PHGPx GENE EXPRESSION IS REGULATED BY ESTROGEN IN THE MALE REPRODUCTIVE ORGANS

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Introduction

Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is an antioxidant that belongs to the superfamily of selenium-dependent peroxidase and interacts directly with peroxidized phospholipid and cholestrol, and cholesteryl ester in biomembrane (Yagi *et al.*, 1996). PHGPx activity is detected in all tissues examined, but is particularly high in testis after puberty (Lei *et al.*, 1995). In testes, PHGPx has been known to be concentrated mainly in spermatids and be influenced on testosterone level during spermatogenesis (Maiorino *et al.*, 1998; Nam *et al.*, 1998). These findings suggest that PHGPx may be associated with spermatogenic cell differentiation rather than a general antioxidative function. On the other hand, Brigelius-Flohe *et al.* (1994) reported that several estrogen responsive elements appear at the transcription regulation site of porcine PHGPx genomic DNA. From this result, although it is conceivable that PHGPx transcription may be regulated by estrogen, no direct evidence is unraveled yet. In this study, to determine whether PHGPx gene expression in male reproductive organs is influenced by exogenous estrogen or not, PHGPx mRNA level was investigated from the testes, epididymes, and prostates of rats treated with 17-β estradiol (E2) or tamoxifen (TAM; an estrogen receptor antagonist) for 1 week throughout Northern blotting, polymerase chain reaction (PCR), and histopathological analyses.

Materials and Methods

After 12-week-old male Sprague-Dawley rats (300-350 g) were injected with E2 (7.5 ug/kg) or TAM (5 mg/kg) for 1 week subcutaneously, all animals were sacrificed under pentobarbital anesthesia and total RNAs were isolated from the testes, epididymides, and prostates of each animals. First strand cDNA for PCR analysis was synthesized using 1 ug of each total RNA. To determine the expression pattern of PHGPx mRNA in epididymis or prostate, 1 ul of the cDNA was amplified by a thermal cycler using the specific primers for PHGPx cDNA (Nam *et al.*, 1997). In addition, β-actin primers were used as an internal standard control. For Northern blotting analysis, 20 ug of total RNAs from each testis were fractionated on an 1 % agarose formalin gel. Hybridization was performed at 47°C for 16 hours with digoxigenin-labeled PCR probe for PHGPx. For histopathological analysis, each organ was fixed in Bouin solution, treated with ethanol and xylene, and stained with hematoxylin and eosin.

Results and Discussion

In porcine PHGPx, the 5'-untranslated region and the first intron contain a variety of putative regulatory elements including estrogen, progesterone, and glucorticoid-responsive elements (Brigelius-Flohe *et al.* 1994). The testicular PHGPx level of rats is kept close to zero by hypophysectomy, but is partially restored by gonadotropin treatment (Roveri *et al.*, 1992). As shown in Figs. 1 and 2, PHGPx mRNA in testes and prostates was greatly increased by E2 treatment, but was remarkably decreased by TAM treatment. These findings indicate that PHGPx transcription may be regulated by steroid hormone such as estrogen in male reproductive organs.

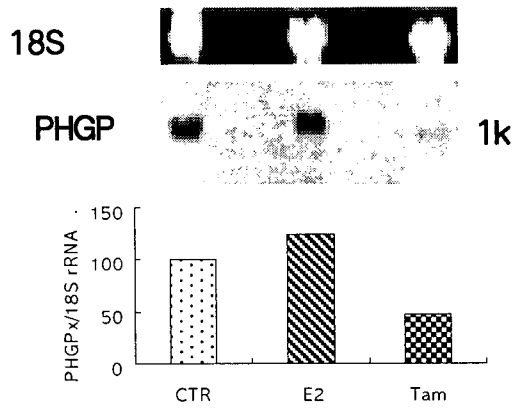

Fig. 1. Northern blot analysis for PHGPx mRNA in rat testes treated with 17 β-estradiol (E2; 7.5 μg /kg) or tamoxifen(Tam; 5 mg/kg) using digoxigenin-labeled cRNA probe. Total RNA samples (20μg) extracted from the testes of each group were loaded onto an 1 % agarose formalin gel. Results were expressed as the ratio of signal density of PHGPx to that of ethidium bromide-stained 18S rRNA transferred into membrane with an image analyzer system (BioDoc-it; UVP, USA). CTR: control.

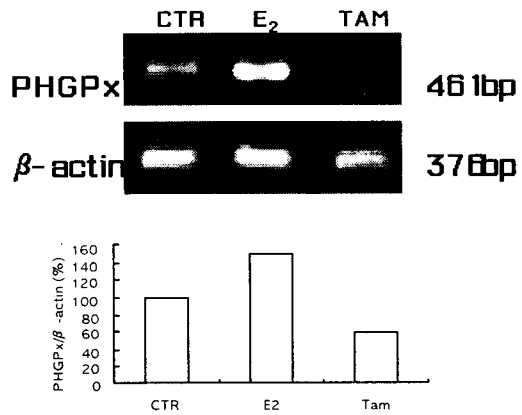

Fig. 2. RT-PCR analysis for PHGPx mRNA in rat prostates treated with 17 β -estradiol (E2; 7.5 μg/kg) or tamoxifen(Tam; 5 mg/kg). PHGPx transcript is presented as a single band (461 bp). PHGPx mRNA level in prostates was analyzed by an image analyzer system (BioDoc-it; UVP, USA) and expressed as

the ratio of density of PHGPx fragment to density of β - actin fragment (376bp). CTR : control.

E2, a natural estrogen in vertebrates, is present in the sperm and its receptor is expressed in various germ cell types during spermatogenesis (Miura *et al.*, 1999). Long period exposure to estrogen can lead to reduced gonad size, feminization of genetic males, low sperm count and /or quality (Sharpe *et al.*, 1993). However, low E2 treatment promoted spermatogonial stem cell renewal, which was suppressed by TAM, a competitive antagonist of estrogen receptor (Miura *et al.*, 1999), suggesting that low concentration of estrogen may be necessary for a normal spermatogenesis. In this study, seminiferous epithelium was slightly proliferated by E2 treatment, but was severely degenerated by TAM treatment daily for 1 week (Fig. 3). However, severe loss and degeneration of germ cells were observed in the seminiferous tubules of male mice treated with the same dose of E2 for 5 weeks daily (data not shown). These findings indicate that estrogen may have a controversial effect on spermatogenesis according to the dosage and exposure duration.

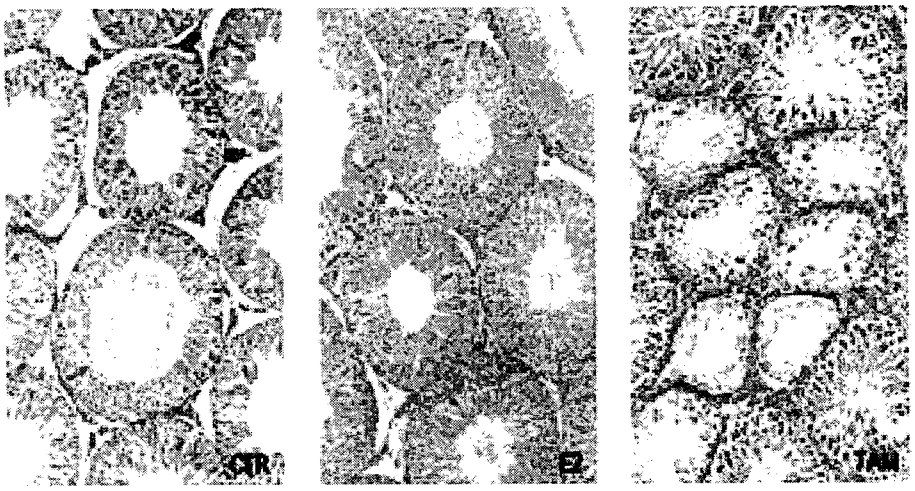

Fig. 3. Histological findings in the testes treated with vehicles (CTR; corn oil), 17- estradiol (E2; 7.5 μg/kg.), or tamoxifen (TAM; 5 mg/kg). Severe necrotic spermatocytes and cell debris are observed in the seminiferous epithelium and lumens. Also, the vacuolization, disruption or partial depletion of the seminiferous epithelium are noticed the testis treated with TAM. However, mild spermatogenic proliferation is observed in the testis of E2 group. H & E, × 100.

The estrogen receptor (ER) exisits as two subtypes, ER alpha and beta, which differ in the C-terminal ligand binding domain and N-terminal transactivation domain. The tissue distribution and the relative expression level of ER alpha and beta seem to be different in tissues. ER alpha is expressed high in uterus, testis, pituitary, ovary, kidney, epididymis, and adrenal gland, while ER beta is greatly expressed in prostate, ovary, lung, urinary bladder, brain, uterus, and testis (Saunders *et al.*, 1998). On the other hand, Fugger *et al.* (2000) suggested that TAM may act as an antagonist against ER beta.

Pettersson *et al.* (2000) also reported that ER beta functions as a dominant regulator of estrogen signaling. In this study, expression of PHGPx mRNA in testis and prostate was greatly increased after E2 treatment, while it was remarkably decreased by TAM treament. However, the level in epididymis after E2 or TAM treatment was not significantly changed compared to that of control (Fig. 4). These indicate that PHGPx gene expression may be related with mainly ER beta.

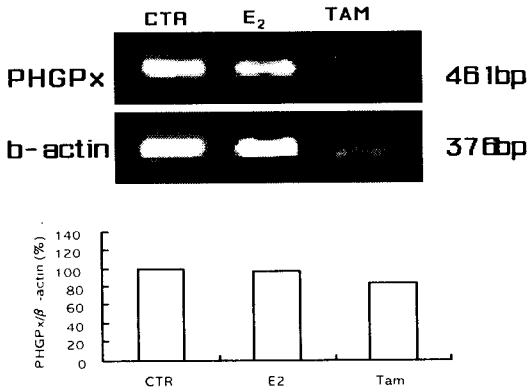

Fig. 4. RT-PCR analysis for PHGPx mRNA in rat epididymides treated with 17β -estradiol (E2; 7.5 μg/kg) or tamoxifen(Tam; 5 mg/kg). PHGPx transcript is presented as a single band (461 bp). PHGPx mRNA level in prostates was analyzed by an image analyzer system (BioDoc-it; UVP, USA) and expressed as the ratio of density of PHGPx fragment to density of β -actin fragment (376bp). CTR : control.

Mouse PHGPx mRNA is expressed stage-specifically during spermatogenesis. The signal first appears in pachytene spermatocytes of Stage X and is mainly expressed in spermatids. PHGPx mRNA is also detected in interstitial Leydig cells (Nam *et al.*, 1998). van Pelt *et al.* (1999) showed that ER beta mRNA and activity were principally found in pachytene spermatocytes and round spermatids. Low ER beta level was observed in all type A spermatogonia, whereas no expression was found in early spermatocytes and interstitial cells. These indicate that PHGPx and ER are directly involved in spermatogenesis. A mild spermatogonial proliferation was observed in seminiferous tubules of rats treated with E2, but the severe loss of germ cells and degeneration of seminiferous tubules were found in those treated with TAM (Fig. 3). Therefore, during spermatogenesis, PHGPx activity may be inhibited by TAM, an endogenous estrogen blocker, but may be stimulated by exogenous E2 treatment. From these results, we hypothesize that exogenous estrogen combines into ER beta and then the complex binds to estrogen-responsive elements of PHGPx gene with unknown coactivators, which regulate the expression of PHGPx. However, TAM would block the binding estrogen with ER beta. To clear the correlation of PHGPx with ER, more detail studies would be performed in future

Acknowledgments

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