Review

Glutathione-Peroxidase (GSH-PO) and Androgen Action in Prostate: An Immunohistochemical Study**Masanori Murakoshi¹, R. Yoshiyuki Osamura² and Keiichi Watanabe³***¹Safety Research Department, Teikoku Hormone Mfg. Co., Ltd., 1604 Shimosakunobe, Takatsu-ku, Kawasaki 213–8522, Japan and ²Department of Pathology, Tokai University, School of Medicine, Bohseidai, Isehara-city 259–1193, Japan and ³Aoba Gakuen, 3–12–19, Setagaya, Setagaya-ku, Tokyo 154–0017, Japan*

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The effect of testosterone and estradiol on the prostate of castrated rats (rat prostatic hyperplasia model after combined treatment with testosterone and estradiol) were investigated by histopathologically and immunohistochemical procedures. The castrated rats were treated for 6 weeks with 1) testosterone 1 mg/animal and 2) testosterone 1 mg/animal plus 17beta-estradiol (estradiol) 10 µg/animal. Histopathologically, glandular hyperplasia of the ventral prostate and glandular hyperplasia with fibromuscular stromal proliferation in the dorsolateral prostate, was clearly observed. Immunohistochemical localization of glutathione peroxidase (GSH-PO) which effectively reduces the lipid peroxides, was predominantly observed in the glandular epithelial cells of the ventral prostate. The intensity of GSH-PO staining in the glandular epithelial cells was remarkably decreased after castration, and that it

was clearly recovered by testosterone or testosterone plus estradiol treatment to the castrated rats. In contrast, GSH-PO was clearly observed in the glandular epithelial cells of the dorsolateral prostate following testosterone alone or testosterone plus estradiol. Androgen receptor (AR) is mainly localized in nuclei. In the dorsolateral prostate, AR was also detected in the nuclei of the proliferated stromal fibromuscular cells. Furthermore, immunodetectable AR rapidly declined after androgen withdrawal and returned to intact levels of staining intensity after androgen replacement. These findings strongly suggest that expressions of GSH-PO and AR in the prostate are testosterone-dependent. It is concluded that immunohistochemical analysis, using GSH-PO and AR, may be a useful method for prediction of the effects of androgen action on the rat prostate.

Key words: androgen, androgen antagonist, immunohistochemistry, prostate, rat**I. Introduction**

Androgen controls the development and function of the prostate, but other steroid hormones, such as estrogen, are also supposed to have an important role in prostate physiology [4]. It is shown that glandular hyperplasia of the prostate

developed in castrated dogs after combined treatment with 5alpha-androstane-3alpha, 17beta-diol and 17beta-estradiol. It is generally accepted that estrogens play a critical role in the etiology of canine and human benign prostatic hyperplasia [22].

It is well documented that the histological appearance of the prostate in the castrated animals treated with testosterone plus 17beta-estradiol, resembles glandular hyperplasia [8]. It is known that 17beta-estradiol alone will induce glandular atrophy of the prostate in normal and castrated animals

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[1, 2]. The suppressive effects of 17beta-estradiol on the male genital organs have been attributed not only to gonadotropin suppression, but also to the direct effect of 17beta-estradiol on the gonads and their accessories [1]. It is generally accepted that 17beta-estradiol exerts a synergistic effect with androgen in promoting prostatic growth in the castrated dog [22], a fact of considerable importance in understanding the pathogenesis of prostatic hyperplasia.

The purpose of the present study was to examine the effect of testosterone plus 17beta-estradiol, on the castrated rat prostate (rat prostatic hyperplasia model after combined treatment with testosterone and 17beta-estradiol), as well as to analytically examine cell proliferation activity using 5-bromo-deoxyuridine (BrdU)-positive cell ratio, and to observe the immunohistochemical localization of androgen receptor (AR), glutathione peroxidase (GSH-PO) and Bcl-2 in the rat prostatic hyperplasia model.

II. Ventral Prostate

It is known that the castration-induced decrease in the number of BrdU-positive cells is reversed by testosterone. The stimulatory effects of testosterone are strongly potentiated by the addition to estradiol resulting in the highest BrdU-positive cell ratio (Table 1). Therefore, glandular hyperplasia is thought to be the main feature of the rat prostatic hyperplasia occurring experimentally as a result of treatment with testosterone in combination with estradiol (Fig. 1).

The intensity of GSH-PO staining in the glandular epithelial cells is remarkably decreased after castration, and is clearly restored by testosterone- or testosterone plus estradiol-treatment of the castrated rats (Fig. 2). It is well recognized that the metabolic and secretory activities of the prostate are regulated by testosterone. The secretory acid phosphatase in the glandular epithelial cells of the

Table 1. Effect of testosterone (T) and T plus 17beta-estradiol (E₂) on ventral prostatic weight and BrdU-positive cell ratio

Group	Preparation	n	Treatment	Prostatic weight (mg)		BrdU-positive cell ratio (%)
				Absolute	Relative	
1	Intact control	10	—	594±81	176±30.2	0.62±0.20
2	Castrated	10	—	572±72	174±32.3	0.02±0.00
3	Castrated	10	T	713±132	197±43.2	2.15±0.82
4	Castrated	10	T+E ₂	936±121	299±41.2	6.08±0.21

Values are the mean±S.D. BrdU, Bromodeoxyuridine
* p<0.05 vs intact (Dunnett's multiple comparison test)
** p<0.01 vs intact (Dunnett's multiple comparison test)
p, 0.01 vs castration+T (Student's *t*-test)

Fig. 1. Ventral prostate of castrated rat following treatment with combined testosterone and estradiol. Glandular hypertrophy/hyperplasia is noted. HE ×100.

Fig. 2. Ventral prostate of castrated rat following treatment with combined testosterone and estradiol. GSH-PO is clearly observed in the glandular epithelial cells. Peroxidase-labeled antibody method, $\times 150$.

Fig. 3. Ventral prostate of castrated rat following treatment with combined testosterone and estradiol. AR is predominantly observed in the nuclei of the glandular epithelial cells. Peroxidase-labeled antibody method, $\times 150$.

prostate appears to be testosterone-dependent, since it disappears after castration and returns after treatment with testosterone [20]. Therefore, an enzyme histochemical or immunohistochemical staining of acid phosphatase has been thought to be a good marker of androgen action in the rat prostate. Based on our data and these facts, GSH-PO in the rat prostate suggests a very close relationship to the

secretory or metabolic status of the glandular epithelial cells.

Immunodetectable AR rapidly declined after androgen withdrawal (2 days after castration) and returned to intact levels of staining intensity after androgen replacement (Fig. 3). This staining pattern is similar to that of GSH-PO. Thus, GSH-PO in the glandular epithelial cells suggests a close relationship to the status of testosterone action of the cells.

Therefore, it is strongly suggested that GSH-PO in the glandular epithelial cells of the rat ventral prostate is considered to be testosterone-dependent [12, 13]. We further speculated that lipid peroxidation is increased in the prostate by testosterone- or testosterone plus estradiol-treatment [9].

The intensity of Bcl-2 staining in the prostatic glandular epithelial cells is greatly decreased after castration, and is clearly restored by testosterone- or testosterone plus estradiol-treatment to the castrated rats. This staining pattern is similar to that of GSH-PO, it suggesting a comparable relationship to testosterone action in the cells. Therefore, it is strongly suggested that expression of Bcl-2 protein in the glandular epithelial cells of the rat ventral prostate is considered to be testosterone-dependent [11].

III. Dorsolateral Prostate

It is known that glandular hyperplasia of the ventral prostate develops in castrated rats after combined treatment with testosterone and estradiol. It is generally accepted that estrogens play a critical role in the etiology of canine and human benign prostatic hyperplasia [2, 21, 22]. The dorsolateral prostate of the rat has been shown to be very sensitive to estrogen [3]. Specific receptors for estrogen have been demonstrated in the dorsolateral prostate [23]. Therefore, it should be a good model for human prostate, since besides androgens, estrogens are supposed to have an important role in physiology and pathology [22].

The weight of the dorsolateral prostate was markedly increased by testosterone plus estradiol to the castrated rats (Table 2). In addition, it is seen that the castration-induced decrease in the number of BrdU-positive cells is reversed by testosterone. The stimulatory effects of testosterone are strongly potentiated by the addition of estradiol, resulting in the highest BrdU-positive cell ratio. Histologically, glandular hyperplasia is clearly seen (Fig. 4). It is well documented that estradiol exerts a synergistic effect with androgen in promoting prostatic growth in the castrated dog or rat, a fact of considerable importance in understanding the pathogenesis of prostatic hyperplasia. Fibromuscular stromal proliferation is also seen (Fig. 4). This feature might be explained by the action of estradiol, since growth of stromal tissue following estrogen administration has been demonstrated in the prostate of non-human primates and dogs [5]. Furthermore,

Rohr *et al.* [18] demonstrated that treatment of castrated dog with estradiol resulted in activation of the stromal tissue in association with smooth muscle cells.

We demonstrated that the intensity of GSH-PO staining in the glandular epithelial cells of the ventral prostate decreased following castration, and could be reversed by testosterone-administration of the castrated rats [9, 15]. We therefore postulated that prostatic GSH-PO may be testosterone-dependent [9, 15]. We found that immunohistochemical staining of GSH-PO in the glandular epithelium of the dorsolateral prostate is clearly responsive to testosterone alone or in combination with estradiol [10]. Therefore, it seems likely that GSH-PO protein synthesis in the glandular epithelium of the dorsolateral prostate can be enhanced (induced?) by sex hormones such as testosterone and estradiol. In fact, the significant increase of GSH-PO protein triggered by lipid peroxides has also been demonstrated in an experimental system of inactivation and reactivation of the arachidonate cascade in rat peritoneal macrophages [7]. These findings indicated that increased levels of lipid peroxides (or peroxidation) enhanced the expression of GSH-PO, or, in other words, the decrease of lipid peroxides might reduce the expression. We suggested that lipid peroxidation is increased in the dorsolateral prostate by testosterone alone, or testosterone plus estrogen-treatment.

AR is decreased 2 days after castration, and is clearly recovered by testosterone-administration of the castrated rats. These findings are in agreement with the results of the ventral prostate. AR was also detected in the nuclei of the proliferated stromal fibromuscular cells (Fig. 5). Human prostate epithelial cells in benign prostatic hyperplasia showed uniformly intense nuclear staining for AR [19]. Furthermore, intense AR staining has been observed in stromal cells of fibromuscular hyperplasia [19]. The intense staining of AR in the epithelium is also in contrast with the abundance of 5 α -reductase in the stroma, which converts testosterone into dihydrotestosterone (DHT) in the prostate. It is possible that the epithelium utilizes DHT supplied by the stroma.

IV. Effect of Antiandrogen

Combined treatment with chlormadinone acetate

Table 2. Effect of testosterone (T) and T plus 17 β -estradiol (E₂) on dorsolateral prostatic weight and BrdU-positive cell ratio

Group	Preparation	n	Treatment	Prostatic weight (mg)		BrdU-positive cell ratio (%)
				Absolute	Relative	
1	Intact control	10	—	298 $\pm$ 48.3	68 $\pm$ 12.1	0.31 $\pm$ 0.12
2	Castrated	10	—	285 $\pm$ 31.5	61 $\pm$ 9.8	0.08 $\pm$ 0.01
3	Castrated	10	T	399 $\pm$ 98.6	88 $\pm$ 42.3	1.35 $\pm$ 0.81
4	Castrated	10	T+E ₂	593 $\pm$ 81.5*	108 $\pm$ 22.3**	3.94 $\pm$ 0.23**

Values are the mean $\pm$ S.D. BrdU, Bromodeoxyuridine

* p<0.05 vs intact (Dunnett's multiple comparison test)

** p<0.01 vs intact (Dunnett's multiple comparison test)

Fig. 4. Dorsolateral prostate of castrated rat following treatment with combined testosterone and estradiol. The glandular epithelial cells exhibit a high columnar shape. In addition, proliferation of interacinar stroma is clearly detected. HE $\times 100$.

Fig. 5. Dorsolateral prostate of castrated rat following treatment with combined testosterone and estradiol. AR is observed in the nuclei of the glandular epithelial cells as well as stromal cells. Peroxidase-labeled antibody method, $\times 150$.

(CMA) as steroidal antiandrogen produced a striking decrease in prostatic weight. Histologically, the combined treatment with CMA produced inhibition of the glandular hyperplasia. Furthermore, we found that the intensity of GSH-PO staining in the glandular epithelial cells of the prostate was remarkably increased after treatment with testosterone plus estradiol to the castrated rats, and was

remarkably decreased after combined treatment with CMA. It is generally accepted that CMA inhibits the uptake of testosterone in the prostate and is selectively incorporated into prostate cells, inhibiting the binding of cytosol 5 α -dihydrotestosterone-receptor [6]. In addition, nuclear immunostaining of AR (both epithelial and stromal cells) is remarkably decreased after combined treatment

with CMA.

The results of this study indicated that CMA binds to the rat prostatic AR and that oral administration caused regression of the hyperplastic prostatic weight. Therefore, CMA is considered to be a steroidal antiandrogen receptor antagonist [14, 16, 17].

V. Concluding Remarks

Immunostainable GSH-PO, AR and Bcl-2 in the rat prostate are testosterone-dependent, and their staining patterns are useful markers for biological testosterone actions in prostatic cells.

VI. References

- Attia, M. A. and Zayed, I. (1989) Thirteen-weeks subcutaneous treated with high dose of natural sex hormones in rats. With special reference to their effect on the pituitary-gonadal axis. *Dtsch. Tierarztl. Wschr.* 96; 438–445.
- Bartsch, G., Bruengger, A., DeKlerk, D. P., Coffey, D. S. and Rohr, H. P. (1987) Light-microscopic stereologic analysis of spontaneous and steroid-induced canine prostatic hyperplasia. *J. Urol.* 137; 552–558.
- Belis, J. A., Adlstein, L. B. and Tarry, W. F. (1983) Influence of estradiol on accessory reproductive organs in the castrated male rats. *J. Androl.* 4; 144–149.
- Cunha, C. R., Donjacour, A. A., Cooke, P. S., Mee, S., Briggsby, R. M., Higgins, S. J. and Sugimura, Y. (1987) The endocrinology developmental biology of the prostate. *Endocr. Rev.* 8; 338–361.
- Ho, S. and Yu, M. (1993) Selective increase in type II estrogen-binding sites in the dysplastic dorsolateral prostates of novel rats. *Cancer Res.* 53; 528–532.
- Ito, Y., Kurosawa, I., Yamanaka, H., Koya, A., Imai, K., Kosaku, N. and Shida, K. (1977) The mechanism of antiandrogenic action of chlormadinone acetate. *Hinyoka Kiyo* 68; 537–552. (in Japanese)
- Murakoshi, M., Ikeda, K. and Watanabe, K. (1986) Immunocytochemical localization of glutathione-peroxidase (GSH-PO) in rat peritoneal macrophages. *Acta Histochem. Cytochem.* 19; 125–133.
- Murakoshi, M., Inada, R., Makino, M., Suzuki, M., Mieda, M., Honma, S. and Yamanaka, H. (1990) Effect of antiandrogen (TZP-4238) on steroid-induced canine prostatic hyperplasia. Light and electron microscopic investigations. *Acta Pathol. Jpn.* 40; 871–879.
- Murakoshi, M., Inada, R., Tagawa, M., Suzuki, M., Mizokami, A. and Watanabe, K. (1993) Histopathological and immunohistochemical studies in the rat ventral prostate. Effects of testosterone and testosterone plus estrogen. *J. Toxicol. Pathol.* 6; 55–64.
- Murakoshi, M., Inada, R., Tagawa, M., Suzuki, M., Mizokami, A. and Watanabe, K. (1994) Histopathological and immunohistochemical studies of the rat dorsolateral prostate. Effects of testosterone and testosterone plus estrogen. *J. Toxicol. Pathol.* 7; 387–395.
- Murakoshi, M., Ikeda, R., Tagawa, M., Suzuki, M., Takekoshi, S. and Watanabe, K. (1996) Immunolocalization of glutathione-peroxidase (GSH-PO) and bcl-2 in the rat ventral prostate. Effects of castration and administration of testosterone. *Acta Histochem. Cytochem.* 29; 283–288.
- Murakoshi, M., Ikeda, R. and Tagawa, M. (1999) A significant relationship between glutathione-peroxidase (GSH-PO) localization and biological action of testosterone in rat ventral prostate. *J. Toxicol. Sci.* 24; 209–216.
- Murakoshi, M., Ikeda, R. and Tagawa, M. (1999) Regulation of prostatic glutathione-peroxidase (GSH-PO) in rats treated with a combination of testosterone and 17beta-estradiol. *J. Toxicol. Sci.* 24; 415–420.
- Murakoshi, M., Tagawa, M., Ikeda, R., Nakayama, T. and Ishimura, K. (2000) Immunolocalization of androgen receptor (AR) and steroid 5alpha-reductase type II (5alpha-reductase type II) in canine prostate. Effect of antiandrogen, chlormadinone acetate (CMA). *Acta Histochem. Cytochem.* 33; 223–229.
- Murakoshi, M., Osamura, R.Y. and Watanabe, K. (2000) Immunolocalization of glutathione-peroxidase (GSH-PO) in the steroid-producing organs and prostate. *Acta Histochem. Cytochem.* 33; 405–412.
- Murakoshi, M., Ikeda, R., Tagawa, M. and Nakayama, T. (2001) Effect of chlormadinone acetate (CMA) on canine spontaneous prostatic hyperplasia. Experimental models for the human benign prostatic hyperplasia (BPH). *Oyo Yakuri/Pharmacometrics* 60; 1–6.
- Murakoshi, M., Ikeda, R. and Tagawa, M. (2001) Immunohistochemistry of the canine prostate. *Acta Histochem. Cytochem.* 34; 147–150.
- Rohr, H. P., Neaf, H. R. and Holliger, O. (1981) The effect of oestrogen on stromal growth of the prostate. *Urol. Res.* 9; 201–207.
- Sar, M., Lubahan, D. B., French, F. S. and Wilson, E. M. (1990) Immunohistochemical localization of the androgen receptor in rat and human tissues. *Endocrinology* 127; 3180–3186.
- Tenniswood, M. P., Brid, C. E. and Clark, A. F. (1976) Acid phosphatases. Androgen dependent markers for rat prostate. *Can. J. Biochem.* 54; 350–357.
- Tunn, S., Hochstrate, H., Habenicht, V. E. and Krieg, M. (1988) 5alpha-reductase activity in epithelium and stroma of prostates from intact and castrated dog treated with androstadiene, the aromatase inhibitor 1-methyl-1,4-androstadiene-3,17-dione, and cyproterone acetate. *Prostate* 12; 243–253.
- Wilson, J. D. (1980) The pathogenesis of benign prostatic hyperplasia. *Am. J. Med.* 68; 745–756.
- Yu, M., Cated, I., Leaw, I. and Ho, S. (1989) Heterogeneity of [³H]-estradiol binding sites in the rat prostate. Properties and distribution of type I and type II sites. *J. Steroid. Biochem.* 33; 449–457.