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# Prostate intraepithelial neoplasia in Noble rats, a potential intermediate endpoint for chemoprevention studies

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**Abstract**

In most prostate chemoprevention studies conducted with animal models, the incidence and multiplicity of tumours have been used as endpoints for efficacy. However, the latency of tumours is usually over 1 year, making these studies costly and time consuming. The main purpose of this study was to assess the utility of prostate intraepithelial neoplasia (PIN), induced in Noble rats by continuous testosterone + oestradiol (T + E) administration, as a potential intermediate endpoint biomarker of efficacy in chemoprevention studies. Noble rats at the age of 12 weeks were treated for 36 weeks with T + E given subcutaneously via Silastic capsules. The incidence and multiplicity of PIN were assessed in various prostate glands by serial sections generated at three separate tissue levels. The efficacy of dehydroepiandrosterone (DHEA) and DHEA 8354 (1000 and 2000 mg/kg diet), difluoromethylornithine (DFMO) (1000 and 2000 mg/kg diet) and oltipraz (125 and 250 mg/kg diet) to inhibit PIN was assessed in two independent sets of experiments. T + E induced multiple PIN in the dorsolateral prostate (DLP) of 80–100% of the animals. DHEA and DHEA 8354 did not affect the incidence but decreased the multiplicity of PIN in the DLP, from $3.2 \pm 1.0$ in control group to $1.5 \pm 1.0$ in the low-dose and to $1.6 \pm 0.6$ in the high-dose group for DHEA ($P < 0.05$ and $P < 0.02$, respectively), and to $1.9 \pm 0.8$ in the high-dose ($P < 0.05$) DHEA 8354. Both agents did not affect PIN in anterior prostate, seminal vesicles or ventral prostate. In a second experiment, DFMO and oltipraz were found not effective in inhibiting PIN. In this study, we provide new evidence that PIN in Noble rats, induced by continuous T + E treatment, is a useful intermediate endpoint for determining the efficacy of DHEA and other potential chemopreventive agents. The hormonal pathogenesis, high multiplicity, short latency, preferential location in the DLP, similarity in morphology and biology to PIN of human prostate, and the sensitivity to agents that suppress prostate carcinogenesis, makes PIN in Noble rats a promising intermediate endpoint for chemoprevention studies.
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*Keywords:* Prostate carcinogenesis; PIN; Noble rats; Chemoprevention

## 1. Introduction

Prostate cancer is the most frequently diagnosed cancer among men and the second leading cause of male deaths in the United Sates [1,2]. That prostate cancer occurs relatively late in life, progresses slowly, is hormone dependent, and in most cases, is preceded by prostate intraepithelial neoplasia (PIN) that could be easy diagnosed and treated, offers excellent perspectives for the development of efficacious chemoprevention strategies [3]. Although good progress has been made in developing novel antitumour and chemopreventive agents and in characterising their mechanisms of action, there is still a lack of a significant breakthrough in the chemoprevention of prostate cancer. One reason for this problem is the lack of appropriate animal models and biomarkers for assessing the efficacy of various chemopreventive agents [4,5]. In most studies, models of

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chemically induced carcinogenesis in rats have been used to evaluate the efficacy of potential chemopreventive agents [6,7]. Lobund–Wistar or Wistar–Unilever rats have generally been employed, because after treatment with carcinogen and continuous hormone stimulation, tumours occur mainly in accessory sex organs [8–10]. However, in these studies, the incidence and frequency of tumours are relatively low, and latency is long (>12–16 months), making efficacy studies costly and time consuming [4]. Furthermore, the carcinogens used for the induction of prostate tumours in animals are apparently not the cause of human prostate cancer, which is considered a hormone-related disease [2]. Most importantly, tumours do not occur in the dorsolateral prostate (DLP), which appears to be relevant to the site of origin of PIN and carcinomas in human prostate. Rather, lesions are observed in the anterior prostate (AP) and seminal vesicles (SV), where human cancer is extremely rare [7,11,12]. Recently developed transgenic mouse models have a high incidence of prostate tumours with a short latency period, but tumours arise in other organs as well, and most importantly, the animals are difficult to breed and maintain for long-term chemoprevention studies [13,14].

In the present study, we employed hormone-induced prostate carcinogenesis in Noble rats to assess the efficacy of several potential chemopreventive agents. Noble rats develop a relatively high incidence of spontaneous and hormone-induced prostate carcinomas, as compared to other rat strains [15–17]. Most importantly, the occurrence of tumours is preceded by multiple dysplastic lesions, which in origin, morphology and biological characteristics appear similar to human PIN [18,19]. Recently, by using Noble rats we found that 9-*cis*-retinoic acid (9cRA) given for 36 weeks reduced the multiplicity of PIN in the DLP in a dose-dependent manner, whereas 4-(hydroxyphenyl) retinamide (4-HPR) was not efficacious [20]. Here, we examined the utility of PIN induced in Noble rats as a potential intermediate endpoint for assessing the chemoprevention efficacy of dehydroepiandrosterone (DHEA), DHEA 8354, difluoromethylornithine (DFMO) and oltipraz. In previous studies, some of these chemoprevention agents have shown inhibitory effects on chemically induced prostate carcinogenesis, as well as activity with other carcinogenesis models [4,21,22]. The above agents were selected because they have differing mechanisms of action: DHEA and its fluorinated analogue DHEA 8354 affect testosterone and oestrogen synthesis and probably their signalling pathways [23]; DFMO is a powerful inhibitor of ornithine decarboxylase activity, a key enzyme in mammalian polyamine biosynthesis [24]; oltipraz is involved in the elimination of carcinogen metabolites and of free radicals by modulating levels of glutathione-*S*-transferase and glutathione reductase activity [25]. Of all the above chemopreventive agents, only DHEA and DHEA 8354 were found efficacious and suppressed the development of PIN in accessory sex organs of Noble rats.

## 2. Materials and methods

### 2.1. Animals

Male Noble rats were obtained from the Biological Testing Branch of NCI. After 1 week of quarantine at the age of 12 weeks, rats were subcutaneously implanted with Silastic tubing for the deliver of steroid hormones. Two, 2-cm long capsules were filled with testosterone propionate (Sigma, T1500; Sigma Chem. Co., St. Louis, MO) and one, 1-cm long capsule was filled with oestradiol-17β (Sigma, E 8875). Control rats received implants of unfilled (empty) Silastic capsules. All Silastic capsules were removed and replaced at 2-month intervals throughout the study (36 weeks).

### 2.2. Chemopreventive agents

DHEA was obtained from Sigma; DHEA 8354 (fluasterone; 16a-fluorinated analogue of DHEA) was supplied by Fort Washington Resources (Hatboro, PA). Both agents were given in the feed, AIN-76A purified mice/rat diet (Teklad, Madison, WI), at 1000 or 2000 mg/kg diet, for 36 weeks. Due to toxicity of the high dose of DHEA 8354, as indicated by decreases in body weight, the high dose was reduced to 500 mg/kg diet at 31 days after beginning hormone treatment. The above doses were selected based of previous studies on prostate carcinogenesis, where they have shown efficacy and suppressed significantly the development of prostate tumours [28].

DMFO was obtained from the NCI Chemoprevention Branch, lot no. 49327. It was given at two concentrations: 1000 and 2000 mg/kg diet.

Oltipraz was obtained from the NCI Chemoprevention Branch, lot no. RP 35972. It was mixed in the diet at 125 and 200 mg/kg diet.

### 2.3. Autopsy and examination of prostate lesions

Animals were killed by $CO_2$ asphyxiation, their abdominal cavities were opened to the pubis, and the VP were dissected first and fixed in 10% buffered formalin. Next, the entire prostate complex, DLP, AP, SV, together with the penis and urinary bladder, were removed from the body and placed in a Petri dish with phosphate-buffered saline for macroscopic examination. The urinary bladder was removed from the entire prostate complex and then the DLP was cut longitudinally into halves through the prostate urethra and between the seminal vesicles. One severed half was placed in the

embedding cassette with the prostate urethral portion lying face down and the lateral part facing up. This tissue was again cut in half along a longitudinal midline plane of the SV. The outer surface of the lateral prostate was placed face down in the embedding cassette. At autopsy, any other tissues of interest (abnormal) were collected for histopathological evaluation. All tissue samples were fixed in 10% neutral formalin and embedded in paraffin. Serial paraffin sections (4 μm), at three separate tissue levels 200 μm apart, were stained with haematoxylin–eosin (H&E) for evaluation of tissue abnormalities. All resulting slides were labelled consecutively. Starting with slide no. 1, every fourth slide was processed for staining. The VP and seminal vesicles were bisected, and then sections were obtained at two different levels and stained with H&E for histopathological evaluation. This method of embedding and sectioning has proved to be essential for the detection of dysplastic lesions and microinvasive carcinomas, and to trace accurately their origin and structural characteristics.

### 2.4. Histomorphology of prostate lesions

The diagnoses of PIN and carcinoma were based on the criteria suggested by Leav and colleagues [18] and Bosland and colleagues [25]. PIN was distinguished from typical hyperplasia based on the multilayers of dysplastic epithelial cells, which frequently formed alveolar or papillary structures. The increased nuclear size, in addition to increased variability in nuclear shape, stainability and nucleolar prominence (anisokaryosis), as well as irregularity in nuclear spacing, cell crowding and cytoplasmic eosinophilia, which sharply contrasted with the palely stained cytoplasm of normal or hyperplastic epithelial cells, were important criteria for characterising PIN in Noble rats. Mitotic figures were also frequently observed in PIN. The repetition of the same lesion within one tubulo-alveolar unit in three consecutive tissue sections was counted as one lesion. In carcinomas, the cells were further dedifferentiated, occupied the entire ductal lumen and infiltrated the surrounding tissue.

### 2.5. Statistical evaluation of data

The differences in the weight of treated vs. control animals were assessed by the two-sided Fisher's $t$ test. The incidence of PIN (animals with PIN vs. those with no PIN) was assessed by comparison of proportions. The multiplicity of PIN (number of PIN per control or treated animal) in control and treated animals were assessed 36 weeks after the start of the experiment, when the animals were killed and all prostate glands examined by serial sections as described above. The differences in the multiplicity of PIN between control and treated animals were evaluated by the Wilcoxon–Mann–Whitney test. Differences with $P < 0.05$ were considered significant [26].

## 3. Results

### 3.1. Testosterone + oestradiol decreased the body weight in Noble rats

In these studies, 220 Noble rats were used. Two sets of experiments were independently performed: one with DHEA and its analogue DHEA 8354 and another with oltipraz and DFMO (Tables 1 and 2). Twenty animals per group were examined. In a preliminary study, five

Table 1
Effect of dehydroepiandrosterone (DHEA) and its analogue DHEA 8354 on prostate intraepithelial neoplasia (PIN) in Noble rats*

| | Basal diet T + E | DHEA 1000 mg/kg diet | DHEA 2000 mg/kg diet | DHEA 8354 1000 mg/kg | DHEA 8354 500, mg/kg | Control placebo |
|---|---|---|---|---|---|---|
| Animals (no): at start | 20 | 20 | 20 | 20 | 20 | 10 |
| Testosterone + oestradiol (T + E) | + | + | + | + | + | – |
| Survival (%) | 95 | 100 | 100 | 90 | 85 | 100 |
| Body weight (g) | 352 ± 5.7 | 347 ± 7.2 | 339 ± 5.1 | 317 ± 7.1* | 328 ± 7.2* | 468 ± 14.9[a] |
| PIN in DLP: incidence (%) | 100 | 80 | 95 | 95 | 90 | 0 |
| Multiplicity | 3.2 ± 1.0** | 1.5 ± 1.0** | 1.6 ± 0.6** | 1.7 ± 0.6** | 2.0 ± 0.9** | 0 |
| AP + SV: incidence (%) | 20 | 20 | 30 | 25 | 25 | 0 |
| Multiplicity | 0.4 ± 0.4 | 0.2 ± 0.4 | 0.3 ± 0.4 | 0.25 ± 0.4 | 0.25 ± 0.4 | 0 |
| VP: incidence (%) | 30 | 30 | 40 | 20 | 20 | 0 |
| Multiplicity | 0.3 ± 0.5 | 0.4 ± 0.6 | 0.5 ± 0.6 | 0.4 ± 0.8 | 0.3 ± 0.7 | 0 |

[a] The body weight of the animals on basal diet only (no hormone implants) was about 30% higher than of the animals with hormone implants, $P < 0.001$, two-sided $t$ test.

* Significant difference in body weight between control animals and those treated with DHEA 8354 ($P < 0.006$ and $P < 0.017$).

** Significant difference in tumour multiplicity between control (T + E) and DHEA-treated animals ($P < 0.001$ and $P < 0.001$ for high and low doses) and between control and DHEA 8354-treated animals ($P < 0.001$ and $P < 0.001$ for high and low doses, respectively) DLP, dorsolateral prostate; AP, anterior prostate, SV, seminal vesicles; VP, ventral prostate; incidence, percent of animals with PIN; multiplicity, number of PIN per treated animal.

Table 2
Effects of oltipraz and difluoromethylornithine (DMFO) on prostate intraepithelial neoplasia (PIN) in Noble rats

| | Basal diet T + E | Oltipraz 125 mg/kg diet | Oltipraz 250 mg/kg diet | DFMO 1000 mg/kg diet | DFMO 2000 mg/kg diet | Control |
|---|---|---|---|---|---|---|
| Animals (no): at start | 20/19 | 20 | 20 | 20 | 20 | 10 |
| Testosterone + oestradiol (T + E) | + | + | + | + | + | – |
| Survival (%) | 97 | 80 | 90 | 95 | 95 | 100 |
| Body weight (g) | 341 ± 7.6 | 351 ± 8.1 | 351 ± 7.4 | 343 ± 4.7 | 343 ± 7.0 | 439 ± 17.3* |
| DLP: incidence (%) | 78.5 | 75 | 80 | 70 | 80 | 0 |
| Multiplicity | 1.4 ± 0.9 | 1.4 ± 1.0 | 1.2 ± 0.8 | 1.3 ± 1.0 | 1.5 ± 0.9 | 0 |
| AP + SV: incidence (%) | 28.5 | 25 | 15 | 10 | 20 | 0 |
| Multiplicity | 0.4 ± 0.4 | 0.3 ± 0.4 | 0.2 ± 0.4 | 0.1 ± 0.5 | 0.2 ± 0.5 | 0 |
| VP: incidence (%) | 31 | 30 | 40 | 20 | 20 | 0 |
| Multiplicity | 0.3 ± 0.4 | 0.4 ± 0.5 | 0.5 ± 0.4 | 0.4 ± 0.3 | 0.3 ± 0.4 | 0 |

See Table 1 for abbreviations.

The body weight of the animals on basal diet only (no hormone implants) was about 30% higher than of the animals with hormone implants, $P < 0.001$, two-sided $t$ test.

animals were killed 20, 24, 26 and 30 weeks after initiation of testosterone + oestradiol (T + E) stimulation to make sure that DLP dysplastic lesions with characteristics of PIN had developed (data not presented). As shown in Fig. 1(a)–(d), the body weight of the animals treated with T + E in all these independent sets of ex-

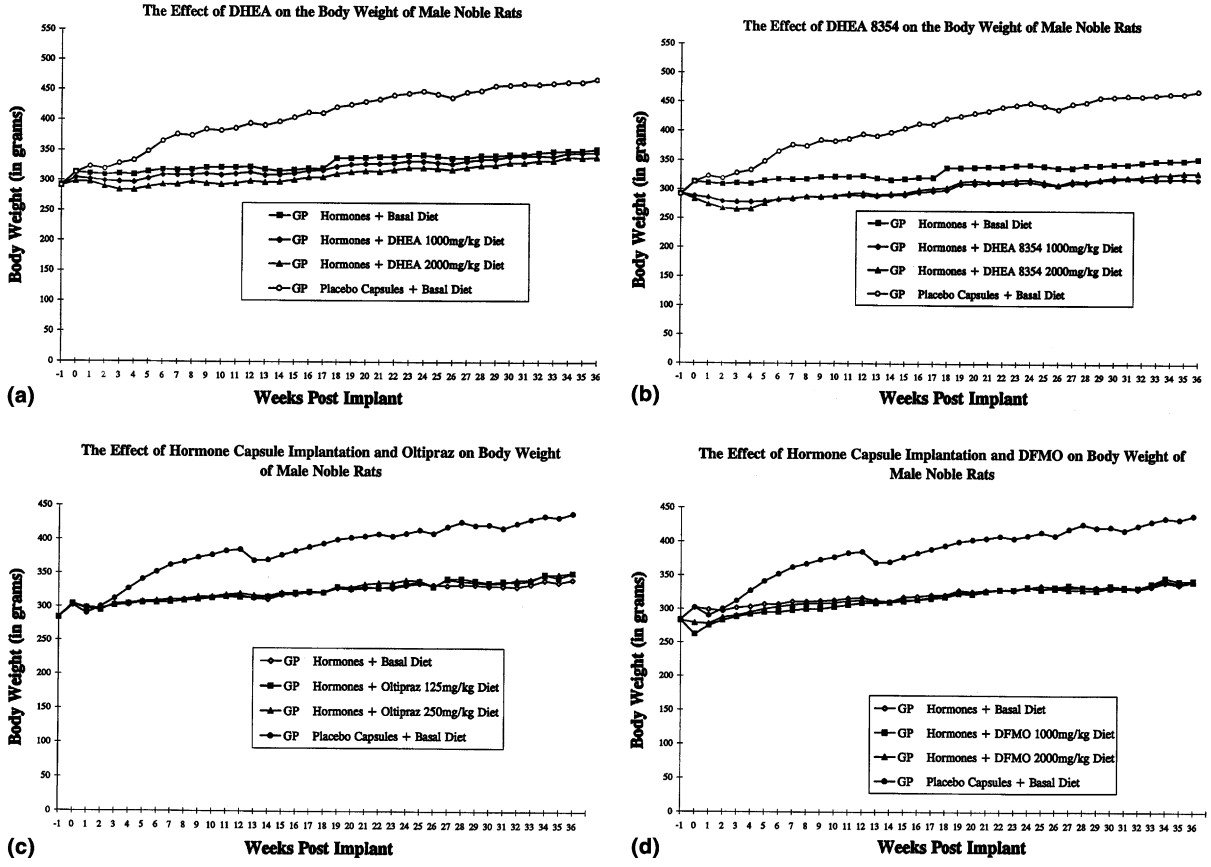


Fig. 1. (a) Body weight growth in control and dehydroepiandrosterone (DHEA)-treated animals. Hormone treatment (testosterone + oestrogen; T + E) of the animals decreased body weight by about 30% ($P < 0.001$). DHEA at both doses did not significantly decrease body weight. (b) Body weight growth of control and DHEA 8354-treated animals. In hormone + basal diet-treated animals, body weight decreased by about 33% as compared to the control (placebo-treated) animals. DHEA 8354 at both dose levels decreased the body weight growth, starting 2 weeks after initiation of treatment ($P < 0.05$). The high dose DHEA 8354 (2000 mg/kg diet) was apparently toxic. Therefore, we decreased it to 500 mg/kg diet, starting 31 days after initiation of treatment and continuing until the end of the experiment. (c) Body weight growth curves of the animals treated with oltipraz. There is a decrease in body weight in T + E-treated animals. Oltipraz, at both dose levels, 125 and 250 mg/kg diet, did not affect the body weight growth. (d) Body weight growth curves of animals treated with difluoromethylornithine (DFMO). The body weight of T + E-treated animals decreased by 30% ($P < 0.01$) as compared to the placebo-treated animals. DMFO at both doses (1000 and 2000 mg/kg diet) did not affect the body weight growth of the animals.

periments was about 30% lower than in control animals. Among all agents examined, DHEA 8354, at both dose levels (1000 and 2000 mg/kg diet), further suppressed body weight gain by 9.9% and 6.9%, respectively ($P < 0.005$ and $P < 0.01$, respectively), as compared to the body weight of the animals treated with T + E only. Since the high dose of DHEA 8354 affected the body weight in the first 4 weeks after beginning treatment, we decreased the dose from 2000 mg/kg diet to 500 mg/kg diet, starting 31 days after start of treatment, until the end of the experiment (Fig. 1(b)).

### *3.2. PIN occurred predominantly in dorso lateral prostate of Noble rats*

PIN was identified mostly in the DLP of control animals and animals treated with chemopreventive agents. The periuretral ductal areas were predominantly affected. One or several ducts were occupied by dysplastic epithelial cells, which frequently formed alveolar or papillary structures (Fig. 2(b)); these figures illustrate typical PIN with multiple layers of dysplastic cells showing increased nuclear size, variability in nuclear

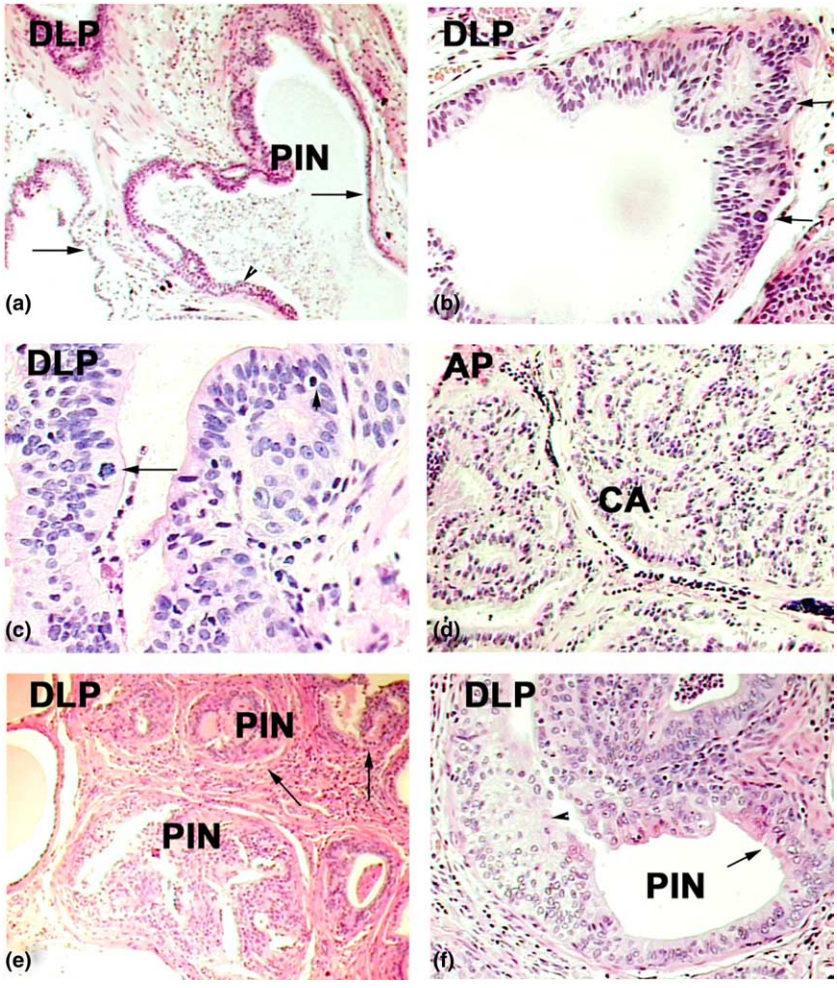


Fig. 2. (a) Prostate intraepithelial neoplasia (PIN) in the dorsolateral prostate (DLP) of a control animal treated for 36 weeks with testosterone + oestrogen (T + E). Dysplastic cells are in multilayers (arrow) or form alveolar or pseudoalveolar (arrowhead) and papillary structures. Haematoxylin & eosin, 100×. (b) PIN in DLP of an animal treated with T + E only. Dysplastic cells form alveolar or pseudoalveolar (lack of defined basal lamina between alveoli) structures. Note the high mitotic activity (arrows) of dysplastic cells, 200×. (c) PIN in a T + E-treated animal. Dysplastic cells display significant atypia with high mitotic activity (arrow) and apoptotic cells (arrowhead), 400×. (d) Carcinoma (CA) in the anterior prostate (AP) of animals treated with T + E. Tumour cells occupy the entire ductal structure and form alveolar structures, 200×. (e) PIN in animals treated with DHEA, 1000 mg/kg diet. One PIN is in the DLP, the upper part of the figure where the arrows are also placed, the other PIN is in the AP, where dysplastic cells form alveolar or papillary structures. Ductal and alveolar structures are surrounded by a severe inflammatory reaction with collagen deposition, numerous blood vessels and fibroblast proliferation, 100×. (f) PIN in the DLP of animals treated with DHEA 8354, 1000 mg/kg diet. Note the dysplastic cells of the most internal ductal layer apparently undergo metaplasia with increased eosinophilic cytoplasm and pyknotic nuclei (arrow). Similar alterations occur in other areas (arrowhead) where the cell cytoplasm became more transparent, 400×.

shape, and chromatin condensation (anisokaryosis), as well as irregularity in nuclear spacing and cell crowding. Mitotic figures (Fig. 2(b) and (c) arrow) were frequently observed in PIN, whereas apoptotic cells were rare (Fig. 2(c), arrowhead). PIN was frequently associated with inflammatory cells (leucocytes, lymphocytes, macrophages) surrounding the lesions or penetrating in the ducts, as well as between the dysplastic cells. In one animal treated with T + E, lesions with characteristics of carcinoma were found (Fig. 2(d)). Note, ducts are occupied by tumour cells forming alveolar structures and that the cells are heterogeneous in form and staining pattern.

### *3.3. DHEA and DHEA 8354 but not DFMO and oltipraz suppressed PIN in Noble rats*

In animals treated with DHEA or DHEA 8354, there were areas in the DLP and AP where alterations in PIN were frequently observed. There were desquamated dysplastic cells in the ductal lumen (Fig. 2(e)) and the stroma surrounding PIN was occupied by collagen deposits (Fig. 2(e), arrows), numerous blood vessels, and inflammatory cells. In Fig. 2(f), the PIN illustrated has dystrophic cells among the most internal cell layer (arrow) or the development of metaplasia in other areas (arrowheads). Oltipraz and DFMO did not induce alterations in PIN morphology.

The incidence of PIN varied between 78.5% and 100% in control animals (treated with T + E). These data are from three independent experiments (Tables 1 and 2). PIN was mostly found in the DLP of 80–100% of animals. The incidence of PIN decreased in AP + SV and VP, where 10–40% of the animals had PIN. DHEA, DHEA 8354, DMFO and oltipraz did not affect the incidence of PIN in any of the glands examined. The multiplicity of PIN per animal was in the range of 1–7, with variable mean values in individual experiments (Tables 1 and 2). Both DHEA and DHEA 8354, at both dose levels, significantly decreased the multiplicity of PIN, from $3.2 \pm 1.0$ in control animals to $1.5 \pm 1.0$ in the low-dose ($P < 0.01$), and to $1.6 \pm 0.6$ in the high-dose ($P < 0.01$) groups of DHEA-treated animals. With DHEA 8354, the multiplicity was $1.7 \pm 0.7$ in the high-dose group (1000 mg/kg diet) ($P < 0.01$) and $2.0 \pm 0.9$ ($P < 0.1$) in the lower-dose group (500 mg/kg diet). Neither, DHEA nor DHEA 8354 was efficacious in inhibiting PIN in AP, VP and SV (Table 1). DFMO and oltipraz did not affect the multiplicity of PIN in any of the prostate glands examined (Table 2).

## 4. Discussion

The main goal of this study was to examine the utility of PIN in Noble rats as a potential endpoint for assessing the efficacy of chemopreventive agents: DHEA, DHEA 8354, DFMO and oltipraz. These agents have different mechanism of action at cellular and molecular levels and, in previous studies they have shown inhibitory effects in mammary, prostate and other carcinogenesis models [23,24,27]. We found that continuous treatment of Noble rats with T + E for 36 weeks induced multiple PIN, located predominantly in the DLP, and that among these chemopreventive agents, DHEA and its analogue DHEA 8354 were efficacious in suppressing the multiplicity of PIN. DFMO and oltipraz were not efficacious inhibitors of T + E-induced prostate carcinogenesis. To the best of our knowledge, this is the first study in which hormone-induced PIN in the prostate of Noble rats has been used as an endpoint to assess the efficacy of the above chemopreventive agents.

Our data support a recent study from McCormick's group [28], where methynitrosurea (MNU) + testosterone-induced prostate carcinogenesis in Wistar–Unilever rats was employed to assess the efficacy of DHEA. In this recent study, DHEA was given at the same doses, 1000 and 2000 mg/kg diet, as in our study, and the incidence and frequency of tumours in various accessory sex organs were examined 60 weeks after the start of treatment. Both doses of DHEA, given 1 week before beginning T + E treatment, 20 or 40 weeks after the initiation of hormone stimulation, when prostate hyperplastic and possibly premalignant lesions occur, suppressed the neoplastic process in the prostate glands. These data support the theory that DHEA may prevent not only the early stages of prostate cancer development, but also the progression of hyperplastic and premalignant lesions towards carcinoma. The precise mechanisms of chemopreventive efficacy mediated by DHEA and DHEA 8354 are unknown. DHEA in rodents is metabolised to 4-androstene-3,17-dione and testosterone. These androgens may also be converted to oestrogens and this is one possible avenue for DHEA to antagonise the stimulatory effects of testosterone on prostate epithelial cells [29]. DHEA may also suppress glucose-6-phosphate dehydrogenase and indirectly affect the proliferation of potentially aberrant cell clones in the prostate [30]. Further, DHEA may affect peroxisome-associated enzymes [31] or farnasyltransferase activity, as has been suggested recently [32]. In addition, using the MNU mammary carcinogenesis model in rats, we have found in preliminary experiments that DHEA induces cell differentiation and inhibits proliferation, and may also potentiate apoptotic cell death (unpublished data). All these data suggest that different molecular mechanisms are involved in mediating the chemopreventive activity of DHEA on prostate and mammary carcinogenesis.

Although the efficacy of DHEA and DHEA 8354 on mammary and prostate carcinogenesis in rodents is promising, it is still an open question if these agents

could be used in human chemoprevention trials. Unlike in rodents, DHEA is synthesised at relative high concentrations in man and a further increase in circulating concentrations may not have the same profound effects on the target cell populations as in rodents. DHEA is also a weak hepatocarcinogen stimulating peroxysome proliferation [21,22]. The potential conversion of DHEA to oestrogens may also yield undesirable hormone-related side-effects in patients, particularly in prolonged chemoprevention trials [29]. DHEA 8354 at both doses suppressed significantly the animals' body weight, suggesting that it is toxic. It is well known that the toxicity and the body weight loss caused by an antitumour agent might have inhibitory effects on tumour development and progression. Therefore, the decrease in the multiplicity of PIN in the animals treated with DHEA 8354 needs to be critically interpreted. The above chemoprevention agents were given in the diet, suggesting relatively constant circulating and tissue concentrations of their metabolites during the whole experiment. On a limited number of animals we also measured the serum testosterone and oestradiol concentrations 5, 15, 25 and 36 weeks after initiation of treatment and found that they were consistently higher as compare to the placebo-treated animals (data not presented).

The employment of PIN in Noble rats as a potential endpoint in prostate chemoprevention studies has some advantages over studies in which tumours have been used as endpoints: (i) PIN is induced by hormone stimulation (T + E) that is apparently involved in the pathogenesis of prostate cancer in men as well; (ii) PIN arises predominantly in the DLP, which is relevant to the area of human prostate cancer origin; (iii) in morphology and hormone dependence, the rat PIN appears similar to PIN in men; (iv) the small size of PIN allows identification of its precise place of origin within various glands and even within specific prostate structures, which is difficult to achieve with tumours because they infiltrate the surrounding tissues; (v) the latency of PIN is much shorter than that of tumours, the first PIN in DLP were identified 20 weeks after initiation of hormone stimulation, whereas the first carcinomas in other carcinogenesis models usually occur after 56 weeks or longer; (vi) the incidence and multiplicity of PIN are much higher than for carcinomas, which makes efficacy studies with PIN as an intermediate endpoint time and money saving; (vii) in a recent study, by using the same model of prostate carcinogenesis and the PIN as endpoint of efficacy, we found that 9-*cis*-retinoic acid but not 4-HPR suppressed the development of PIN in Noble rats [33]. All these features of PIN in Noble rats make it an attractive model for efficacy studies with potential chemoprevention and/or antitumour agents. The main obstacle in considering PIN as an endpoint in chemoprevention studies is that it is not known whether PIN will progress toward malignant tumours. PIN may spontaneously regress and disintegrate [34]. However, the fact that testosterone treatment alone was able significantly to increase prostate cancer in Noble rats as compared to non-treated animals, and that tumours occurred preferentially in the DLP, where PIN was also most frequent [18,19], supports the hypothesis that most PIN will progress into malignant tumours.

In conclusion, in this study we obtained data indicating that PIN, induced in Noble rats by T + E stimulation, is a feasible model for assessing the efficacy of potential chemoprevention agents. Their hormone pathogenesis, multiplicity, short latency, preferential location in the DLP, similarity in morphology to human PIN, and sensitivity to chemoprevention agents that have shown inhibitory effects on chemically induced prostate carcinogenesis make PIN in Noble rats a promising intermediate endpoint for prostate chemoprevention studies.

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