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Expression of Prostatic Glutathione-Peroxidase (GSH-PO) in The Rat Treated With A Combination of Testosterone and 17ß-Estradiol

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In order to confirm the relationship between sex hormone administration and glutathione-peroxidase (GSH-PO) in the rat ventral prostate, the levels of GSH-PO mRNA, GSH-PO activity, and lipid peroxide (Thiobarbituric acid: TBA) value in the ventral prostate were investigated. Male Crj: CD (SD) IGS rats were divided into six experimental groups. Group 1 consisted of intact controls. In group 2, rats were sacrificed two days after castration. In groups 3 and 4, rats were subcutaneously administered 1 mg/animal of testosterone daily for three- or seven-day administration two days after castration, respectively. In groups 5 and 6, rats were subcutaneously administered 1 mg/animal of testosterone plus 0.01 mg/animal of 17ß-estradiol (E_2) daily for three- or seven-day administration two days after castration, respectively. GSH-PO activity of the ventral prostate homogenate for testosterone or testosterone plus E_2 administration to the castrated rat was increased and the TBA value was remarkably decreased. The prostatic GSH-PO mRNA level was diminished in the castrated rat ventral prostate, but was increased by testosterone or testosterone plus E_2 administration. In particular, the GSH-PO mRNA level of testosterone plus E_2-treated animals was higher than that of testosterone-treated animals. These findings strongly suggest that expression of GSH-PO in the rat ventral prostate is testosterone- or E_2-dependent. We speculate that the transcription of prostatic GSH-PO mRNA was regulated by testosterone or E_2 and de novo synthesis of GSH-PO would thus be regulated at transcription level by testosterone or E_2.

Key words : Glutathione-peroxidase (GSH-PO), Lipid peroxidation, mRNA, Testosterone, 17ß-estradiol

INTRODUCTION

Glutathione-peroxidase (GSH-PO) is a selenium-dependent enzyme that exists as a homotetramer with each 22-kDa subunit containing a selenium atom incorporated within a catalytically active selenocysteine residure [4]. There are three other members of the selenium-dependent GSH-PO family, although cytosolic GSH-PO is the predominant form [6]. The gene encoding GSH-PO was mapped on chromosome 3q11-13 [6]. Because GSH-PO decomposes hydrogen peroxide and organic hydroperoxides produced during normal metabolism and after oxidative insults, GSH-PO prevents peroxide-induced DNA damage, lipid peroxidation, and protein degradation [10, 26].

In our previous report [19], immunocytochemical localization of GSH-PO, which effectively reduces lipid peroxides was demonstrated in the rat ventral prostate under castration and testosterone administration. As the result, the intensity of GSH-PO staining was decreased by castration and it was clearly recovered by testosterone administration to the castrated rats. Therefore, we postulated that GSH-PO in the glandular epithelial cells of the rat ventral prostate was testosterone-dependent [19].

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The development and differentiation as well as the maintenance of structure and secretory functions of the prostates are dependent on androgens, but other steroid hormones, such as estrogen, are also supposed to have an important role in prostate physiology [8, 16, 21].

In the present study, in order to confirm the relationship between sex hormone administration and the biological significance of GSH-PO in the prostate, we used a biochemical approach (northern blotting, GSH-PO activity and lipid peroxide value) to study the ventral prostate of normal, castrated and testosterone or testosterone plus 17ß-estradiol-administered rats.

MATERIALS AND METHODS

Animal and tissue preparation

Male Crj: CD (SD) IGS rats were purchased from Charles River Japan Inc. (Atsugi, Japan) at the age of seven weeks. The animals were kept in a barrier-sustained animal room, which was maintained at a temperature of 22 ± 2 ˚C with a relative humidity of 55 ± 15 %. The room was ventilated twenty-one times per hr and provided with 12 hr of light (from 8:00 to 20:00). Solid food (CE-2, CLEA Japan Inc.) and tap water were given *ad libitum*. The animals were kept for a one-week acclimation period under laboratory conditions.

Five animals served as controls (Group 1). In group 2, five rats were sacrificed two days after castration. In groups 3 and 4, five rats were subcutaneously administered l mg/animal of testosterone-propionate (testosterone, Sigma Chemical Co., St. Louis, MO) daily for 3- or 7-day administration two days after castration, respectively. In groups 5 and 6, five rats were subcutaneously administered l mg/animal of testosterone plus 0.0l mg/animal of 17ß-estradiol (E_2, Teikoku Hormone, Mfg. Co. Ltd., Kawasaki) daily for 3- or 7- day administration two days after castration, respectively. Testosterone and E_2 were dissolved in dimethyl sulfoxide. Each rat was killed by decapitation and the ventral prostates were removed immediately.

The animals were cared for according to the principle outlined in the guide for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our institution.

Biochemical examination

1. Measurement of lipid peroxide levels

One gram of fresh prostate was homogenized with a potter type homogenizer in 10 ml of 0.25 M sucrose solution containing 1 mM $MgCl_2$ and 0.7 mM 2-mercaptoethanol. The homogenates were centrifuged at 700 g for 10 min and pellets were removed. Then the lipid peroxide level of the homogenate was determined by the thiobarbituric acid (TBA) method according to the method of Yagi [28] and expressed in terms of malondialdehyde (n mol/ml).

2. Measurement of GSH-PO activity

One hundred microliters of homogenate obtained were pre-incubated with 2.5 ml of reaction medium containing 0.1 M Tris/borate buffer, pH 8.5, 3 mM EDTA/Tris, 0.12 mM NADPH (Oriental Yeast Co., Tokyo), 0.25 mM reduced glutathione (Sigma Chemical Co., St. Louis, MO) and 2.5 μg of glutathione reductase (Sigma Chemical Co., St. Louis, MO) for 2 min at 37 ˚C. The reaction was then started by the addition of 0.1 ml of cumen hydroperoxide (Mathen Coleman and Bell Manufacturing Chemist, USA). The decrease in the absorbance at 340 nm was measured after 5 min incubation using a Hitachi spectrophotometer model 210. One unit of enzyme oxidized 1 μmol NADPH to NADP/min at 37 ˚C.

3. Northern blotting

The GSH-PO mRNA was measured in extracts prepared by rapidly homogenizing tissues with guanidinium isothiocyanate solution [5]. Total mRNA was prepared by a standard cesium chloride-ultracentrifugation method [22]. Fifteen micrograms of total RNA were electroseparated and nick-transferred onto nitrocellulose filters. Hybridization was performed, as reported previously, using a [^{32}P]-labeled complementary deoxyribonucleic acid (cDNA) probe for GSH-PO [7], while a cDNA probe for chicken beta-actin [4] was used as an internal positive control (house-keeping gene mRNA).

Statistical analysis

The data were expressed as means ± SD. Homogeneity of variance was tested by Bartlett's method, and when the assumption of homogeneity of variance was met,

one-way layout analysis of variance was performed. When a significant difference was observed, Scheff's or Dunnett's multiple comparative test [30] was performed between the castrated group and the other experimental groups.

RESULTS

TBA value and GSH-PO activity in the ventral prostate homogenates are summarized in Table 1. The prostatic GSH-PO mRNA levels are shown in Fig. 1. In group 1 (Intact control), lipid peroxide levels expressed as TBA values of the ventral prostate

Table 1 Lipid peroxides (TBA) value and glutathione-peroxidase (GSH-PO) activity

Group	Treatment	TBA value (n mol/mL)	GSH-PO activity (unit/g)
1	Intact	4.97 ± 1.12	12.91 ± 0.25
2	Castration	4.07 ± 1.20	7.81 ± 1.01
3	Castration + T 3 days	4.91 ± 0.83	12.88 ± 0.35*
4	Castration + T 7 days	2.73 ± 0.60*	19.82 ± 1.15**
5	Castration + T 3 days + E_2 3 days	4.56 ± 1.88	12.75 ± 0.35*
6	Castration + T 7 days + E_2 7 days	2.79 ± 1.09*	20.05 ± 0.98**

T: Testosterone, E_2: 17ß-estradiol
Values are the mean ± S.D. (n = 5).
Significantly different from castration (*: $p < 0.05$, **: $p < 0.01$).

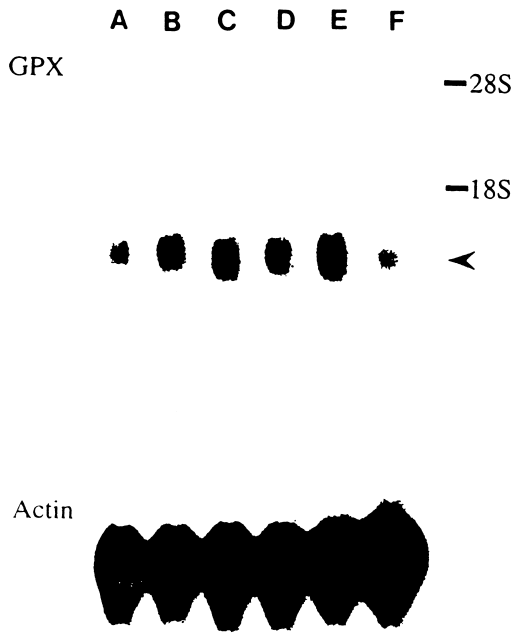

Fig. 1 Northern blot analysis of prostatic total RNA. It is hybridized with cDNA for glutathione-peroxidase (GPX) mRNA, or that for chicken beta-actin (Actin) mRNA. lane A: Intact control. lane B: Castration + Testosterone 3 days. lane C: Castration + Testosterone 7 days. lane D: Castration + testosterone 3 days + E_2 3 days. lane E: Castration + Testosterone 7 days + E_2 7 days. lane F: Castration.

homogenates from control rats were 4.97 ± 1.12 nmol/ml. GSH-PO activity was 12.91 ± 0.25 unit/g ventral prostate. In group 2 (Castration), GSH-PO activity was decreased, but TBA value was similar to that of group 1. The prostatic GSH-PO mRNA level was much lower than that of group 1. In group 3 (Castration plus Testosterone 3 days), both GSH-PO activity and TBA value were similar to those of group 1. The prostatic GSH-PO mRNA level was higher than that of group 1. In group 4 (Castration plus Testosterone 7 days), GSH-PO activity was higher than that of group 1. TBA value was much lower, being only about 50 % of group 1. The prostatic GSH-PO mRNA level was comparable to that of group 3. In group 5 (Castration plus Testosterone 3 days and E_2 3 days), both GSH-PO activity and TBA value were similar to those of group 3. The prostatic GSH-PO mRNA was comparable to that of group 3. In group 6 (Castration plus Testosterone 7 days and E_2 7 days), GSH-PO activity was higher than that of group 4. TBA value was similar to that of group 4. The prostatic GSH-PO mRNA level was higher than that of group 4.

DISCUSSION

In our previous study [19], we demonstrated that the intensity of GSH-PO staining in the glandular epithelial cells of the rat ventral prostate was remarkably decreased after castration, and that it was clearly recovered by testosterone administration to the castrated rats. Therefore, it seemed that GSH-PO in the glandular epithelial cells of the rat ventral prostate could be considered testosterone-dependent, so we further speculated that GSH-PO staining pattern in the glandular epithelial cells of the ventral prostate was thought to be a useful marker for biological testosterone action [19].

In the present study, we found that GSH-PO activity of the ventral prostate homogenate from testosterone or testosterone plus E_2 administration to the castrated rat was increased and the TBA value remarkably decreased. In addition, the prostate GSH-PO mRNA level was diminished in the castrated rat ventral prostate, but increased by testosterone or testosterone plus E_2 administration. In particular, the GSH-PO mRNA level of testosterone plus E_2-treated animals was higher than that of testosterone-treated animals.

E_2 alone will induce glandular atrophy of the prostate in normal and castrated animals [1, 2]. The suppressive effects of E_2 on the male genital organs have been attributed not only to gonadotropin suppression, but also to the direct effect of E_2 on the gonads and their accessories [1, 3]. On the other hand, it is generally accepted that E_2 exerts a synergistic effect with androgen in promoting prostatic growth in the castrated dog, a fact of considerable importance in understanding the pathogenesis of prostatic hyperplasia [27]. Moore *et al.* [17] demonstrated that treatment of castrated dogs with 17ß-estradiol results in a two-fold increase in the cytosolic androgen receptor content. Based on this observation they put forward the hypothesis that enhancement of the androgen receptor activity may be responsible for the synergism of estradiol and androgen in experimentally induced prostatic hyperplasia. Trachtenberg *et al.* [25] developed this concept and gave new insight into the mechanism of steroid-induced prostatic hyperplasia by measuring the nuclear and cytosolic androgen and estrogen receptor contents; in castrated dogs administration of estradiol plus 3 alpha-androstanediol synergistically increased the nuclear androgen receptor content to an extent that exceeded that found in castrated dogs treated with 3 alpha-androstanediol only.

GSH-PO is selenoenzyme and its active center consists of selenocystein [9]. In addition, the disappearance of GSH-PO in selenium-deficient rat liver was immunochemically and immunocytochemically proved by us [26] and others [23]. We found that only a negligible amount of GSH-PO mRNA was exhibited in the case of selenium deficient rat liver, while the mRNA coding for beta-actin [29] was outstandingly increased with selenium deficiency. Therefore, we postulated that the transcription of GSH-PO mRNA was regulated by selenium and the *de novo* synthesis of GSH-PO would thus be regulated at transcription level by selenium [29]. In the prostate, testosterone is irreversibly converted to dihydrotestosterone (DHT) by an enzyme, 5 alpha-reductase. DHT, the major androgen of the prostate, has a higher affinity for androgen receptors than testosterone [11], and is required for the normal development and function of the gland. Based on our data and these facts, it is suggested that the

transcription of prostatic GSH-PO mRNA was regulated by testosterone (DHT) or E_2, and *de novo* synthesis of GSH-PO would thus be regulated at transcription level by testosterone (DHT) or E_2.

GSH-PO is a selenium-dependent enzyme that exists as a homotetramer with each 22-kDa subunit containing a selenium atom incorporated within a catalytically active selenocysteine residure [4]. There are three other members of the selenium-dependent GSH-PO family, although cytosolic GSH-PO is the predominant form [6]. The gene encoding GSH-PO was mapped on chromosome 3q11-13 [6]. Because GSH-PO decomposes hydrogen peroxide and organic hydroperoxides produced during normal metabolism and after oxidative insults, GSH-PO prevents peroxide-induced DNA damage, lipid peroxidation, and protein degradation [10, 26]. P53, after being activated by DNA-damaging reagents, has been shown either to induce G1 growth arrest or apoptosis [14]. The p53 target genes that mediate or associate with p53-induced apoptosis include Bax [13], Fas/APO 1 [10], as well as those involving generation of reactive oxygen species [20]. Recently, Tann *et al.* [24] have identified and characterized GSH-PO, an antioxidant enzyme, as also induced by p53. It appears paradoxical that p53, on one hand, induces the gene responsible for reactive oxygen species generation, which mediates apoptosis [20], and on the other hand, induces expression of a protective antioxidant enzyme, GSH-PO, which protects cells from oxidative damage and apoptosis [12, 15]. It is known that p53-induced reactive oxygen species generation is a rather later event [20]. Thus, p53 may regulate cellular redox status in a time-dependent manner: it increases antioxidant synthesis at an early stage followed by an increase in reactive oxygen species generation.

It has been shown that rat prostatic glandular epithelium undergoes apoptosis within hours of castration and results in involution of the gland within 7 days [13]. In our previous report [18], castration induced apoptosis in the prostatic glandular epithelial cells and apoptosis was reduced by testosterone administration to the castrated rats. Furthermore, we found that the intensity of the staining for bcl-2, a proto-oncogene that blocks apoptosis in multiple contexts, in the prostatic glandular epithelial cells was greatly decreased after castration, and that it was clearly recovered by testosterone administration to the castrated rats [18].

The relationship between GSH-PO expression, apoptosis, and testosterone or testosterone plus estrogen stimulation of the ventral prostate requires further study.

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