atrophy or postatrophic hyperplasia, occurring in association with inflammation often locating in the peripheral prostate (De Marzo et al., 1999b). PIA has been postulated to arise in the setting of increased oxidative stress, most likely derived from the close inflammatory cells (De Marzo et al., 1999b). PIA is suggested to give rise to prostatic carcinoma either directly or indirectly via development into PIN (De Marzo et al., 1999b; Putzi and De Marzo, 2000). However, recent study by Woenckhaus & Fenic (2008) does not find a clear role for PIA in the development of prostate cancer and authors concluded that more examinations of genetic or epigenetic aberrations are needed.

In a follow-up study using prostate needle biopsies MacLennan et al. (2006) demonstrated that a history of chronic inflammation is associated with an increased risk for prostate cancer. Interestingly, androgen ablation treatment of patients with prostate cancer triggered T-cell mediated inflammation within the prostate (Mercader et al., 2001). A number of mechanisms contibuting to T cell response was suggested by the authors; apoptosis and injury fo hormone-dependent prostate tumor and epithelial cells; disruptor of prostate tumor vessels or modulation of locally produced cytokines. On the other hand, absence of inflammatory cells close to adenocarcinoma may be caused by tumor immunosuppression (Blumenfeld et al., 1992; Miller and Pisa, 2007).

It is noteworthy that histopathological studies on inflammation and prostate cancer have mainly focused on characterizing lymphocytes (Lucia and Torkko, 2004). Tumor associated macrophages (TAM) have been considered to particularly promote tumor invasion and metastasis (Mantovani et al., 2006). In addition, mast cells have also been identified in prostate tumors and implicated in the growth of tumors by increasing tumor vascularization and metastasis (Haverkamp et al., 2008). Indeed, in addition infiltrating cells, more data should be obtained to address the function of infiltrating inflammatory cells or the impact they may have on prostate carcinogenesis (Haverkamp et al., 2008).

2.3.3.3 Pathology

The most common type of prostatic carcinomas are malignant tumours of glandular epithelium; adenocarcinomas (Montironi et al., 2006). They may exhibit ductal-, mucinous-, or signet ring cell features. Prostatic adenocarcinoma originates in the peripheral zone in approximately 75% of cases, with the remainder originating in the transition zone. Transition zone tumours are usually smaller and better differentiated compared to peripheral zone tumours (Hamdy et al., 2004). The central zone accounts smaller number of prostatic adenocarcinomas (Mai et al., 2008).

The diagnostic criteria for prostatic carcinoma include findings in the architectural and cytological features as well as luminal, stromal and immunohistochemical findings. Architectural features specify the spacing, size and shape of the acini. Malignant acini are usually small or medium, have irregular arrangement and are randomly scattered in the stroma in clusters or singly. One important diagnostic feature is also the absence of basal cell layer. The cytologic features include nuclear and nucleolar enlargement which occurs in most malignant cells. The most important immunohistochemical markers are lack of basal cell markers, such as high-molecular weight keratin 34βE12 and p63. α-Methyl-coenzyme A racemase (AMACR) is often intensively stained in prostatic adenocarcinomas and can thus be used as a confirmatory stain (Montironi et al., 2006).

2.3.3.4 Histological grading and tumour staging

For grading of prostate cancer, the Gleason grading system is recommended (reviewed by Humphrey 2004, Epstein et al., 2005; Lopez-Beltran et al., 2006). The system is based on the degree of glandular differentiation and the architectural pattern of growth. The Gleason score is composed of a primary and secondary pattern that are each analyzed on a scale of 1 to 5, resulting in a total score between 2 to 10. Prostate cancer is often multifocal. Foci may have different Gleason scores and thus it is important to find as many foci as possible to eliminate sampling error (Crawford et al., 2001) and to include the highest grade in scoring of biopsies. In addition to the Gleason grading system, the World Health Organization (WHO) nuclear grading system can be used. In this method, nuclear anaplasia is scored as 1, 2 and 3 corresponding to slight, moderate, and marked nuclear changes, respectively (Montironi et al., 2006).

For tumour staging, the TNM (Tumour, Nodes, Metastasis) system is most commonly used (Sobin et al., 2002; Schröder et al., 1992). Prostate cancer often spreads to the pelvic lymph nodes, bones and lung. The TNM classification of prostate cancer defines tumours in a scale of Tx to T4 (primary tumour can not be assessed to tumour is fixed or invading into neighboring organs); nodes in a scale of Nx to N1 (regional lymph nodes cannot be assessed to regional lymph node metastases) and metastasis in a scale of Mx to M1 (no distant metastases to distant metastates present) (Hamdy et al., 2004).

2.3.3.5 Overview on medical management and prevention

As androgens have long been recognized to be required for normal prostatic development, androgen deprivation is an established treatment for advanced prostate cancer (Nelson, 2004). Androgen deprivation may be achieved by physical means (castration) or by biochemically (gonadotropin-releasing hormone (GnRH) agonists, estrogens, antiandrogens or androgen receptor agonists or antagonists) (Damber & Aus 2008). However, despite treatment with androgen deprivation and/or anti-androgens, most men with advanced prostate cancer ultimately suffer hormone-refractory prostate cancer (HRPC) (Autorino et al., 2003). At present, the treatment options for HRPC include chemotherapeutic regimens such as taxanes (Autorino et al., 2003) but new therapeutic options are needed especially for this final stage of prostate cancer.

In general, it has been estimated that even 1/3 of all cancer deaths can be prevented by changes in diet and lifestyle and/or through the use of preventive drug therapy (Crawford 2003b). However, concerning prostate cancer not all suspected risk factors (age, ethinicity, and family history) are modifiable. Suggested candidate chemopreventive agents for prostate cancer include hormonal agents (5-α-reductase inhibitors, antiandrogens, aromatase inhibitors and selective estrogen receptor modulators, SERMs), non-steroidal anti-inflammatory drugs (NSAID), specific cyclooxygenase-2 (COX-2) inhibitors, antioxidants (vitamin E, lycopene, selenium and carotenoids), and phytoestrogens (isoflavonoids) (reviewed by Crawford 2003b; Etminan et al., 2004; Harris et al., 2005; Hussain et al., 2003; Thomas et al., 2008). Interestingly, also the cholesterol-lowering drugs, statins, may have a role in the prevention of prostate cancer (Murtola et al., 2008).

The recognition that prostate inflammation may contribute to prostate carcinogenesis might give new possibilities for prevention. Epidemiological studies have achieved e.g. a modest protective effect of non-steroidal anti-inflammatory drug intake on prostate cancer incidence and progression (De Marzo et al., 2004). In the future, as the process of inflammation in the prostate and the pathogenesis of precancerous lesions become better defined, more specific targets can be identified for prostate cancer prevention (De Marzo et al., 2004).

2.4 The lower urinary tract (LUT)

2.4.1 The structure and function of the LUT

The lower urinary tract (LUT) consists of bladder and urethra. The bladder itself consists of three layers; an epithelial layer, a muscular layer and an adventitial layer. The smooth muscle layer of the bladder, detrusor, forms the thickest layer of the bladder wall. In the bladder, between the two ureters, is triangular region called trigone and below this, the bladder neck that forms the proximal sphincter mechanism. The distal sphincter mechanism consists of periurethal musculature (levator ani), urethal smooth muscle and a striated muscle component called the rhabdosphincter. It is oriented predominantly anteriorly in a signet ring shape (Mundy, 2004).

The male urethra is divided into three parts; prostatic, membranous and spongy. The prostatic part begins at the apex of the trigone of the bladder and descends through the prostate. The wall of the prostatic urethra has a groove on each side, called the prostatic sinus in which most prostatic ductules open while some open along the sides of the urethral crest. In the middle part of the crest is the seminal colliculus the opening of which leads to the prostatic utricle (remnant from embryonal stage) and to the opening of an ejaculatory duct. The bladder wall as well as ureters above and prostatic urethra below are lined by urothelium; multilayered transitional epithelium. Urothelium is somewhat impermeable to fluids and solutes but it is not still inert since drugs can be absorbed from the bladder and it contains sensory nerves (Mundy, 2004).

The innervation of the bladder and urethra is from three separate neuronal pathways: sympathetic (hypogastric nerves), parasympathetic (pelvic nerves), and somatic (pudendal nerves) and they contain both afferent and efferent pathways. Micturition is fundamentally a spinal reflex facilitated and inhibited by higher brain centers, and subject to voluntary facilitation and inhibition. Briefly, during bladder filling, intravesical pressure changes very little until compliance is achieved and awareness on bladder filling. Efferent activity is initiated and mediated through pelvic parasympathetic nerves (cholinergic nerve fibers). In the detrusor, acetylcholine works as transmitter by binding to a muscarinic receptor on the smooth muscle membrane. Another transmitter, extracellular ATP, binds to a specific purinergic receptor ($P2X_1$). By binding to its receptor, a series of cellular events lead to the rise of intracellular Ca^{2+} and initiates the process of detrusor smooth muscle contraction and synchronous coordinated reciprocal relaxation of the urethal sphincter until the bladder is empty (Fry, 2004; Mundy, 2004).

2.4.2 Urodynamical measurements

Urodynamics means a study which aims to measure and determine the relationship between bladder volume, bladder pressure (cystometry) and urine flow under physiological conditions. Though there may sometimes appear a lack of correlation between symptoms and urodynamic measurements, urodynamics can lead to important findings when making diagnosis (Craggs and Knight, 2004).

In standard urodynamic testing, pressure, flow and volume are usually measured. In general, micturition consists of the storage and voiding phases. During normal bladder filling, bladder pressure stays almost unchanged and intraurethal pressure appears to slightly rise. Just before voiding, there is a decrease in the intraurethal pressure matched by a cessation of the electromyographic activity of the intrinsic rhabdosphincter. After this bladder pressure exceeds urethral pressure and voiding occurs. The rise in bladder pressure is maintained until the bladder is empty; then bladder pressure returns to normal and the bladder filling-voiding cycle starts again. The urine flow rate is dependent on the driving force (bladder) and resistance to flow. Resistance depends on the active and passive factors in the urethra e.g. urethal lumen shape and length and active contraction of the sphincter (Craggs and Knight, 2004; Mundy, 2004).

2.4.3 Lower urinary tract symptoms (LUTS)

Lower urinary tract symptoms (LUTS) are common in men and they tend to increase with age. In Finland, the prevalence of at least one symptom was 89% studied in population-based survey (Koskimäki et al., 1998). LUTS are related to storage symptoms (urgency, frequency, nocturia, incontinence), voiding symptoms (hesitancy, intermittency, slow stream) or postmicturition symptoms (feeling of incomplete emptying, postmicturition dribbling) (Rosenberg et al., 2007; Patel and Chapple, 2008). LUTS may also be related to pain (perineal, prostate, or pain on ejaculation) or both urination and pain (dysuria). Assessment of the symptoms and bother is based on different scores such as International Prostatic Symptom Score (IPSS) and the Danish Prostatic Symptom Score (DAN-PSS-1); the latter being in clinical use in Finland (Häkkinen et al., 2007).

2.4.3.1 LUTS and BPH

Symptoms are a manifestation of clinical diseases of the bladder (ageing, obstruction, neurogenic related bladder conditions) and prostate (BPH, prostatitis, prostate cancer) (Nickel, 2006b). Age-related endocrine changes, especially in androgens and estrogens, are considered to facilitate LUTS as well as development of BPH (Schatzl et al., 2000). BPH refers to LUTS by causing bladder outlet obstruction (BOO). The two considered components of obstruction are prostatic enlargement (static component) and

increased smooth muscle tone in the bladder neck and prostate (dynamic component) (Nickel, 2006b). However, since all LUTS are not prostate related, the evidence for a direct link between BPH, BOO and LUTS is weak (Andersson, 2007). Thus, LUTS in men might be due to BOO secondary to non-prostatic cause or related to neither BOO nor prostatic disease (Patel and Chapple, 2008).

2.4.3.2 LUTS and prostatitis

Another prostate disease that is common cause of LUTS is prostatitis. As clinical BPH is characterized by voiding LUTS, prostatitis is characterized primarily by pain but also with voiding disorders of various degrees (Hochreiter and Z'Brun, 2004). Indeed, a recent study concerning the relationship between prostate inflammation and LUTS assessed with IPSS, found evidence for the association of the degree of LUTS and the degree of chronic inflammation (Nickel et al., 2007c). The pain and voiding symptoms in patients with CP/CPPS may be caused by structural or functional obstruction of the lower urinary tract. Another cause may be bladder outlet dysfunction, bladder neck hyperplasia, or spasm of the pelvis floor muscles (pseudodyssynergia). These could lead to changes in flow and pressure and possible reflux of urine into prostatic ducts (Hochreiter and Z'Brun, 2004). Kaplan et al., (1996) studied men at 50 years of age or younger diagnosed to have chronic prostatitis. However, videourodynamic studies revealed that 24% had pseudodyssynergia, 49% had detrusor instability and 54% had primary bladder neck obstruction. The conclusion was that young men with pelvic pain associated with chronic voiding dysfunction are frequently misdiagnosed as having chronic non-bacterial prostatitis. Thus urodynamic studies are recommended for accurate diagnosis, especially when voiding symptoms coexist with perineal or pelvic pain (Gonzalez and Te 2006).

2.4.3.3 Medical treatment for LUTS

Since the prostate is no longer considered as only the cause for LUTS, medical treatment has also moved from the prostate to the bladder and other extraprostatic sites (Andersson, 2007). LUTS includes symptoms of overactive bladder (OAB) and in this case anticholinergics drugs that block muscarinic receptors in the bladder are used alone or in combination with α-adrenoceptor antagonists (α-blockers) (Andersson, 2007; Patel and Chapple, 2008). LUTS suggestive BPH have traditionally been treated with α-blockers and 5α-reductase inhibitors that decrease ‘dynamic’’ and ‘static’ components of bladder outflow resistance, respectively (Andersson, 2007; Patel and Chapple, 2008). Significant improvement in LUTS IPSS score was recorded recently with the use of PDE5 inhibitors that have commonly been used in the treatment of erectile dysfunction. The mechanism of action might be based on relaxation of prostatic and urethal smooth muscle (Patel and Chapple, 2008). Other promising medical treatments of LUTS associated or suggestive with BPH include e.g. subtype

selective α_1-AR antagonists, purinoceptor antagonists, and inhibitors/antagonists of endothelin receptors (Andersson, 2007).

2.5 Estrogens, prostatic diseases and LUTS

2.5.1 General overview in the mechanisms of action

Androgens have long been known to be the major sex hormones influencing the development, maturation and carcinogenesis of the prostate. However, evidence has been obtained to show that estrogens also regulate prostatic development and function (Griffiths, 2000; Ho SM 2004; Ricke et al., 2007). They are also considered to be potent inductors in the development of prostatic diseases (reviewed by Risbridger et al., 2003; Ho SM 2004; Ellem and Risbridger, 2007; Prezioso et al., 2007). Thus the functions of estrogens in the prostate are complex, as they appear to have both beneficial and adverse effects. The effects of estrogens on the prostate can be mediated indirectly via action on the hypothalamus-pituitary-gonadal axis or via prolactin. Estrogens have also direct effects on the prostate which are elicited by an external hormone or by estradiol produced in the prostate by local aromatization of testosterone (Härönen and Mäkelä, 2004).

The estrogen action in the prostate is mediated by estrogen receptor (ER) subtypes named ERα and ERβ (Heldring et al., 2007). The ERα and ERβ have distinct tissue-specific expression and variation of physiological activities in several tissues. In the classical, genomic mechanism of estrogen action, estrogens diffuse into the cell and bind to ER, which is located in the nucleus. This nuclear estrogen-ER complex binds to estrogen response element sequences, resulting in associated protein production, and physiological response. This genomic mechanism occurs in hours. There are also non-genomic mechanisms in which response occurs within seconds or minutes. These non-genomic actions are mediated through ER-like proteins located in or adjacent to the plasma membrane, or through other non-ER plasma membrane-associated estrogen-binding proteins (Deroo and Korach, 2006; Heldring et al., 2007).

2.5.1.1 Expression of the estrogen receptors in the LUT and prostate

In the human male bladder, ERβ is found in the nuclei of epithelial and smooth muscle cells (Saunders et al., 1997; Taylor and Al-Azzawi, 2000) without expression of ERα (Saunders et al., 2001). In contrast, both ERα and ERβ, are expressed in the adult human prostate. ERα generally locates in the stromal compartment and it has been associated with adverse effects of estrogens; aberrant proliferation, inflammation and the development of malignancy. Conversely, ERβ is expressed predominantly in the prostatic epithelia and associated with beneficial effects of estrogens; anti-proliferation, differentiation and apoptosis (reviewed by Ellem and Risbridger 2007).

Corresponding locations for ERs in the rat LUT and prostate have been described. In the epithelium of the urinary bladder, ERβ but not ERα, has been detected. In addition ERβ in the rat bladder body has been found in the bladder neck, in the striated muscle cells of proximal urethra (Salmi et al., 2001) and in the prostatic ganglia (Mäkelä et al., 2000). Interestingly, ERβ in these sites was found to be co-expressed with androgen receptor (Salmi et al., 2001). In the rat prostate, ERβ is strongly expressed in the prostatic epithelium, while ERα is expressed in the stroma (Mäkelä et al., 2000).

2.5.2 Age-related changes in the sex steroid hormone contents

While considering the role of sex steroid hormones in the development of prostatic diseases, the age factor with concomintant hormonal alterations is interesting. It is well established that aging is associated with a decline of serum total and free testosterone. Decrease is due to changes in testicular function, altered regulation of Leydig cell function and increase of plasma sex hormone binding globulin (SHBG) capacity (Kaufman & Vermeulen 2005). Since testosterone is converted to estradiol by an aromatase enzyme one could expect significant decrease also in the estradiol level. However, the decrease in precursor level is compensated by an increase of fat mass and tissue aromatase activity and giving rise to stable estradiol concentrations (Prezioso et al., 2007; Kaufman & Vermeulen 2005). Other reasons for increased serum estradiol may be decreased estrogen metabolism, displacement of estrogen from sex hormone binding globuline (SHBG) or exogenous sources (Braunstein, 1999). It is interesting that serum estrogen levels in elderly men are higher than in postmenopausal women (Khosla et al., 2002).

The kind of imbalance in the androgens to estrogens, ‘relative estrogen excess’ is considered as a cause of stromal hyperplasia and prostate enlargement (Bosland, 2005; Prezioso et al., 2007). The estrogen-androgen imbalance has also been considered to cause gynecomastia; benign proliferation of the breast glandular tissue, that can be detected in up to 60% of boys during puberty (Braunstein, 1999; Shulman et al., 2008).

2.5.3 Estrogens and prostatic inflammation

Interestingly, estrogens have anti-inflammatory but also proinflammatory properties. The immunomodulating effects of estrogens are dependent on various factors including the cell types, target organ, timing and concentration of estrogens (Straub, 2007). Estrogens have been strongly linked to autoimmune processes in women, who are much more predisposed to autoimmune diseases than men (De Marzo et al., 2007). Concerning the rodent prostate, the findings of a number of experimental studies suggests that estrogens have proinflammatory effects depending on the animal species used, hormone exposure time and dosage used (discussed in detail in section 2.6.3.1.1).

In humans, the theory that hormonal disorders could promote prostatic inflammation is much less studied and explored. The hormonal abnormalities in men with CP/CPPS have been identified but no difference was found in estradiol concentrations in patients compared to controls (Dimitrakov et al., 2008). The authors concluded there may have been some limitations in the study design and follow-up studies should assess whether reported hormonal findings improve or worsen in parallel with symptom severity (Dimitrakov et al., 2008). Interestingly, estradiol has been found in association with an increased risk of having LUTS, but unfortunately CPSI was not assessed in that study (Rohrmann et al., 2007).

The most evident support for the role of estrogens in prostatic inflammation in human is obtained from the study in which patients with CP/CPPS were treated with mepartricin (De Rose et al., 2004). When CP/CPPS patients were treated with mepartricin, a decrease in the total NIH-CPSI score was observed and a statistically significant decrease with regard to pain. In addition, serum estradiol levels were significantly lower compared to patients treated with placebo (De Rose et al., 2004). It was concluded that the capability of mepartricin to lower estrogen levels may account for its clinical improvement.

Taken together these experimental and clinical findings, it is quite plausible that chronic inflammation in the adult human prostate reflects an autoimmune reaction caused, at least in part, by estrogens (De Marzo et al., 2007).

2.5.4 Estrogens and prostate cancer

Epidemiological and experimental data establish a clear link between estrogens and prostate cancer (reviewed by Härkönen and Mäkelä, 2004; Ho SM 2004; Bosland, 2005; Ellem and Risbridger, 2007; Prezioso et al., 2007; Ricke et al., 2007). The evidence includes (1) genes in the estrogen metabolism pathways have been found in association with familial prostate cancer (2) in species susceptible to prostate cancer (man and dog) the estradiol to androgen ratio increases during aging when prostate cancer manifests and is diagnosed (3) African Americans who have the highest incidence of prostatic cancer, have elevated levels of both free plasma estradiol and testosterone (4) experimental data indicates that excessive exposure to estrogens facilitates prostatic changes and malignancies (reviewed by Härkönen and Mäkelä (2004); Bosland (2005); Ricke et al. (2007)). In addition, recent molecular studies have identified an aggressive form of prostate cancer to express TMPRSS2-ERG gene fusion regulated by estrogen receptor signaling pathways (Tomlins et al., 2005; Setlur et al., 2008).

Though elevated blood estrogen in the presence of high testosterone has been considered as an important risk factor contributing to prostate carcinogenesis (Ho E et al., 2004), many epidemiologic studies have failed to show a clear association between circulating plasma steroids and prostate cancer (Carruba, 2007; Sampson et al., 2007). However, there are several issues related to measurement of both androgens and

estrogens which may explain inconsistent and inconclusive data. Prostate cancer requires 20-30 years until clinical manifestation, and thus the impact of timing should be considered in measurements. On the other hand, estrogen exposure early in uterine or perinatal life may lead to permanent changes in the prostate. In addition, circulating steroids may not represent intraprostatic concentrations and the enzyme metabolism in the prostate may have been changed (Carruba, 2007).

As the normal prostate expresses ERα in the stromal compartment and ERβ in the epithelium, one could expect differences in prostate cancer. However, though widely studied, no confirmatory evidence has been obtained that they would be differently expressed or have their own physiological role in prostatic malignancies (Härkönen and Mäkelä, 2004). However, preclinical as well as clinical evidence exist that antiestrogen and SERM treatment would be of value in prostate cancer treatment and prevention. In the transgenic adenocarcinoma mouse model (TRAMP model) SERM, toremifene, has been shown to inhibit development and progression of experimental prostate cancer and its precancerous lesions (Raghow et al., 2002). Phase II data from trials using toremifene in the treatment of men with HGPIN validate the use of SERMs as a rational and provocative strategy for the prevention of prostate cancer (Steiner and Pound, 2003; Taneja, 2005; Price et al., 2006). At present, the mechanistic anti-carcinogenic effects of toremifene in the prostate are not, however, known. More information on the function of prostatic ERα and ERβ is needed to develop novel, prostatic specific SERMs (Härkönen and Mäkelä, 2004).

2.5.5 Estrogens and LUTS

Given that the ERs are found not only in the prostate, but also in the bladder and other parts of the urinary tract, it is obvious that estrogens have an influence on LUT. Estrogen effect in the rodent LUT has been studied in different experimental models as intoduced in section 2.6.3.2. In men, two recent studies have explored the correlation of estradiol and LUTS. Schatzl et al. (2000) studied estradiol levels in serum in elderly men with LUTS and found that estradiol correlated with IPSS. Rohrmann et al. (2007) studied estradiol levels in symptomatic, 60 years old or older men and concluded estradiol to be associated with an increased risk of having LUTS. Thus, studies using drugs targeted at estrogen action or metabolism are warranted.

2.5.6 Selective estrogen receptor modulators (SERMs)

2.5.6.1 General overview and clinical indication for the SERMs

Selective estrogen receptor modulators (SERMs) are nonsteroidal phenylethylene derivatives that bind with high-affinity estrogen receptors. They mimic the effects of estrogens in some tissues (agonistic effects), but have antiestrogenic effects in others

(antagonistic effects) (Weryha et al., 1999). SERMs provide treatment opportunities in the treatment and prevention of breast cancer and secondly, prevention of postmenopausal complications such as osteoporosis (Weryha et al., 1999).

The first SERM, tamoxifen, was developed in the 1970s (Robertson 2004). It is nonsteroidal antiestrogen approved for the treatment of estrogen-dependent breast cancer. However, tamoxifen has an estrogen-agonistic effect on the endometrium and thus there has been a rationale to develop new SERMs (Weryha et al., 1999). Toremifene that is structurally similar to tamoxifen has been in clinical use since 1995 principally for the indication of advanced hormone-sensitive breast cancer (Kangas, 1990a; Kangas, 1990b; Harvey et al., 2006). Fulvestrant (ICI 182,780), pure estrogen receptor antagonist, without known agonistic effects binds, blocks and degrades estrogen receptor (Robertson, 2004; Howell, 2005). It has also been approved for the treatment of breast cancer (Shelly et al., 2008). At present, the SERM class includes more than seventy molecules of which many are under clinical research, and only a few are in clinical use (Diez-Perez, 2006). The ideal SERM in women would include reduction in a risk of breast cancer and cardiovascular disease, and prevention of osteoporosis, without stimulation of the endometrium, induction of hot flushes or an increased risk of venous thromboembolism (Palacios, 2007).

2.5.6.2 SERMS and prostatic diseases

Based on the evidence that estrogens play a role in the genesis of prostatic diseases, it has been suggested that a targeted approach for treating prostate cancer and BPH through the use of SERMs, and more specifically ERα antagonist or ERβ agonist, would be of benefit (rewieved by Steiner et al., 2001; Ellem and Risbridger, 2007; Risbridger et al., 2007; Prins and Korach, 2008). Indeed, studies have provided important data that antiestrogens may effectively inhibit development and progression of experimental and even clinical prostate cancer. In a TRAMP mouse model toremifene decreased development of prostate tumors as well as appearance of HGPIN lesions (Raghow et al., 2002). In a phase II trial, toremifene prevented progression to prostate cancer in men with HGPIN (Price et al., 2006; Taneja et al., 2006). Recent finding of TMPRSS2-ERG gene fusion positive prostate cancer regulated by estrogen receptor-dependent pathways opens new possibilities for clinical implications. Fusion positive prostate cancer patients may benefit from SERM chemopreventive treatment compared to men who have TMPRSS2-ERG- negative prostate cancers (Setlur et al., 2008).

2.6 Prostate and LUT of the rat

2.6.1 Structure, blood supply and innervation of the rat prostate

The rat prostate surrounds the urethra and is composed of several distinct lobes: the ventral (VP), lateral (LP), dorsal (DP), and anterior (coagulating gland) lobes. In general, the prostate morphology is identical in the mouse and rat. Lobes are connected to the urethra by connective tissue and a series of ducts. Prostatic tissue is tubuloalveolar and consists of acini surrounded by a stromal matrix. Smooth muscle layer surrounds each prostatic acinus. However, lobes and their ducts differ from each other morphologically as well as biochemically. In general, lateral and dorsal prostatic lobes (DLP) resemble each other more than they do the ventral lobe. In addition, DLP is considered to structurally resemble human peripheral zone more than VP.

The VP consists of two lobes. The acini of the VP are quite tightly packed in the stroma and have a large degree of infolding. The epithelium is mostly columnar with some cuboidal cells that have basally located nuclei. The secretions in the acini of VP are pale and slightly eosinophilic. The acini in the LP are loosely arranged in the stroma. They are generally large, they vary in size and have highly infolded walls. The epithelium is cuboidal or columnar and the nucleus is located centrally. Secretions found in the acini of LP stain intensively with eosin and this maybe the clearest sign to microscopically separate the lateral lobe from DP and VP. As in the LP, the acini in the DP are quite large and loosely arrange in the stroma. They are less convulated than either in the VP or DP. The epithelium is lined by mostly cuboidal cells with centrally placed nuclei. Secretions of DP acini stain with eosin by an intensity between that of the LP and VP (Jesik et al., 1982).

The ducts draining secretion from the prostate to the urethra are long and lined mainly with columnar epithelium. Walls of the ducts are highly convoluted and they consist of connective tissue surrounded by several layers of smooth muscle (Jesik et al., 1982). The prostatic ducts of each lobe differ from each other both developmentally and in arrangement and attachment. The main difference is in the draining of the ducts. The VP is drained by subducts that drain into four of five main channels, which in turn empty into the urethra. Instead, the drainage of the LP and DP is via 7 to 8 and 10 to 14 ducts that empty two to four acini directly into the urethra (Jesik et al., 1982). Ductal morphogenesis and adult ductal branching patterns in the rat prostate have been examined by microdissection methods (Hayashi et al., 1991). The time course for protruding of primary ducts differ between the lobes as well as timing of ductal branching. Especially, the branching pattern of DP differs from other lobes. Interestingly, prostatic development in the mouse has been described to be dependent of androgens, but elongation and branching of prostatic ducts are minimally dependent upon continued androgenic stimulation (Sugimura et al., 1986).

The arterial blood supply to the prostate is from the internal iliac artery through the superior vesical artery and its branches. Branches to the DP and LP follow their ducts; in the VP also branching freely. The inferior vesical artery arises from the superior vesical artery and it supplies blood to the dorsal surface of the prostate. Venous blood is returned by the superior and inferior vesical veins, which generally follow the corresponding arteries (Jesik et al., 1982).

The rat prostate is innervated by postganglionic neurons located in the pelvic plexus (Nadelhaft et al., 2003). The adrenergic fibers are found in the rat prostatic acinus muscle layer and in addition, the existence of cholinergic fibers and muscarinic receptors in the rat prostate has been established (Nadelhaft et al., 2003).

2.6.2 LUT and urodynamical measurements in rodents

The male rat lower urinary tract consists of the bladder, bladder neck and urethra. The striated muscle of the urethra is called the rhabdosphincter (RB). The RB extends without interruption from the bladder neck to the pelvic floor in all species but its relation to the prostate varies; in the rat prostatic glands locate outside of the RB while in human the prostate is partially overlaid by RB fibers (Lehtoranta et al., 2006). In addition, the rat RB can be divided structurally into three different parts and functionally into two parts (Lehtoranta et al., 2006). The lower urinary tract of the rat is supplied by the pelvic and hypogastric nerves and lumbosacral sympathetic chain (Steers, 1994). Motoneurons innervating the bladder originate in the major pelvic ganglion lying on the lateral prostate (Steers, 1994). The rat RB is innervated by autonomic nerves in addition to somatic innervation.

In general, voiding occurs in response to activation of tension receptors in the bladder wall and reflex bladder activity relies on the pontine micturion center (PMC) in the brainstem. Descending input from the PMC activates preganglionic neurons in the spinal cord which send axons in the pelvic nerve that convey excitatory input to the bladder (Steers, 1994). However, the urination of the male rat is more complex process (Streng et al., 2001).The bladder pressure increases and urine flow takes place during a period of high-frequency oscillations of intraluminal pressure (IPHFOs) as short on-and-off flow rate peaks (Streng et al., 2001). The electromyogram (EMG) activity of the proximal RB indicates that the proximal urethra contracts during voiding helping the urine flow to maintain its peak-like character and high flow rate speed through the urethra during the transient, negative-directed, repolarization (TRP) (Streng et al., 2001).

Urodynamical measurements have been performed successfully in mice and rats under anesthesia as well as in conscious animals (Maggi et al., 1989; Pandita et al., 1998; Streng et al., 2002b). Anesthesia is known to have an effect on voiding e.g. reflex micturition is abolished in barbiturate-anesthetized rats though a variety of reflexes of bladder motility can be demonstrated in urethane-anesthetized rats (Maggi et al., 1989). These preclinical urodynamical models have given important insight to the details and

mechanistical studies concerning voiding. In rats, transvesical recording of bladder pressure has been preformed under urethane anesthesia (Maggi et al., 1989). Thereafter, more sophisticated technology has been used to measure alterations at a wider scale of urodynamical parameters. In these studies, bladder pressures during different voiding phases, maximal and average flow rates, voiding time as well as micturion times, and volume of residual urine has been recorded in mice and rats under anesthesia and in addition, the amplitude of EMG activities of the proximal RB in the rat have been recorded successfully (Streng et al., 2002b).

In conscious animals, cystometric investigations can be performed without any anesthesia by catheterization of the bladder. By this method the bladder pressures, bladder capacity, micturition volume, and residual volume can be investigated (Pandita et al., 1998). This recording method may not still be as sensitive as urodynamical measurements using anesthetized rodents.

2.6.3 Experimental models of prostatic diseases and voiding dysfunction

Reliable animal models of human diseases are critically important for the discovery of molecular pathways, genetic influences, environmental factors, and successful management strategies for humans. Furthermore, they offer a possibility to study disease associated pathways that would be impossible to cover in man (Vykhovanets et al., 2007). Prostatic inflammation and prostate cancer have widely been studied using several approaches and species. Recently, transgenic animal models have also given new insights into disease mechanisms. As indicated earlier, the etiology of prostatic inflammation is unknown, as well as its relation to prostate carcinogenesis. Thus, rational models of prostatic diseases resembling human pathogenesis are needed to evaluate mechanisms and to give possibilities for drug discovery.

2.6.3.1 Prostatic inflammation models

In recent past years, different ways to induce prostatic inflammation have been applied to explore etiological factors. Inflammation in the prostate has been induced e.g. by infectious agent (E. Coli) (Seo et al., 2003), by irritant agent (ethanol / dinitrobenzenesulfonic acid) (Lang et al., 2000), or by mechanical obstruction (Melman et al., 2005). Inflammation induced by these methods has been suggested to resemble the acute form of inflammation (Vykhovanets et al., 2007).

Prostatic inflammation mimicing human chronic non-bacterial inflammation has been obtained e.g. by an autoimmune based method (using male accessory gland homogenate, MAG, with Freund's adjuvant, CFA) (Moron et al., 2000; Vykhovanets et al., 2007). In rats subjected to experimental stress, prostatic inflammatory changes of chronic type (stromal inflammation) have also been found (Aronsson et al., 1988).

Spontaneous, age-dependent and hormone-responsive prostatic inflammation has been observed in different rat strains including Lewis, Copenhagen, and Wistar rats (Muntzing et al., 1979; Lundgren et al., 1984; Robinette, 1988). Spontaneously developing rat prostatic inflammation has been noticed to have a similarity to human chronic prostatitis (Muntzing et al., 1979). Furthermore, a recent study showed that age-related changes in the rat intraprostatic lymphocyte phenotype and importantly, immunological changes in spontaneous prostatic inflammation resemble those in estradiol induced prostatic inflammation (Vykhovanets et al., 2006).

2.6.3.1.1 Estrogen-induced prostatitis models

Estrogen-induced lateral lobe-specific prostatic inflammation has been a widely studied rodent model of chronic non-bacterial prostatitis and represents category III prostatitis (Vykhovanets et al., 2007). The etiology of estrogen-induced prostatic inflammation has been considered to be comparable to that in humans, who often develop non-bacterial prostatic inflammation in life when androgen levels are downregulated (Vykhovanets et al., 2007). The effect of estradiol (E_2) treatment for different periods has been studied using both intact and castrated animals as well as different rat strains (Lundgren et al., 1984; Naslund et al., 1988; Robinette, 1988).

Castration itself has been observed to increase the incidence and severity of both stromal and luminal prostatic inflammation (Lundgren et al., 1984; Naslund et al., 1988). Robinette (1988) and Naslund (1988) showed estradiol treatment alone for 14 to 30-days, induces prostatic inflammation but Leav et al. (1989) failed to confirm this. Interestingly, Naslund reported that the estradiol effect in intact animals is blocked by concomitant administration of testosterone (T) but not administration of the same dose of dihydrotestosterone (DHT) (Naslund et al., 1988). Thus, it seems that estrogen-induced prostatic inflammation is dependent on estrogen and androgen content both, treatment time and rat strain.

Despite the method used for estrogen-induced inflammation, histological analysis of the sections has revealed severe, multifocal, predominantly chronic inflammation locating mainly in the lateral prostatic lobe (Naslund et al., 1988; Robinette, 1988). The inflammatory cell picture has consisted of an accumulation of neutrophils in the lumina and mononuclear cells in the stroma (Robinette, 1988; Tangbanluekal and Robinette, 1993). By longer hormonal treatment times (for more than five months) fibromuscular proliferation consisting of fibroblast, smooth muscle cells and collagen has also been reported (Robinette, 1988). A more detailed study of estradiol induced inflammation in the lateral prostate revealed that estrogen upregulated proinflammatory cytokines such as IL-6 and IL-10 well before any inflammatory cells were observed in the prostate, pointing towards T-helper (Th2) cell response (Harris et al., 2000). It has also been reported that estradiol induced prostatic inflammation has an autoimmune nature (Seethalakshmi et al., 1996). Interestingly, recent study by Rudick et al. (2008) showed that experimental autoimmune prostatitis in mice, induced by

immunization with rat prostate homogenates, causes chronic pelvic pain originating from the prostate in addition to prostatic inflammation.

In experimental estrogen-induced prostatitis models, increased prolactin concentrations in serum with elevated pituitary weights have been observed. The administration of bromocriptine (dopamine D2 agonist that inhibits pituitary prolactin release) to E_2+DHT treated rats have been shown to suppress pituitary weight and hyperprolactinemia (Tangbanluekal and Robinette, 1993). Furthermore, it attenuated prostatic inflammatory response suggesting that hyperprolactinemia may at least partly mediate inflammation.

The use of antiestrogen ICI 182,780 has been shown to reduce prostatic inflammation in the LP induced by T+E2 treatment (Thompson et al., 2002). Interestingly, antiestrogens, aromatase inhibitor or antibiotics have not been able to reduce spontaneous rat prostatitis (Lundgren et al., 1984; Naslund et al., 1988).

2.6.3.2 Experimental models of obstructive voiding induced by estrogen action

Neonatally estrogenized rats show high mean maximal bladder pressure and decreased mean flow rate indicating bladder outlet obstruction (Streng et al., 2001). A lack of transient repolarization (TRP) peak in the electrical activity (EMG) recorded from proximal rhabdosphincter as well as muscular atrophy suggest that obstruction is at least in part due to the failure of rhabdosphincter to relax (Streng et al., 2001; Streng et al., 2002a). The mice overexpressing human aromatase gene (AROM+) develop similar urodynamical changes (Streng et al., 2002c). These mice have elevated serum estrogen and reduced serum testosterone, and rhabdosphincter atrophy. Interestingly, the alterations in urodynamics and rhabdosphincter size and function can be reversed in developmentally estrogenized male rodents by aromatase inhibitor treatment at adulthood (Streng et al., 2002a).

The exact site and mechanism of estrogen action in the LUT has not been established but there are multiple ways how estrogens may influence on bladder function e.g. through modifications in muscarinic receptors and by inhibiting the movement of extracellular calcium ions into muscle cells (Robinson & Cardozo 2003).

2.6.3.3 Prostate cancer models

There are several rat models used to study experimental prostate carcinogenesis and to investigate chemoprevention possibilities. Incidence of spontaneous prostate tumors in rats is low and they are observed only at very old ages (Shirai et al., 2000; Shirai, 2008). Tumors can be implanted or induced by hormones (T+E_2 treatment in Noble rats), or by chemical agents [such as N-methyl-N-nitrosourea (MNU); 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine(PhIP) or by 3,2`-dimethyl-4-amino-biphenyl

(DMAB)] or by combination with two or more carcinogens/hormones (MNU+testosterone or DMAB+testosterone) (Shirai et al., 2000). A transgenic rat model named 'transgenic rats with adenocarcinomas of the prostate' (TRAP) has also been created (Asamoto et al., 2001). In the TRAP model carcinomas develop even at the age of 15 weeks, which is a short time when compared to 60 weeks exposure time in DMAB and PhPI models (Shirai, 2008).

There are differences in the site of origin (VP; DLP; seminal vesicles) of tumors in both spontaneous and inducible models, depending on the strain of rat and the induction protocol (Lucia et al., 1998; Shirai et al., 2000). Despite of various rodent models of prostate cancer, at present there is no ideal model for all aspects of prostatic carcinogenesis. Each model has its strengths and weaknesses, and acquired characteristics should be carefully considered (Lucia et al., 1998). The most effective models would be those that can be used to follow cancer development and progression and in addition, models to be used in testing of therapeutic options.

2.6.3.3.1 Noble rat model

Noble rats typically have a very low incidence of spontaneous prostatic carcinoma. In 1977 Dr Robert Noble treated adult, male Noble rats with sex hormone pellets implanted subcutaneously. Pellets were convenient for long-term experiments, and it was possible to remove and replace them when necessary (Noble, 1977; Noble, 1982). Testosterone treatment for more than 27 weeks, usually more than one year, increased incidence of prostatic carcinomas of the dorsal prostate (incidence 20%). When testosterone and estradiol were used together, tumors appeared at a younger age with corresponding incidence. Tumors were observed to arise in the base of greatly enlarged dorsal lobes of the prostate and coagulating gland (Noble, 1977). Since then, Noble rat model has widely been used in prostatic carcinoma studies with modifications in androgen and estrogen amounts and exposure times. It has been observed that Noble rats may be more sensitive, but not specific, to hormone treatment since corresponding hormonal treatment of Sprague-Dawleys leads to lower prostatic adenocarcinoma incidence (Bosland et al., 1995).

Taken together, prostatic dysplasia and adenocarcinoma has been shown to develop after T+E_2 treatment of intact, adult male Noble rats for 16 weeks and for 52 weeks, respectively (Leav et al., 1988; Leav et al., 1989; Bosland et al., 1995; Lane et al., 1997; Wang and Wong, 1998). Interestingly treatment with DHT alone, or in combination with E_2, did not induce dysplasias (Leav et al., 1988; Leav et al., 1989). The dysplastic foci were mainly described to locate throughout the DLP (Leav et al., 1988), but they have also been observed to locate in the periurethal ducts of the DLP (Leav et al., 1989). This observation is noteworthy since adenocarcinomas locate mainly in the same periurethal area ducts of the DLP. Thus hyperplasia of the periurethal ducts, but not peripheral duct-acinar dysplasia, appears to be a likely precursor of induced carcinomas (Bosland et al., 1995). However, many Noble rat studies have concentrated to describe early histopathological changes only in the DLP

(Lane et al., 1997; Thompson et al., 2002; Tam et al., 2007). Unfortunately, this makes no relevance on true carcinogenesis since carcinomas develop to other region.

The dysplastic as well as carcinogenic changes seem to be dependent on the treatment time as well as the hormone dosages used. Noble (1977) reported that a single testosterone pellet did not cause carcinoma, and the minimum dose of testosterone was at least two or three testosterone pellets at a time. The use of high doses of testosterone and estradiol treatment has also been shown to produce high incidence of prostate carcinogenesis in a relatively short time (at 12 month incidence 91%) by Wang & Wong (1998). Unfortunately, in earlier Noble rat studies 'home-made' silastic capsules have been used which leads to variability in the packed hormone amounts and releasing properties. Probably for this reason, the difference in the reported incidences and inducing times varies. The serum hormone concentrations have not been repetitively studied but some studies has reported that in T+E_2 treated animals testosterone has been slightly elevated while estradiol and prolactin concentrations were significantly elevated compared to controls (Leav et al., 1988; Lane et al., 1997; Tam et al., 2007).

Dysplasias as well as adenocarcinomas have been reported to morphologically resemble prostatic intraepithelial neoplasia or PIN in human. Adenocarcinomas have ranged from highly differentiated to poorly differentiated (Wang and Wong, 1998). However, no metastases have been observed (Bosland et al., 1995; Wang and Wong, 1998). An important consequence of chronic T+E_2 treatment of Noble rats is the increase in pituitary weight and concomitant development of hyperprolactinemia. This has been considered to drive development of prostatic inflammation and dysplasia. In general, only common observations concerning prostatic inflammation has been reported. Inflammation has been noted to be present in DLP, and often to be associated with areas of dysplasia (Leav et al., 1988; Leav et al., 1989; Lane et al., 1997; Thompson et al., 2002; Tam et al., 2007). The possible association between inflammation and periurethal area adenocarcinomas has not been studied.

In conclusion, strengths of the Noble rat model include the possibility to study hormonally induced precursor lesions as well as adenocarcinomas. The limitations are that adenocarcinomas rarely metastase, and on the other hand, pituitary tumors develop (Lucia et al., 1998). Development of precursors and adenocarcinomas make the Noble rat model suitable for drug testing, but in fact only a small number of medical options have been tested using this model. Dopamine agonist (bromocriptine) and pure anti-estrogen (ICI 182,780) have shown lower incidence of the inflammation and dysplasia in the DLP of T+E_2 treated Noble rats, and concomitanly in decreasing prolactin concentrations in serum (Thompson et al., 2002; Tam et al., 2007). However, the effect of these agents on the development of prostatic adenocarcinomas in the periurethal area has not been reported.

3 AIMS OF THE PRESENT STUDY

The main objective of this study was to explore prostatic inflammation and its relationship with voiding dysfunction and prostate carcinogenesis by developing an experimental model based on estrogen and androgen treatment. A novel selective estrogen receptor modulator (SERM), fispemifene, was tested for the prevention and treatment of prostatic inflammation in this model.

The specific aims were:

1. To induce non-bacterial prostatic inflammation with combined testosterone and estradiol treatment in the Noble rat, and to develop valid microscopic methods for the categorization and quantification of the inflammatory changes.

2. To study the impact of the testosterone and estradiol concentrations and altered T-to-E_2 ratio in the development of prostatic inflammation and voiding symptoms in the Noble rat.

3. To study the association of the inflammation with prostate cancer and its precursors.

4. To study anti-inflammatory and antiestrogenic properties of the novel SERM, fispemifene, in the established model.

4 MATERIALS AND METHODS

4.1 Experimental animals and surgical procedures (I-IV)

Adult male Noble rats (NBL/Cr) were obtained from our own breeding colony (I-III) and from Charles River (IV) (Raleigh, NC). All animals were housed (two rats per cage) under a 12 hr light–12 hr dark lighting cycle. They were given free access to tap water and food (soy-free rodent pellet diet; SDS, Witham, Essex, England). The experimental procedures were reviewed by the local Ethics Committee on Animal Experimentation at the University of Turku and approved by the local Provincial State Office of Western Finland.

At the age of 10-12 weeks (250-300 grams), the Noble rats were divided into experimental groups. In studies I-III no castration was performed. In study IV, animals in two groups were castrated via the scrotal route by removing epididymal fat pads with testes under general anesthesia before hormone implantation. The pellets were inserted in subcutaneous pockets formed over the scapular area through a 1 cm incision. The incised area was then disinfected and sutured. Anesthesia for implantation and castration was induced with xylazine (Rompun; Bayer, Leverkusen, Germany) and ketamine (Ketalar; Pfizer Inc, Espoo, Finland).

4.2 Hormonal treatments (I-IV)

Testosterone (T), dihydrotestosterone (DHT) and estradiol (E_2) implants were used in the hormone treated animals and placebo implants were used in the control animals. Implants were purchased from Innovative Research of America, Inc. (Sarasota, Florida, USA). These pellets work by the double process of erosion of the pellet and diffusion of the active product providing a constant release rate (Levine and Levine, 1989). Release times for implants were 21-day, 60-day- or 90- day. To maintain hormone levels, implants were replaced by new pellets in long-term studies (I-IV). In studies I and IV, the irreversibility of the histopathological changes was studied by removing implants after three weeks- (I) and 15-weeks T+E_2 treatment for the following three week period. Hormonal treatment time, implant replacement intervals, hormone dosages released per day, studied test compounds and the number of animals in each study is shown in Table 2. In all studies, placebo animals were treated by corresponding schedules with implants without a biologically active compound.

Table 2. Summary of the hormonal procedures used in the animals

Treatment time	Implant releasing time and replacement intervals	T	DHT	E_2	Test compound	n
3 wk (I)	21-day	238 μg	-	71 μg	ICI 182,780 Removal of T/E_2	32
3 wk (I)	21-day	238 μg	-	5.0 μg		5
3 wk (IV)	21-day	-	-	5.0 μg	Fispemifene	38
3 wk (IV)	21-day	-	140 μg	71 μg	Fispemifene Tamoxifen	70
6 wk (I)	21-day (21+21)	238 μg	-	71 μg		10
13 wk (II, III)	60-day (45+45)	833 μg	-	83 μg		8
13 wk (II)	90-day	278 μg	-	83 μg		8
18 wk (III) (IV)	60-day (45+60+21) (45+45+36)	833 μg	-	83 μg	Removal of T/E_2 Fispemifene	21 20
26 wk (III)	60-day (60+60+60)	833 μg	-	83 μg		10

Abbreviations used in the Table: T= testosterone released per day; DHT= dihydrotestosterone released per day; E_2= estradiol released per day; n= indicates total number of hormone- and test compound treated animals per study

4.3 Test compounds (I, IV)

4.3.1 ICI 182,780 (I)

Pure antiestrogen, ICI 182,780 (Tocris Cookson), was dissolved in absolute ethanol and diluted in rapeseed oil to a final concentration of 3.3 mg/ml. The final volume of ethanol did not exceed 1%. Rats were treated for three weeks with T+E_2. In the beginning of the third week, animals were divided into two groups. Half of the animals received ICI 182,780 s.c. in the flank twice per week at a dose 3 mg /kg. The injections were given on days 14 and 17 after hormone implantation. Another half served as controls and received injections of absolute ethanol dissolved in rapeseed oil without ICI 182,780.

4.3.2 Fispemifene and tamoxifen (IV)

Fispemifene (synthesized by Hormos Medical, Oulu, Finland) is a novel selective estrogen receptor modulator (SERM). The chemical name is Z-2-{2-[4-(4-Chloro-1,2-diphenylbut-1-enyl)phenoxy]ethoxy}-ethanol. Fispemifene binds to both ERα and ERβ receptors and has antiestrogenic or estrogenic actions, or both, depending on the target cells and tissues. Fispemifene has either no or only slight effects on the safety pharmacology parameters studied and it has been well tolerated in all *in vivo* toxicological studies performed (Savolainen-Peltonen et al., 2004; Kangas et al, unpublished data). Fispemifene and tamoxifen (Sigma-Aldrich, Inc., St. Louis, MO, USA) were dissolved in corn oil (Fluka, BioChemika, Buchs, Switzerland) and administered daily by gavage. Fispemifene (Fis) was given in three different doses (3, 10, and 30 mg/kg/day). The dose of tamoxifen (Tam) was 1 mg/kg/day (Fitts et al., 2004). The control animals received an equal volume of the vehicle (corn oil).

4.4 Urodynamical recordings (I, II)

After T+E_2 treatments for three-, six, and 13- weeks, urodynamical recordings were performed. The urodynamical measurements and data analysis were performed by Dr. Tomi Streng and the methodological details of the measurements have been described by Streng et al., (2002b). Briefly, the rats were anaesthetized using an i.p. injection of chloral hydrate (0.9 g/kg, Sigma Chemical Co., St. Louis., MO 63178, USA), and i.v. injections of urethane (0.32 g/kg, Sigma Chemical Co., St. Louis., MO 63178, USA) were used to maintain anesthesia for urodynamical measurements. The bladder and distal urethra were exposed. A 20G i.v. cannula was inserted through the bladder apex into the lumen for saline (0.9% NaCl) infusion and bladder pressure measurements. Micturition was evoked by saline infusion (10 ml/h). Transvesical cystometry was recorded by using the Statham pressure transducer (P23BC, Hato Ray, Puerto Rico). The urine flow rate was measured by using a flow meter (model T206) with an ultrasonic flow probe (# 2,5SB178; Transonic Systems, Inc. Ithaca, NY, USA) at the distal part of urethra. In addition, extracellular electrical activity of the proximal rhabdosphincter was recorded by using a flexible suction electrode. Continuous recordings were obtained by using the Acq Knowledge 3.5.3 program (Biopac Systems Inc., Santa Barbara, CA, USA). When the micturitions had illustrated several similar and consecutive features for the rodent, three representative voidings were chosen for further analysis. The urodynamical parameters measured are summarized in Table 3. In study II, all the presented urodynamical parameters were recorded, while no rhabdosphincter activity was measured in study I.

Table 3. Parameters used in the urodynamical recordings of placebo- and hormone treated animals

Abbreviation	Description	Unit
BP	Maximal bladder pressure at different phases	cmH_2O
BC	Bladder capacity (infused volume before voiding)	ml
FR	Flow rate	ml/min
MT	Micturition time	sec
M Int	Micturition interval	min
RU	Residual urine (infused volume minus voided volume)	ml
RB ampl	Rhabdosphincter EMG (electromyography) amplitude	mV
RB TRP	Rhabdosphincter transient repolarization amplitude	mV

4.5 Histological sample preparation (I-IV)

At the end of the treatment period or after the urodynamical recordings, animals were weighed and sacrificed by CO_2 suffocation, and blood collected by heart puncture before neck dislocation. The testes, seminal vesicles (after removal of the coagulating glands and secretory material), bladder and the urethra-prostate complex (consisting of the ventral and dorsolateral lobes (DLP), and the transverse section of the proximal urethra containing the sections of urethra, the prostatic collecting ducts and deferent and seminal vesicle ducts), and pituitary gland were rapidly excised and weighed. Thereafter, samples were collected and fixed in neutral 10% formalin solution for 18-20 hours at room temperature, dehydrated in ethanol, cleared in xylene and embedded in paraffin. Paraffin embedded sections were cut perpendicular to the longitudinal urethral axis at 5 μm thickness. Slides were incubated overnight at +37 °C and sections for immunohistochemical stainings were stored at +4 °C; others at room temperature. Subsequently, they were either stained with hematoxylin and eosin (H&E) or with immunohistochemistochemical methods. The stained sections were photographed with an Olympus DP-10 camera under an Olympus SZX9 microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

4.6 Serum hormone measurements (I-IV)

Serum samples were stored at -70°C until the measurement of hormone concentrations by enzyme linked immunosorbent assay (ELISA). In order to measure the concentrations of total unconjugated estradiol and testosterone, samples were extracted twice with diethyl ether, and the remaining organic solution was evaporated under nitrous gas in a warm water bath. Hormones were re-dissolved in zero-standard serum, and concentrated when necessary. Thereafter, an enzyme immunoassay for the in-vitro-diagnostic quantitative determination of estradiol and testosterone was conducted according to the instructions given by the manufacturer (17β-estradiol ELISA for

human serum and plasma, testosterone ELISA for human serum and plasma, IBL-Hamburg, Germany). Serum prolactin was measured with the rat prolactin enzyme immunoassay kit without pre-treatment (Rat prolactin EIA kit, Spi-Bio, BERTIN group, Montigny Le Bretonneux, France). The detection range for estradiol, testosterone, and prolactin was 25 to 2.000 pg/ml, 0.2 to 16 ng/ml, and 0.39 to 50 ng/ml, respectively.

4.7 Histopathological examination of the prostate samples

Paraffin embedded urethroprostatic complex was cut in serial cross-sections (5 μm thickness) to cover the whole prostate. 15-20 sections at 200-500 μm intervals were obtained depending on the prostate size. Inflammation assessment was done specifically from the dorsolateral prostatic lobes (DLP). Three to six representative sections that contained DLP were then further selected from the serial sections for the assessment of inflammation in each animal. The sections were selected carefully and systematically to represent the dorsolateral prostatic lobes to their full extent at each level of cutting. Normally, one section gave a good indication of the inflammation status, but more sections were selected to improve the confidence of inflammation assessment. In studies II and IV, sections were screened by two independent observers who were blind to the treatment groups.

4.7.1 Inflammation assessment (I-IV)

A standardized histopathological classification and counting system of inflammation infiltrates in the Noble rats was established based on classification of human prostate inflammation sections (Nickel et al., 2001b). It was used in the assessment of the inflammation after hormonal treatments for three to six weeks (I, IV). Inflammation infiltrate in the DLP was considered to be perivascular when more than ten inflammatory cells were found around the capillary, stromal when inflammatory cells were scattered in the stroma or periglandular space, and glandular when inflammatory cells were found in the epithelium or lumen. Inflammatory infiltrates in each category were counted from the serial sections made from the dorsolateral lobes of the prostate. A computer-based quantitative method was used to study inflammatory responses to anti-estrogen (ICI 182,780) treatment (I). In addition to the total amount of inflammation, the mean size of the infiltrates was assessed.

In studies II-IV with longer hormonal treatments, it was not possible to count individual perivascular and stromal infiltrates since inflammatory cells were distributed over wider stromal areas and did not form infiltrates of high cell density with clear borders. Instead of counting the three categories, the location, extent and grade of the inflammation infiltrates were assessessed in study III.

The aggressiveness of the inflammation in the DLP was analyzed in studies II and IV according to the method developed for the human prostate (Irani et al., 1997). Briefly, inflammation was analyzed on a 4-point grading scale on the basis of its relation to the epithelium. Grade 0 meant no contact between inflammatory cells and epithelium; grade 1 some contact; grade 2 periglandular infiltrates adjacent to partially destroyed epithelium and grade 3 the number of these acini was more than 25 %.

The number of inflamed acini was counted for the whole DLP area by using the same sections. Glandular inflammation was confirmed by immunohistochemical neutrophil staining, but counting was done based on H&E stained sections. The counting method for inflamed acini is based on findings in rat and mouse showing that postpubertal elongation and branching of prostatic ducts are minimally dependent upon continued androgenic stimulation (Sugimura et al., 1986). Furthermore, in a study using human prostate sections, no difference was seen between control and benign prostatic hyperplasia (BPH) tissues when the total number of acini was evaluated (Babinski et al., 2003). Based on these findings, the number of the cross-sections of tubuloacinar structures should remain the same in adult animals. This was confirmed by counting the total number of tubuloacinar structures in four sections per animal from the hypo-T and hyper-T group. A summary of the assessment methods used is shown as Table 4.

Table 4. Summary of histopathological prostatic inflammation assessment used

Method based on	Description	Study
Location	Number of perivascular, stromal, and glandular infiltrates	I, IV
Area	Mean area of the perivascular/stromal infiltrate	I
Extent	Focal, multifocal, diffuse inflammation (scale 1-3)	III
Grade	Mild, moderate, severe inflammation (scale 1-3)	III
Aggressiveness	Contact between inflammation and epithelium (scale 0-3)	II, IV
Glandular inflammation	Number of inflamed acini	II, IV

4.7.2 PIN-like lesions and adenocarcinomas (III)

To analyze the PIN-like lesions and adenocarcinomas, sections were made as previously described and different levels of the DLP and periurethal area were selected. For PIN-like lesions, low-grade (LGPIN) or high-grade (HGPIN) were distinguished by the degree of cellular atypia in epithelium in the pre-existing ducts or acini, applying criteria used for LGPIN and HGPIN in human specimens (Sakr and Partin, 2001; Montironi et al., 2006). PIN-like lesions consisted of focal areas of

hyperplastic epithelium displaying distinct variation in size, shape, and staining properties of cells and nuclei. Compared to epithelial changes in the LGPIN-like lesions, HGPIN-like lesions had more evident cytologic atypia and nuclear enlargement, and even early signs of invasiveness in some lesions.

Carcinomas were locally invasive lesions characterized by abnormalities in glandular architecture, cytological atypia, and immunohistochemical findings (lack of basal cells). The tumors were classified as, moderately- or poorly-differentiated adenocarcinomas. In well-differentiated carcinomas, the glandular structure was retained, whereas in moderately- and poorly-differentiated cancers no glandular structure was apparent. Nuclear pleomorphism was distinctly evident in moderately differentiated tumors and poorly-differentiated cancers were anaplastic tumors. One section from the periurethal area, where PIN-like lesions or adenocarcinomas were present to their fullest extent, was selected for further analysis. Computer-based analysis with the aid of a Digital Virtual Microscope (Digital Virtual Microscope, Soft Imaging System, Olympus Finland Oy, Vantaa, Finland) was used to measure the areas of the cancer lesions. Using this virtual overview image, it was also possible to count the number of cancer lesions and the mean area of the each lesion per section. The same method was used to measure cell proliferation using Ki-67 immunostained sections (i.e., the number of Ki-67 positive cells/mm^2).

4.7.3 Assessment of immunohistochemical stainings (PR, Fra2) (IV)

The number of the cells with progesterone receptor (PR) and fos-related antigen-2 (Fra2) positive nucleus in the DLP epithelium was analyzed by a semi-quantative grading system. Immunohistochemically stained sections were analyzed under a 10 x objective. Values from zero (0) to four (4) were given depending on the number of positive nuclei. Value 0 was given when there were no cells with positive nuclei, whereas value 4 was given when all nuclei were positive. Values 1, 2, and 3 were given according to the number of positive cells found in the DLP. Sections were screened by two independent observers in a blinded fashion.

4.8 Immunohistochemical stainings (I-IV)

Sections were deparaffinized by xylene and alcohol solutions (100%, 96% and 70%), followed by rehydration with distilled water. Antigen retrieval was performed by boiling sections for 15 minutes in citric acid buffer (10 mM, pH 6.0). Endogenous peroxidase activity was blocked with 1% H_2O_2 solution for 20 minutes at room temperature. For cytokeratin 5/6, 20 minutes heat-induced epitope retrieval in 1 mmol/L EDTA, pH 9.0 was used. Sections were then incubated overnight at + 4ºC with the primary antibody diluted in PBS/3%BSA + 0.05% Tween (neutrophil without tween); sections stained for cytokeratin 5/6 were incubated for 30 minutes at room temperature without tween. The primary antibodies and dilutions used are listed in Table 5.

The following day, the sections were washed with PBS and incubated at room temperature for 30 minutes with secondary antibody (HRP-conjugated anti-rabbit secondary antibody or HRP-conjugated anti-mouse secondary antibody: DAKO EnVision Systems; DakoCytomation, Glostrup, Denmark), and then rinsed with PBS. The color was developed with diaminobenzidine substrate (Dako EnVision System). The sections were then slightly counterstained with Mayer's hematoxylin, dehydrated and mounted.

Table 5. Primary antibodies and dilutions used in the immunohistochemical stainings

Code	**Primary antibody**	**Dilution (study)**
AR	Androgen receptor (polyclonal rabbit anti-human) Santa Cruz Biotechnology, Santa Cruz, Canada	1:200 (III)
ERα	Estrogen-receptor alpha (monoclonal mouse anti-human) DAKO A/S, Glostrup, Denmark	1:50 (III,IV)
PR	Progesterone receptor (polyclonal rabbit anti-human) DAKO A/S, Glostrup, Denmark	1:400 (III,IV)
CD3	T-lymphocyte (monoclonal mouse anti-rat) Caltag Laboratories, Burlingame, Canada	1 :100 (I,III,IV)
CD8	Cytotoxic T-lymphocyte (monoclonal mouse anti-rat) Caltag Laboratories, Burlingame, Canada	1 :100 (I,III,IV)
Neutrophil	Neutrophil (myeloperoxidase MPO; polyclonal rabbit anti-human) Hycult Biotech, Netherlands	1:400 (II,IV)
Macrophage	Macrophage (CD68; polyclonal mouse anti-human) AbD Serotec, UK	1:2000 (IV)
Ki-67	Proliferation marker (monoclonal mouse anti-rat) DAKO A/S, Glostrup, Denmark	1:100 (III)
Cytokeratin 5/6	Basal cell marker (monoclonal mouse anti-human) DAKO A/S, Glostrup, Denmark	1:20 (III)
Fra2	Fos-related antigen 2 (polyclonal rabbit anti-Fra2) Santa Cruz Biotechnology, Santa Cruz, Canada	1:400 (IV)

4.9 Statistical analysis (I-IV)

The normal distribution of the data was checked with Shapiro-Wilk`s W normality test. In normally distributed data One-Way ANOVA (with Dunnett's or Tukey's post test) or the T-test (I-IV) was used. If the data were not normally distributed, the Mann-Whitney U-test or Kruskal-Wallis test was used (I-IV) (Statistica, version 5.1, Stat Soft, Inc. Tulsa, Oklahoma, USA; GraphPad Prism Software, version 4.00, San Diego, California, USA; SAS software, version 9.1., system for windows). For analysis of

incidence data, the X^2 and Fisher exact tests were used (III) and the Pearson Product-Moment Correlation Test was used to explore possible correlations between the urodynamical parameters and the number of inflamed acini (II). All data presented as group means ± SD. P- values less than 0.05 were considered statistically significant.

5 RESULTS

5.1 Changes after T+E_2 treatment for three to six weeks (I)

5.1.1 Hormonal changes and organ weights

Hormone concentrations in serum were monitored in all studies (I-IV) for assuring hormonal changes. Selected organ weights were used as biomarkers of hormone action and compared with hormone concentrations. The treatment of Noble rats with E_2 alone or T+E_2 attenuated body weight gain, which is in agreement with earlier studies (Robinette, 1988; Leav et al., 1989). Therefore, relative organ weights were calculated in all studies.

Serum hormone concentration measurements indicated significantly increased estradiol concentrations in serum after the T+E_2 treatment (testosterone 240 μg per day and estradiol 70 μg per day) for three and six weeks. Prolactin concentration and relative weights of the pituitary gland followed estradiol concentrations. Serum testosterone concentration was increased above the control level at six weeks and a corresponding increase was observed in the relative weights of the seminal vesicles.

5.1.2 Gradual development of the inflammation

An increased number of inflammatory cells were seen in the DLP at three weeks after T+E_2 implantation. The inflammatory cells, mainly lymphocytes, were first located around the capillaries. When the T+E_2 treatment time was extended to six weeks, inflammatory cells were also seen in the stroma and the periglandular space and finally in the epithelium and lumen of acini (I, Figure 1). Perivascular, and stromal inflammation consisted mainly of lymphocytes and mast cells, glandular inflammation consisted of lymphocytes and polymorphonuclear leucocytes, mainly of neutrophils. The majority of the lymphocytes in the stroma were T-lymphocytes. Cytotoxic T-cells were found among stromal inflammatory cells and intraepithelially. Glandular inflammation was seen predominantly in the lateral prostatic lobes at six weeks. There was a clear shift from the perivascular foci to more advanced forms, stromal and glandular foci, between three and six weeks of the hormone treatment. No signs of inflammation were seen in the periurethal and periductal areas. Neither obvious signs of inflammation were observed in the other organs studied, including bladder and kidneys.

5.1.3 Urodynamical changes

No statistically significant differences were seen in the urodynamic parameters recorded between the placebo and hormone-treated animals at three weeks. At six weeks, the bladder capacity and the relative bladder weight were significantly increased in hormone-treated rats that reflected by significantly less frequent micturition, prolonged micturition time and a trend of decreased urine flow rate.

5.1.4 The dose-dependency and reversibility of estrogen action and effect of an antiestrogen

The effects of two different daily doses of estradiol were compared (5 μg or 70 μg per day), while using the same dose of testosterone (240 μg per day) for three weeks. Estradiol concentration in serum and the relative pituitary weight showed a dose-dependent increase as expected. The number of the inflammation infiltrates was dependent on the dose of estradiol used e.g. it was smaller in the low dose estradiol group. Reversibility of the estrogen-induced inflammation was studied by removing estradiol and testosterone implants after treatment for three weeks. The animals were analyzed following a three-week period without hormone exposure. Histology of prostates showed a fading of the inflammation. There was no glandular inflammation left and also the number of perivascular and stromal inflammation infiltrate was diminished.

Antiestrogen (ICI 182,780) treatment concomitantly used with T+E_2 significantly attenuated prostatic inflammation at three weeks compared to T+E_2+vehicle treatment. The total number of the inflammation infiltrates relative to the prostate area was decreased. The mean size of the inflammation infiltrates remained unaltered. No major changes were seen in serum hormone concentrations or in the organ weights between the groups.

5.2 Effect of altered T-to-E_2 ratio on prostatic inflammation and voiding (II)

5.2.1 Hormonal changes and organ weights

Adult male Noble rats were treated with one dose of estradiol (83 μg per day) and two different doses (278 or 833 μg/day) of testosterone for 13 weeks. Testosterone doses were selected to cause hypoandrogenic and hyperandrogenic states, respectively. Testosterone concentration in the rats of the hypoandrogenic state (referred as hypo-T group) was significantly below the level of the placebo group. In the rats with hyperandrogenic state (referred as hyper-T group), testosterone concentration was

increased significantly above the level of the placebo group. The relative weights of the seminal vesicles followed these changes in serum testosterone concentrations.

Estradiol concentration in both groups was significantly increased compared to the placebo group and the prolactin concentrations in serum followed the estrogen concentrations. In the hyper-T group, the estradiol concentration was even higher than in the hypo-T group. This may reflect increased rate of aromatization. The relative weight of the pituitary gland was significantly increased in both hormone-treated groups, though it was higher in the hypo-T group. The T-to-E_2 ratios in serum were 300 for placebo group and 10 and 50 in the hypo- and hyper-T groups, respectively.

5.2.2 Inflammatory changes in the DLP

In the hypo-T group, the diameter of the acini was decreased compared to the hyper-T group, indicating diminished secretory activity. A corresponding increase of inter-acinar space occupied by stromal structures was noted. However, there were isolated inflammatory cells and confluent sheets of inflammatory cells in the DLP of the rats in both groups (II, Figure 2). The perivascular, stromal and periglandular inflammation was mostly composed of lymphocytes as described in study I.

Glandular inflammation was analyzed further, and two different types of inflamed acini were seen (II, Figure 3). There were acini were the lumen was filled with secretory material, cell debris, neutrophils and macrophages. The lining epithelium of these acini was partially destroyed and infiltrated by lymphocytes and thus resembled the chronic form of glandular inflammation. The other type of inflamed acini represented the acute type of inflammation having intact epithelium and mainly neutrophils in the lumen. Still, there were intermediate forms of glandular inflammation types containing varying numbers of periglandular and intraepithelial lymphocytes. No differences between the groups were observed in the distribution of different types of inflamed acini. However, the total number of inflamed acini was significantly higher in the hypo-T group than in the hyper-T group. Respectively, there was more contact between the inflammation and the epithelium in the hypo-T group than in the hyper-T group, which gave a slightly more aggressive score to the hypo-T group.

5.2.3 Urodynamical changes

When hypo-T group animals were compared to placebo-treated animals, bladder pressure at different phases was non-significantly decreased. The maximal and average flow rates, the micturition time and micturition intervals, and the bladder capacity were all significantly increased. Volume of residual urine was unchanged. There was a significant increase in total and transient repolarization (TRP) amplitude in the EMG of the proximal rhabdosphincter.

In the hyper-T group animals, basal bladder pressure was significantly increased compared to placebo-treated animals. The maximal and average flow rates were significantly decreased and the micturition time and intervals were significantly prolonged. The increases of bladder capacity and volume of residual urine were not significant. Both the total and TRP amplitudes were significantly decreased.

5.3 Inflammation and prostate carcinogenesis (III)

5.3.1 Hormonal changes and organ weights

When the animals were treated with testosterone (833 μg per day) and estradiol (83 μg per day) for 13, 18 and 26 weeks, testosterone, estradiol and prolactin concentrations were all significantly higher than placebo values. The relative weights of the seminal vesicles, the whole prostate and pituitary gland followed alterations in serum hormone concentrations.

5.3.2 Inflammation in the DLP

Stromal and periglandular and glandular inflammation were observed in the DLP, particularly in the LP, in all animals treated with T+E_2 for 13 to 26 weeks. At 13 weeks, inflammation was mainly focal, involving approximately 10-20% of the DLP area. After 26 weeks of treatment, inflammation was more abundant and diffusely distributed involving approximately 50% of the DLP area. The inflammation changed during hormone exposure from individual inflammatory cells and foci of inflammatory cells to more pronounced, diffuse distribution of inflammatory cells with tissue destruction and reactive changes in prostatic epithelial cells. Thus, it was not possible to count individual perivascular and stromal infiltrates in these long-term experiments since inflammatory cells were distributed over wider stromal areas and did not form infiltrates of high cell density with clear borders.

5.3.3 PIN-like lesions

LGPIN- and HGPIN-like lesions were observed in the prostatic ducts emerging from the LP and DP in all animals treated for 13 weeks with T+E_2 (III, Figure 1E). These lesions located in the periurethal area, inside the striated external sphincter muscle (rhabdosphincter). The proliferation activity in PIN-like lesions was increased compared to ducts without these lesions and there was often a discontinuity in the cytokeratin 5/6 staining. Stromal inflammation, mainly T-lymphocytes, was seen adjacent to these PIN-like lesions after T+E_2 for 13 weeks (III, Figure 1E).

HGPIN-like lesions were also observed in the glands of the lateral lobes in some of the animals treated for 26 weeks with T+E_2 (III, Figure 1C). No glandular or periglandular inflammation was seen associated with these acini.

5.3.4. Ductal adenocarcinomas

Prostatic adenocarcinomas were found in all animals treated with T+E_2 for 18 and 26 weeks, but not after 13 weeks of treatment. These multifocal adenocarcinomas located exactly in the same area where the majority of PIN-like lesions first developed; in the periurethal area inside the rhabdosphincter (III, Figure 2). The PIN-like lesions in the DLP area did not progress to adenocarcinomas.

The proliferative activity was increased in cancers and basal cell marker cytokeratin 5/6 was totally lost. In the adenocarcinomas as well as in the PIN-like lesions, AR was strongly expressed, but no specific staining for PR or ERα was seen in these lesions (III, Figure 3). There was no inflammation present adjacent to these adenocarcinomas observed at 26 weeks.

5.3.5 Implant removal study

To evaluate the hormone dependency of the ductal adenocarcinomas, the rats were treated first with T+E_2 for 15 weeks and the three following weeks without E_2 or without both, T and E_2. Moderately-differentiated prostatic adenocarcinomas in the periurethral area were seen in all animals treated continuously with T+E_2 for 18 weeks. When the E_2 was removed, three out of five of the animals developed small, well-differentiated adenocarcinomas. When both implants were removed, only two out of seven of the animals developed adenocarcinomas. The number of PIN-like lesions decreased when hormone implants were also removed.

5.4 The effects of fispemifene in the Noble rat model (IV)

In order to test the antiestrogenic and anti-inflammatory properties of fispemifene, five study designs were used. In the first part, in which the antiestrogenicity was assessed, animals were castrated and treated with E_2 plus vehicle or fispemifene for three weeks. In the second part, inflammation was induced in castrated animals with DHT+E_2 treatment and animals were treated with three different doses of fispemifene or one dose of tamoxifen for three weeks. In the third part, intact animals were treated for 13 weeks with T+E_2 and inflammation was evaluated. In the fourth part, animals were treated with T+E_2 for 18 weeks and during the last five weeks (weeks from 13 to 18) vehicle or fispemifene was administered. In the fifth part, the effect of E_2 removal on inflammation was examined as control treatment to fispemifene treatment. All the hormone dosages used are given in section 4.2, table 2.

The immunohistochemical expression of PR and Fra2, prolactin concentration in serum, the weights of seminal vesicles and pituitary glands were used as endpoints of estrogen action. Inflammation was assessed by counting perivascular and stromal infiltrates (short-term studies) or the number of inflamed acini (long-term studies). Furthermore, the aggressiveness of the inflammation was assessed on the basis of the relation of lymphocytes to acinar epithelium (long-term studies).

5.4.1 Hormonal changes and organ weights

5.4.1.1 Short-term experiments (3 weeks studies with castrated animals)

In the first short-term experiment animals were castrated and no concomitant androgen treatment was used. In these animals, estradiol treatment alone significantly increased the serum estradiol and prolactin concentrations. Fispemifene had no statistically significant effect on estradiol concentration, but it decreased the prolactin concentrations in serum dose-dependently when used simultaneously with estradiol. Estradiol treatment alone also increased the weight of the pituitary gland and the weight of the seminal vesicles which were reversed dose-dependently by fispemifene.

In the second short-term experiment, animals were castrated and treated with DHT+E_2. Treatment increased the serum estradiol level. Fispemifene had no significant effects on serum estradiol while tamoxifen significantly increased it. Both fispemifene and tamoxifen reduced the estradiol-induced increase of the prolactin level and the relative weight of the pituitary gland.

When fispemifene or tamoxifen were administered alone, both significantly increased serum prolactin levels but they did not influence the estradiol level, or the relative weights of the pituitary gland or seminal vesicles.

5.4.1.2 Long-term experiments (13 and 18-weeks studies with intact animals)

Serum testosterone, estradiol, and prolactin concentrations were all significantly increased at 13 and 18 weeks in noncastrated, T+E_2 treated animals compared to placebo animals**.** The relative weights of the seminal vesicles and the pituitary glands followed the testosterone and prolactin concentrations, respectively.

In the animals in which the E_2 implant was removed after T+E_2 treatment for 15 weeks for the following 3-weeks period, no changes were seen in testosterone concentration, but estradiol and prolactin concentrations were significantly decreased compared to animals treated with T+E_2 for 18-weeks. Correspondingly, no changes were seen in the relative seminal vesicle weights, but removal of E_2 decreased significantly and the weight of the pituitary gland also decreased.

Fispemifene had no effects on testosterone or estradiol concentration when compared to animals treated with T+E_2+Oil for 18-weeks. However, it significantly decreased the prolactin concentration, which was followed by a decreased relative weight of the pituitary gland. No changes were seen in relative seminal vesicle weight between the fispemifene and vehicle groups.

5.4.2 Antiestrogenic effects of fispemifene (short-term study)

The antiestrogenic activity of fispemifene was evaluated by its effect on immunohistochemical staining of PR and Fra2 in acinar epithelium. Normally in castrated rats, there were no cells with PR- or Fra2-positive nuclei in the DLP. When castrated animals were implanted with E_2, the number of Fra2- and PR-positive was increased. PR-and Fra2-positive nuclei were predominantly localized in the acinar epithelium of the lateral prostate. Some stromal cells in the lateral prostate were positive for both PR and Fra2. Fispemifene decreased the number of cells with PR and Fra2-positive nuclei and did not induce any positive cells when used alone.

5.4.3 Inflammation assessment; effect of fispemifene and tamoxifen (short-term study)

There was no perivascular or stromal accumulation of lymphocytes in any castrated animal treated singly with estradiol or fispemifene. To induce inflammation in the DLP in castrated rats, androgen is needed in addition to estrogen. Low-doses of DHT have a pro-inflammatory action and high-doses of DHT have an anti-inflammatory action in the prostate. The dose of DHT in the three-week experiment was chosen to induce a maximal extravasation of lymphocytes at the 71 μg/day dose level of estradiol (Yatkin et al., 2008).

DHT+E_2 treatment for three-weeks increased the number of the perivascular and stromal infiltrates and only a small number of inflamed acini were seen. Fispemifene caused a dose-related non-significant decline on the total number of the infiltrates. When fispemifene was administered alone to DHT-maintained animals, there was no significant increase in the number of inflammatory infiltrates. In contrast, tamoxifen significantly increased the total number of perivascular and stromal inflammatory infiltrates but had no significant anti-inflammatory effect in the presence of estradiol.

5.4.4 Anti-inflammatory effects of fispemifene (long-term study)

In the non-castrated animals treated with $T+E_2$ for 13- and 18 weeks, inflammation in the stroma varied from solitary infiltrates to confluent areas and only some perivascular foci could be observed. All animals had inflamed acini locating mostly in the lateral lobes. The total number of inflamed acini as well as the aggressiveness of the inflammation was analyzed. At 18 weeks, inflammation was significantly more aggressive than at 13 weeks meaning more contact between infammatory cells and epithelium. The number of the inflamed acini was higher at 18 weeks than at 13 weeks. Fispemifene, given concomitantly with $T+E_2$, showed only a slight effect on aggressiveness at 18 weeks. However, fispemifene significantly decreased the total number of inflamed acini (IV, Figures 6 and 7). The decrease was equivalent to that seen after removal of estradiol implants. Tamoxifen was not tested in this study design due to its inflammatory properties observed in short-term studies.

6 DISCUSSION

6.1 Development of prostatic inflammation, obstructive voiding and prostate cancer in hormone-treated adult Noble rats

6.1.1 Hormonal requirements

Estrogen-induced prostatic inflammation is well-described phenomenon in the adult rat and mouse (Naslund et al., 1988; Robinette, 1988; Bianco et al., 2002). Further, it has repeatably been shown that the combined treatment of adult Noble rats with testosterone and estradiol results in the development of precancerous lesions and adenocarcinomas (Noble, 1977; Bosland et al., 1995; Thompson et al., 2002). However, the development of prostatic inflammation and cancer in the same animal species and strain after the same hormonal treatment has not been studied in any of the previous studies. This is also true for obstructive voiding and prostatic inflammation which develop in rats and mice after neonatal estrogen treatment (Pylkkänen et al., 1991; Streng et al., 2001) and after the testosterone treatment at adulthood (Maggi et al., 1989; Pandita et al., 1998). Serum hormone concentrations have not been reported systematically in any of these studies nor has a rationale seldomly been given for the selected doses. The possible role of the inflammation in prostate carcinogenesis or in development of obstructive voiding has not been adequately discussed, either. In conclusion, no attempts have been made to study the possible causal interrelationships of these diseases when they appear as consequence of the treatment of experimental animals with androgen and estrogen.

In the present study, the testosterone and estradiol concentrations in serum were systematically monitored to find out the hormonal requirements for the induction of inflammation, prostate carcinogenesis and obstructive voiding. Figure 1 summarizes their development as a function of time and hormone concentrations. The present findings support the concept that increased estradiol concentration combined with a decreased T-to-E_2 ratio in serum plays a significant role in induction and maintenance prostatic inflammation. Supraphysiologic concentrations of both testosterone and estradiol and a decreased T-to-E_2 ratio were needed for the development of obstructive voiding, precancerous lesions and adenocarcinomas.

The doses of testosterone and estradiol used in the long-term studies resulted in serum concentrations that were higher than reported previously in Noble rat studies by others (Leav et al., 1988; Ofner et al., 1992; Lane et al., 1997). This may account for the much shorter time required for PIN-like lesions and carcinomas to appear in the present study compared to earlier studies. While Wang and Wong (1998) used a high dose strategy similar to ours, the time required to induce a high prostate carcinoma incidence was much longer in their study (10-12 months). In most other studies, including the study by Wang and Wong (1998), silastic tubing implants were used for hormone delivery. In contrast, commercially available slow-release hormone pellets

were used in the present study to ensure constant hormone release and to improve reproducibility of the disease models. A significant increase in estradiol concentrations was seen at 13 and 26 weeks of treatment even though same dose of estradiol was used. This is likely due to altered estrogen metabolism during the course of the treatment. Possibly, aromatization of exogenous testosterone and/or altered balance between estrone and estradiol or between conjugated and non-conjugated forms contributed significantly to the increased estradiol concentration.

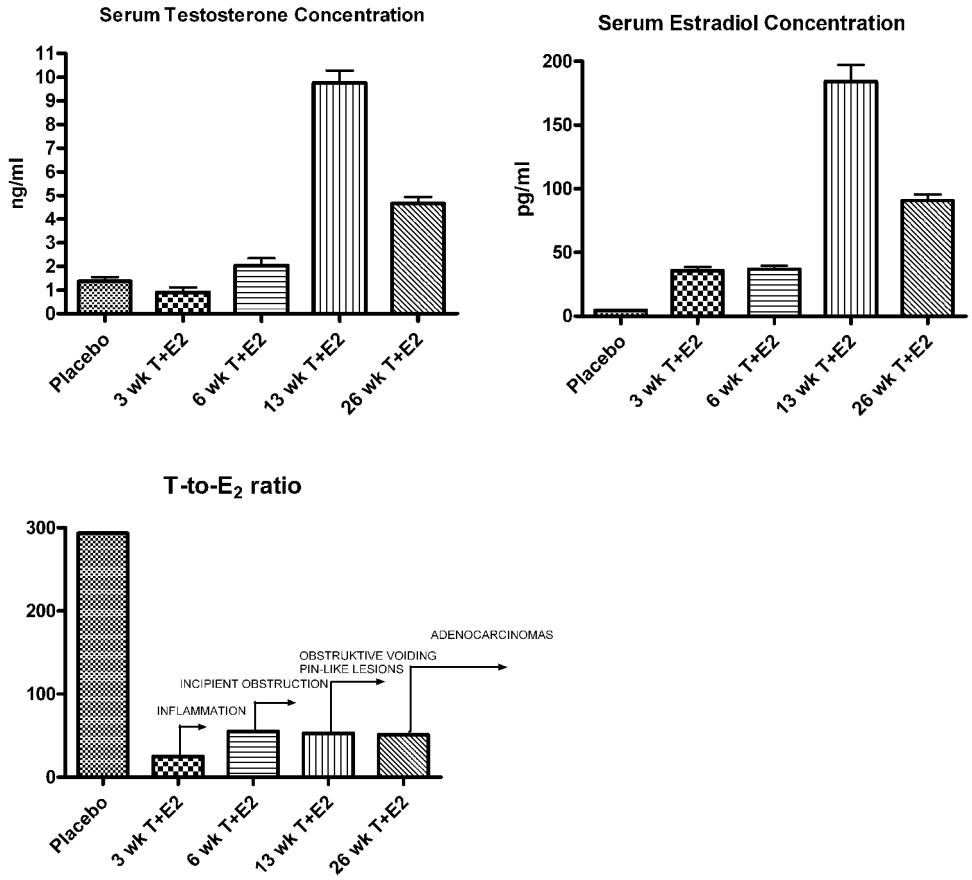

Figure 1. Serum hormone concentrations (upper panel) and the testosterone-to-estradiol (T-to-E_2) ratios (lower panel) promoting development of prostatic inflammation, obstructive voiding, PIN-like lesions and adenocarcinomas in the present model (13 week treatment refers to hyper-T group)

Another confounding factor which may account for the difference between the studies may be the diets fed to the animals. Soy-free diet was used during breeding, growth, and maintenance of the animals which assured an almost complete absence of estrogenic dietary stimuli; others typically used standard natural ingredient diets or did not even describe the diet used. It is known that soy contains components that may have anti-inflammatory, antiestrogenic or anti-carcinogenic properties (Sharma et al., 1992; Bosland et al., 2001; Chen & Anderson 2002; Kurahashi et al., 2007; Yatkin et al., 2007). Thus the use of a soy-free diet may possibly make rats more susceptible to hormonal prostate carcinogenesis.

6.1.2 Histopathology of prostatic inflammation

Prostatic inflammation developed in the DLP after T+E_2 treatment for three weeks. Lymphocyte-predominant infiltrates and the inflammation pattern (perivascular, stromal/periglandular and glandular) seen in animal studies are similar to those described in human chronic non-bacterial prostatitis and in BPH patients (Boag and Young, 1999; Gumus et al., 2004). When the T+E_2 treatment time was extended to 13 weeks, stromal infiltrates occupied an increasing stromal proportion of the DLP due to the increased number and the migration of the cells. The periacinar accumulation of lymphocytes and the number of inflamed acini increased simultaneously.

Two different types of inflamed acini were recognized in both treatment groups after the T+E_2 treatment for 13 to 26 weeks. Acini with signs of chronic inflammation had partially destroyed epithelium with intraepithelial lymphocytes and intraluminal neutrophils and macrophages. These acini resembled the segregated inflamed glands described by Kohnen and Drach (1979) in the human prostate. Anim et al., (2006) used the term ‘active chronic prostatitis’ to describe the corresponding pattern of inflammation in the human prostate. Intact epithelium and luminal neutrophils were characteristic of the other type of inflamed acini which formed the largest group of inflamed acini. These acini resembled the acute type of prostatic inflammation in the human prostate (Alexander, 1999). There were intermediate forms of inflamed acini showing various numbers of neutrophils, macrophages and lymphocytes and varying degrees of epithelial alterations, giving the impression that acute acinar inflammation gradually turns chronic. As in humans, the aggressiveness of the inflammation was assessed (Irani et al., 1997). Both the aggressiveness of the inflammation and the total number of the inflamed acini increased in the present study with treatment time as shown in Figure 2. A corresponding gradual development has not been described in human.

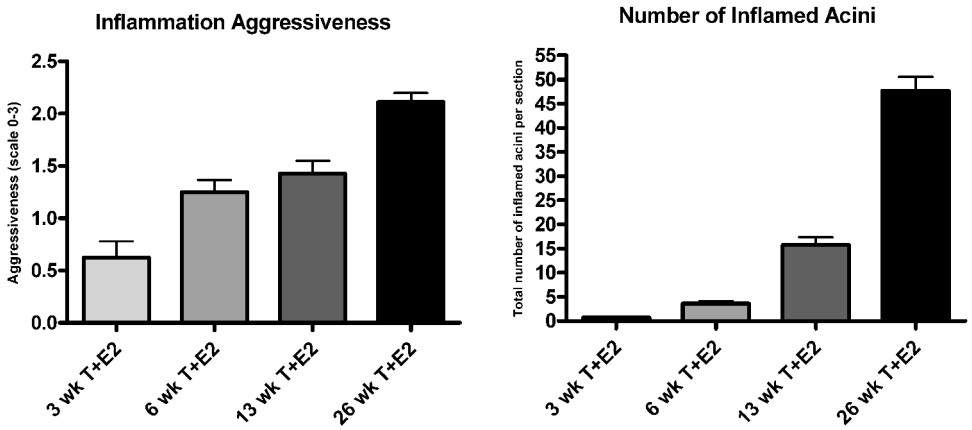

Figure 2. Aggressiveness of the inflammation and the number of inflamed acini increased as a function of testosterone and estradiol ($T+E_2$) treatment time (13 week treatment refers to hyper-T group)

In the present study, different parameters were established to describe and quantitate inflammatory response (presented in Table 4, section 4.7.1). Parameters based on the location, area, extent, grade and aggressiveness were adopted from human prostate section analysis (Irani et al., 1997; Nickel et al., 2001b; Sciarra et al., 2008) and modified for the present animal study. The counting of the inflamed acini has not been described in the inflammation assessment earlier but it was succesfully used in the assessment of anti-inflammatory properties of fispemifene in the present study.

6.1.3 Altered voiding

In the present study, urodynamical measurements were performed after $T+E_2$ treatment for three, six- and thirteen weeks. No changes were observed after treatment for three weeks, and only incipient signs of obstructive voiding were observed after treatment for six weeks. To better understand hormonal conditions required for the development of obstructive voiding the impact of testosterone concentration and decreased T-to-E_2 ratio was studied.

The hypoandrogenic animals with a decreased T-to-E_2 ratio developed prostatic inflammation and non-obstructive voiding. When the testosterone concentration was increased above control and T-to-E_2 ratio still remained below control, the animals developed obstructive voiding in addition to inflammation. Thus, it was concluded that prostatic inflammation and voiding function differed in their responses to the increase of the testosterone and estradiol concentrations and the T-to-E_2 ratio, which is not in accordance with the hypothesis that prostatic inflammation would be a sufficient condition for the development of obstructive voiding.

6.1.4 Development of precancerous lesions and carcinomas

In the present study, adenocarcinomas were found in the proximal parts of the collecting ducts located in the posterior periurethal region; no carcinomas were observed in acinar or ductal epithelium outside the rhabdosphincter. This confirms earlier findings of Bosland et al. (1995). The PIN-like lesions have not previously been reported in this posterior periurethral region. Dysplastic and other histopathological changes have earlier been described in the dorsolateral lobes containing the acini (Lane et al., 1997; Thompson et al., 2002; Tam et al., 2007). However, no carcinomas have been found. This suggests that PIN –like lesions seem develop into carcinomas only in prostatic ducts.

The specific location for the adenocarcinomas is noteworthy. In human, prostate cancer mainly arises in the peripheral zone. The central zone accounts smaller number of prostatic adenocarcinomas but interestingly these central zone prostatic adenocarcinomas have a high prevalence of ductal carcinoma (Mai et al., 2008). The difference in the zone specificity maybe due to different embryonic origin; the central zone likely shares the same origin with Wolffian ducts and the remaining prostate develops from urogenital sinus mesenchyme. Furthermore, hormonal control also differs; central zone development is under the control of testosterone and development of the rest of the prostate is under the control of dihydrotestosterone (Emberton and Mundy, 2004). This developmental difference and formation of the estrogen-responsive sites in the lower urinary tract (Pylkkänen et al., 1991; Pylkkänen et al., 1993) may explain the particular location for adenocarcinomas also seen in the present study.

The adenocarcinomas were classified to three grades: well-, moderately- or poorly differentiated as earlier described in the Noble rat model (Wang & Wong et al., 1998). The Gleason grading system has not been applied to the Noble rat model; thus far only one experimental study has used the ‘Gleason analogous grading system’ utilizing a knock-in mouse adenocarcinoma prostate model (Wu et al., 2006). The majority of the adenocarcinomas were moderately-differentiated and multiple cancers were present. These adenocarcinomas resembled human prostate cancer in their increased proliferative activity, detected by Ki-67 immunostaining, and the absence of a basal cell layer identified by cytokeratin 5/6 immunostaining. Unlike human prostate cancer that often forms metastasis, no clearly detectable metastases were observed in the present study. Metastasis has not been found in other earlier Noble rat studies (Bosland et al., 1995; Wang and Wong, 1998).

The PIN-like lesions and adenocarcinomas had a strong expression of androgen receptor, a marker of androgen responsiveness. The high serum testosterone concentrations in this study probably account for the high proliferation activity observed in the epithelial cells of the ducts. The reduced tumor incidence following removal of testosterone implants confirmed the androgen-dependency of these ductal adenocarcinomas. Human prostate cancer often turns from androgen-dependent to androgen-independent but they express androgen receptor (De Marzo et al., 2004).

6.2 Site and mechanism of estrogen action in the prostate

6.2.1 Proinflammatory action of estrogen

Prostatic inflammation developed gradually after the start of estradiol administration. Accumulation of T-lymphocytes in the perivascular space was the earliest sign of estrogen action. This suggests that an estrogen-induced increase in vascular permeability has an important role in the prostatic inflammatory response as in other autoimmune diseases. In addition to the direct effects of estrogens on the endothelial cells, estrogens may also have an influence on vascular smooth muscle cells. They contain estrogen receptors and are therefore potential targets for estrogen action (Koh, 2002). In the stromal smooth muscle cells and epithelial cells, estrogens may further increase synthesis of chemokines e.g. MIP-2 which, in turn, attracts inflammatory cells towards acini (Harris et al., 2000).

When the treatment time was prolonged from 3 to 6 weeks glandular inflammation, a more advanced form of prostatic inflammation developed. Glandular inflammation was composed of inflamed acini containing mostly neutrophils. A role of estrogen in recruitment of neutrophils into inflamed sites has also become evident in studies using the hypogonadal (hpg) mouse which features complete postnatal deficiency of pituitary gonadotropins, and subsequently of sex steroids (Bianco et al., 2002). A strong recruitment of neutrophils and macrophages (but not lymphocytes) into the mouse uterus after the treatment of the mouse with estradiol at pregnancy levels offers another example of these organ-specific estrogenic effects on neutrophils (Tibbetts et al., 1999). The mechanism of estrogen action is not known but estrogen may control chemotactic factors and their receptors (Straub, 2007). Epithelial cells may also be directly involved in the recruitment of inflammatory cells.

6.2.1.2 Prolactin and prostatic inflammation

Estrogen-induced inflammation in the DLP has been associated with increased prolactin levels and increased weight of the pituitary gland (Tangbanluekal and Robinette, 1993). This was confirmed in the present study. Earlier studies have suggested that prolactin at least partly mediates the estrogen-induced inflammatory action in the prostate (Tangbanluekal and Robinette, 1993). In the transgenic mouse with prostate-specific expression of prolactin, chronic inflammation (primarily lymphocytes and macrophages) was frequently observed in the prostate but also an increased distribution of ERα in stromal cells was evident (Kindblom et al., 2003). Therefore, it is difficult to gain unequivocal evidence to support the idea that either prolactin or estrogen alone would cause inflammation since blocking of prolactin release by bromocriptine only partially prevented estradiol-induced inflammation (Lane et al., 1997). In addition to pituitary release, prolactin can be produced in the prostate itself (Nevalainen et al., 1997), and it may act not only as an hormone but also

as a cytokine (Ben-Jonathan et al., 2002), which further complicates the interpretation of the findings.

6.2.2 Mechanism of estrogen carcinogenicity

The number of PIN-like lesions and carcinomas decreased after removal of estogen implant indicating that the development of the cancers was dependent on estrogen. However, no ERα– or PR-positive cells could be detected in the ductal epithelial cell or cancer cells. No specific role has either been found for ERα in human prostate cancer (Härkönen and Mäkelä, 2004). This means that the action is mediated by ERβ or it is indirect or mediated through non-receptor mechanisms.

Estogens present in excessive amounts, may also be carcinogenic through their metabolites. An increased local conversion of estradiol to catecholestrogens and further reactive metabolites which acts as DNA damaging carcinogens has been demonstrated at the sites of prostate carcinogenesis in Noble rats (Bosland 2006; Carruba 2007). In addition, carcinoma sites may lose active protective enzymes against these reactive estrogen metabolites (Cavalieri et al., 2002).

6.3 Relationship of inflammation with obstructive voiding and prostate carcinogenesis

6.3.1 Altered voiding

The association of lower urinary tract dysfunctions with non-bacterial prostatitis has not been studied in animal models until recently. The role of increased urethal pressure has been tested in rats with partial urethral obstruction caused by nylon ligature on uretha (Takechi et al., 1999). This model is not relevant for the discussion of the possible consequences of prostatic inflammation on voiding, because of obvious changes in bladder function by the ligature narrowing the urethral lumen. Nakano and colleagues (Nakano, 2006) injected hydrochloric acid beneath the prostatic capsule of the rat inducing tissue damage and consequently a non-bacterial prostatitis and lower urinary tract disorders. In agreement with the findings of Nakano et al., non-bacterial prostatitis induced with hormonal treatments was followed by alterations of the lower urinary tract in the present study.

There are several possible explanations for the development of urodynamic alterations simultaneously with prostatic inflammation. Inflammatory cells secrete cytokines that can exert their effects locally or systemically (Jang and Schaeffer, 2003). Another possibility is that urodynamic changes are caused by direct, non-inflammatory hormonal effects on the LUT. It should also be emphasized that in the present study the urodynamical recordings and inflammation analysis were performed at the same time

and only at the end of the hormone treatment. The histopathological end-points and voiding dysfunctions do not necessarily reflect or correlate immediately to the course of acute and chronic inflammation. It is known that the clinical course of prostatitis syndromes varies; patients with CP/CPPS have chronic and episodic symptoms without normalization while acute prostatitis patients have sudden and passing symptoms (Alexander, 1999).

6.3.2 Prostate carcinogenesis

The present findings support the concept that inflammation plays a significant role in the early steps of prostate carcinogenesis. There were no inflammatory cells in the posterior periurethral area before the development of PIN-lesions predominantly in ducts coming from the lateral lobes. PIN-like lesions preceeding the development of ductal adenocarcinomas were surrounded by lymphocytes. Neither neutrophils nor macrophages were present. Lymphocytes have also been found close to human PIN-lesions (Blumenfeld et al., 1992).

The function of lymphocytes adjacent to precancerous lesions remains unknown. There are many possibilites e.g. inflammatory cells secrete several cytokines and other factors that promote epithelial cell proliferation and stimulate angiogenesis (Lucia and Torkko, 2004; De Marzo et al., 2007). Reactive oxygen and nitrogen radicals produced in inflammation (De Marzo et al., 2007) may be involved in malignant transformation of epithelial cells located in proximity to lymphocytes, causing tissue damage and making the ductal epithelium vulnerable to carcinogenic insults. In addition to local effects, it is possible that stimulatory factors produced by lymphocytes and neutrophils in the acini are transferred in secretions that flow down the prostatic ducts.

The absence of lymphocytes or other leucocytes e.g. macrophages around adenocarcinomas suggest that these cancers have escaped from immunological surveillance. This kind of reduction of cell-mediated immunity has been shown to be associated with some invasive cancers (Lucia and Torkko, 2004). Such cancer-related immunosuppression may be caused by local tumor effects (e.g., tumor cells inhibiting T-cell function in the tumor microenvironment) or by systemic effects (e.g., induction of systemic impairment of tumor-reactive T cell function) (Kim et al., 2006; Adler, 2007). In human prostate cancer, the function of different inflammatory cells and the impact they may have on prostate carcinogenesis should also be clarified (Haverkamp et al., 2008).

6.4 Treatment of prostatic inflammation with SERM

6.4.1 Rationale for the use of SERMs

As presented in this study, as well as in the literature, both sex steroid hormones, androgens and estrogens, contibute to the development of prostatic diseases. The incidence of prostatic diseases increases with age, concomitanly with declining testosterone and normal or even slightly increased estradiol concentrations. In theory, estrogen induced prostatic inflammation could be considered to be prevented by testosterone replacement therapy. However, high androgen concentrations to antagonize estrogen action overstimulate prostate growth (Yatkin et al., 2008). Thus other approaches should be addressed. Androgen and estrogen receptors, or enzymes metabolising these hormones, could be considered as a reasonable drug targets for the prevention and treatment of prostatic diseases.

Aromatase is an enzyme converting androgens to estrogens, and it plays an important role in the production of estrogens in men (Braunstein, 1999). Aromatase activity has been demonstrated in men in the testis, adipocytes, and also in the prostate. Thus the use of aromatase inhibitor to prevent estrogen action could in theory work. However, clinical trials with aromatase inhibitors have failed to show efficacy in prostate cancer therapy (Santen et al., 2001; Smith et al., 2002).

A more targeted approach with SERM, toremifene, showed prevention of progression to prostate cancer in men with HGPIN (Price et al., 2006). In addition, toremifene has been shown to prevent osteoporosis and other adverse effects resulting from androgen-ablation therapy for prostate cancer (Smith et al., 2008). Considering the different location and function of ERα and ERβ in the prostate there is also sensible to evaluate specific ERα-antagonists or ERβ-agonists for the prevention and treatment of prostate diseases (Ellem and Risbridger, 2007; Setlur et al., 2008). In fact, selective ERβ-agonists have recently received attention as an anti-inflammatory agent and their effect has been proven e.g. in the experimental models of chronic intestinal and joint inflammation (Harris et al., 2003). In addition, DPN, a specific ERβ agonist, has been shown to prevent prostatic inflammation in testosterone treated luteinizing hormone receptor knockout (LuRKO) mice, which may be caused by local or systemic effects (Savolainen et al., 2007). However, no clinical data has yet been published to show the efficacy of any SERM or ERβ-agonists on human prostatic inflammation.

6.4.2 Fispemifene and prostatic inflammation

Fispemifene is a novel tissue-specific SERM that acts as an antiestrogen in the breast, mainly as an estrogen in the bone and as a partial estrogen/antiestrogen in the endometrium. At the level of the hypothalamic-pituitary axis, fispemifene acts as an antiestrogen by inhibiting the negative feedback of estrogen. This results in an

enhanced production of luteinizing hormone (LH) and subsequently increased testosterone levels. Fispemifene has been safe and well-tolerated in Phase I single and repeated dose studies in healthy men and in a Phase II study in hypogonadal men (Hormos Medical database).

In the present study, the pro-and anti-inflammatory actions of fispemifene in the hormone treated rat prostate were assessed. Histopathological results showed that fispemifene decreased the number of inflamed acini, but did not significantly decrease the number of perivascular and stromal lymphocyte infiltrates in the DLP of the Noble rat. Fispemifene itself was devoid of estrogenic effects in the prostate and no inflammatory effects were seen in the DLP when fispemifene was administered alone or together with DHT. In contrast, tamoxifen exhibited pro-inflammatory properties when administered together with DHT, but had no significant anti-inflammatory action on lymphocyte infiltration. Due to this pro-inflammatory action, tamoxifen was not tested in the 18 week experiment.

The lack of the anti-inflammatory effect on lymphocyte influx was surprising. The role of estrogen in the control of the lymphocyte migration through the vascular wall is well established even though the sites and mechanisms of estrogen action remain unclear (Straub, 2007). Inhibition of lymphocyte accumulation by ICI 182,780 compound was consistent with the estrogen receptor-mediated mechanism in the DLP of the intact, testosterone and estradiol treated Noble rat (Study I). The lower binding affinity of fispemifene to estrogen receptors compared to ICI 182,780 (Savolainen-Peltonen et al., 2004) may explain the weak action of fispemifene on extravasation of lymphocytes. The extent of estrogen receptor blockade and reduction in estrogen signaling produced by ICI 182,780 is also unique and differentiates it from SERMs (Howell and Abram, 2005).

In the present study, fispemifene exhibited simultaneous antiestrogenic action as evidenced by decreased serum prolactin concentration and pituitary weight as well as decreased PR and Fra2 immunohistochemical expression in the acinar epithelium of the DLP suggesting that the attenuation of glandular inflammation may be due to the antiestrogenicity of the compound. It is possible that these estrogen-induced, androgen-antagonized events may be activated with the changes in the ratio between the estrogen and androgen. The same mechanism could account for down-regulation of the expression of multiple other estrogen-responsive genes and proteins in the prostate (West et al., 1990; Risbridger et al., 2001; Nellemann et al., 2005). Normally, the antigenic proteins would be sequestered or suppressed but an increased ratio of estrogen-to-androgen would expose them or induce their expression. The inflammatory response may thus represent a host response to the acinar epithelium that is antigenically distinct from normal epithelium (Blumenfeld et al., 1992).

Taken together, the findings of the present study suggest that SERMs could be considered as a new therapeutic possibility in the prevention of chronic prostatic inflammation; treatment started on newly-diagnosized CP/CPPS patients before

episodic phases begin and in addition, SERMs could be considered in the treatment of chronic active prostatic inflammation.

6.5 Relevance of the findings in the understanding of human diseases and drug discovery

This established preclinical model is readily available for the screening of drug candidates for the prevention and/or treatment of chronic prostatic inflammation, obstructive voiding and prostate cancer. The established animal models mimick the human diseases in many respect. Lymphocyte-predominant infiltrates and inflammation pattern (perivascular, stromal/periglandular and glandular) seen in animal studies are similar to those described in human chronic non-bacterial prostatitis (Boag and Young, 1999). The adenocarcinomas resembled human prostate cancer in their increased proliferative activity, detected by Ki-67 immunostaining, and the absence of a basal cell layer identified by cytokeratin 5/6 immunostaining.

The Noble rat model may be relevant also for the study of urethral obstruction in men. Urodymical findings indicated a non-prostatic mechanism in the development of obstructive voiding in hormone-treated Noble rats. This is in accordance with the clinical findings implying that the LUTS may be caused by urethral obstruction and/or abnormal detrusor contractility (under- or overactive bladder) in men with or without enlarged prostate. However, one has to be cautious when interpreting the present data or when applying the data to man. The study was exploratory using an animal model with extreme hormone concentrations and ratios of the T-to-E_2 to detect clear responses in prostatic inflammation and voiding in a reasonable time. The information supporting the role of the T-to-E_2 ratio in the development of chronic non-bacterial prostatitis and obstructive voiding in man is limited and indirect. The T-to-E_2- ratio in serum declines in non-obese men from 160 to 100 (at the age of 25 to 34 years and at the age of 85 to 100 years, respectively) (Vermeulen, 1996). Among men with LUTS, the corresponding ratios were 170 and 120 (at the age of 40 to 59 years and at the age of 80 years or more, respectively) (Schatzl et al., 2000). The ratio may drop to 60 or even less in massively obese men (Schneider et al., 1979; Kley et al., 1980). The possibility that the age-related decrease in the testosterone concentration and the decrease in the ratio of T-to-E_2 may be critical for the development of chronic prostatitis and LUTS cannot be excluded in man (Schatzl et al., 2000).

7 SUMMARY AND CONCLUSIONS

The present study aimed to explore the action of estradiol and testosterone on the development of prostatic diseases and voiding dysfunction by establishing an experimental model. Prostatic inflammation was suggested as a key factor in the genesis of lower urinary tract symptoms and prostate cancer. In this case, new medication efforts, e.g. based on SERMs anti-inflammatory action, could be considered in the prevention and treatment of human prostate diseases. The main conclusions of the present experimental study are as follows:

1. Elevated estradiol concentration in the presence of normal or increased testosterone was essential for the gradual development of prostatic inflammation in the DLP in three weeks. Estrogen-dependency was supported by the findings, in which removal of estrogen implant or treatment with antiestrogen diminished inflammation. Microscopic methods were successfully established to quantitate inflammatory infiltrates.

2. The significance of absolute hormone concentrations and imbalance in the testosterone to estradiol ratio (T-to-E_2) on prostatic inflammation and voiding was shown. Histopathological and urodynamical analyses indicated that the decreased T-to-E_2 ratio from >300 to 10 combined with a hypoandrogenic state and an elevated serum estradiol level developed prostatic inflammation and non-obstructive voiding. The supraphysiologic concentrations of testosterone and estradiol with the T-to-E_2 ratio of 50 an had weak anti-inflammatory effect, but caused obstructive voiding. Diverse responses did not support the hypothesis that non-bacterial prostatic inflammation would be a sufficient condition for the development of obstructive voiding.

3. 13-weeks treatment with testosterone and estradiol resulting in supraphysiologic hormone concentrations maintained inflammation in the DLP. In the periurethal area, where no pathological changes were observed at earlier timepoints, PIN-like lesions appeared at 13 weeks with adjacent lymphocyte infiltration. These lesions progressed to adenocarcinomas at 18 and 26 weeks of continuous testosterone and estradiol treatment. No inflammatory cells were observed any more adjacent to adenocarcinomas. The results thus point towards an initiator role for inflammation in the early steps of prostatic carcinogenesis.

4. Fispemifene, a novel SERM, showed anti-inflammatory action in this experimental model by significantly attenuating the number of inflamed acini in the DLP. Fispemifene also exhibited antiestrogenic properties by decreasing expression of estrogen-induced biomarkers in the acinar epithelium. SERMs could thus be considered as a new therapeutic option for chronic prostatic inflammation.

8 ACKNOWLEDGEMENTS

This study was carried out in the Department of Cell Biology and Anatomy, Institute of Biomedicine, University of Turku during the years 2003-2008. Professor Kalervo Väänänen is warmly acknowledged for providing the opportunity to work in the Department of Cell Biology and Anatomy.

I owe my deepest gratitude to my supervisor, Professor (Emeritus) Risto Santti. His vast store of knowledge, careful phrasing on questions and thorough attention to findings has guided me through this project. I truly appreciate his committment to research work and I am fortunate for all the support and encouragement he has given me during these years.

I want to thank Professor Mika Scheinin for enabling me to be a student in the Drug Discovery Graduate School (DDGS). I warmly thank Eeva Valve for coordinating all the practical issues related to DDGS.

I want to thank Docent Paula Kujala and Professor Olavi Lukkarinen for their valuable comments and suggestions when reviewing this thesis. I also want to thank Michael Nelson for his careful work with revising the language of the thesis.

I warmly thank all of my co-authors for their contribution to this work. I want to thank Liisa Pylkkänen for her profound preclinical as well as clinical expertise in cancer biology; Tomi Streng for his special proficiency in urodynamics and carrying out the urodynamical measurements; Emrah Yatkin whose support and skilful assistance in several things I have always been able to trust, Markku Kallajoki for expertise in pathology and Maarten Bosland for his valuable knowledge of the Noble rat model. I also thank Katherine Vega for her skills with the Noble rat model. I express my deep thanks to Eva-Maria Talvitie, Yvonne Konkol, Arto Laakso and Mikael Anttinen for carefully carrying out numerous analysis and modellings.

I am truly grateful for all the people who have worked in the ‘prostatitis research group’. You all have been so dignified in the lab as well as outside the lab sharing friendship. In addition to my supervisor and co-authors I deeply thank Riku Anttila, Jorma Paranko, Mari-Liinu Kauppila, Natalia Habilainen-Kirillov, Pauliina Koivunen, Milla-Riitta Kuronen, Juho Mäkelä, Minna Rauhala, Leena Ruotsalainen and Tuula Tanner. Tuula is especially acknowledged for her patience in histological sectioning and Natalia for her comprehensive work with the animals, in the lab and office.

I am grateful for the National Technology Agency of Finland (TEKES) for the financial support and collaboration during years 2003-2006. I want to thank Merja Hiltunen from TEKES, and representatives from attendant companies; Risto Lammintausta from Hormos Medical and Pekka Kallio from Orion Pharma. Also other people related to this collaboration are warmly acknowledged. Valuable collaboration with Hormos Medical after the TEKES project has been essential for the continuation

of the project and I am especially thankful to Risto Lammintausta, managing director, for giving me challenging opportunity to study new SERM compound.

I want to thank Professor Juha Peltonen, Professor Pirkko Härkönen, Professor Sari Mäkelä and numerous other present and former members of the Department of Cell Biology and Anatomy. I especially wish to thank Sanna Auer, Iris Dunder, Teuvo Hentunen, Soili Huhta, Teija Hurmerinta, Krista Hänninen, Johanna Järvi, Marja-Liisa Kostian, Anneli Kurkela, Tiina Laitala-Leinonen, Taina Malinen, Niina Saarinen-Aaltonen, Saija Savolainen and Annikki Vaisto. Sirpa Saarinen is acknowledged for the skilful computer assistance. I also thank Ulla-Marjut Jaakkola, director of the central animal laboratory, for providing proper facilities and expert staff for collaboration.

I want to thank all my friends and especially Ruusa Hilakari, Katariina Nuoranne, Salla Rinne and my colleagues from The Degree Program of Health Biosciences, Tbiot98, for friendship.

I warmly thank my parents, Sirpa and Vesa Ämmälä, my sister Erika Helin and my parents-in-law, Heta and Markus Bernoulli, for support.

Finally, I devote dearest thanks to my husband Leo and to our sons, Daniel and Niklas.

This study was financially supported by the National Technology Agency of Finland (TEKES) and attendant companies, Hormos Medical Ltd, Subsidiary of QuatrX Pharmaceuticals and Orion Oyj, Orion Pharma, and the Drug Discovery Graduate School.

Turku, September 2008

Jenni Bernoulli

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