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·*Original Article*·

# Glutathione peroxidase activity in cell cultures from different regions of human epididymis

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**Abstract**

**Aim:** To study the secretory activity and androgen regulation of glutathione peroxidase (GPx) in epithelial cell cultures from human epididymis. **Methods:** Tissue was obtained from patients undergoing therapeutic orchidectomy for prostatic cancer. Epithelial cell cultures were obtained from the caput, corpus and cauda epididymides. Enzymatic activity was measured in conditioned media by colorimetric methods in absence or presence of 1, 10 or 100 nmol·L$^{-1}$ testosterone. The effect of 1 μmol·L$^{-1}$ flutamide was also evaluated. **Results:** GPx activity was higher in cultures from corpus and cauda than caput epididymidis. The presence of different concentrations of testosterone increase enzyme activity in cell cultures from all epididymal regions. Addition of flutamide reverses the androgen dependent increase of GPx activity. **Conclusion:** GPx activity is secreted from human epididymal cells in a region dependent manner and is regulated by androgens. *(Asian J Androl 2005 Mar; 7: 33–37)*

**Keywords:** glutathione peroxidase; human epididymis; cell culture; androgen regulation

## 1 Introduction

Mammalian spermatozoa acquire their motility and fertilizing capacity during epididymal transit [1]. Also, the epididymis is believed to play an important role in the protection of maturing spermatozoa especially from reactive oxygen species (ROS) [2, 3]. The main scavenging system for ROS are glutathione related enzymes, including glutathione peroxidases (GPx) [4]. In the epididymis of rats, mice and other species, two GPx isoforms have been reported: plasma GPx (GPx3), a selenocysteine-containing enzyme, and an epididymis specific isoform (GPx5), which is not dependent on selenium [5–9]. In humans, GPx3 activity has been detected in seminal plasma, being decreased in infertile men [10]. GPx5 activity has not been found in human tissues, probably because its transcript is incorrectly spliced [11]. To our knowledge, no reports of GPx activity secreted by epididymal cells have been published. In previous works, we have established and characterized an *in vitro* system in which epithelial cell cultures obtained from human caput, corpus and cauda epididymidis are run in parallel [12, 13]. Using this system, in the present report we studied the activity of GPx in conditioned media from epithelial cell cultures of human caput, corpus and cauda epididymides. The influence of different concentrations of testosterone and flutamide was also evaluated.

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## 2 Materials and methods

### 2.1 Reagents

All reagents, unless otherwise indicated, were obtained from Sigma Chemical Co. (St. Louis, USA).

### 2.2 Human tissue

Epididymal tissue was obtained from six patients undergoing therapeutic orchidectomy for prostatic carcinoma (age range 62–75 years) who had not undergone a previous therapy. Epididymides were received in cold culture medium (Dulbecco's modified Eagle and Ham F-12, 1:1) and brought to the laboratory within 60 min. Only epididymides with motile spermatozoa and normal histology at the level of light microscopy were included in this study.

### 2.3 Cell isolation and culture

Cell isolation and culture were carried out according to a previously described method [12, 13]. Briefly, epididymides freed from most connective tissue were cut in three segments corresponding to caput, corpus and cauda. After a first enzyme digestion with 0.3 % trypsin, 0.2 % hyaluronidase, 0.1 % bovine seroalbumin (BSA) and 0.01 % DNAse for 2–3 hours at 35 °C, the segments were washed and separately incubated in a culture medium containing 0.35 % collagenase, 0.2 % hyaluronidase, 0.1 % BSA, 0.01 % DNAse and 5 % FBS for 8–10 hours at 35 °C in a shaking water bath. The supernatant of this first enzymatic digestion was centrifuged, washed and the resulting cells (mostly stromal) were plated for control purposes. Resulting tubule fragments were cut in small pieces, washed and pipetted in the presence of 5 % of FBS, until cell aggregates (20–25 cells) were obtained. The aggregates of each epididymal segment were plated on Matrigel coated, multi-well culture plates (24-well) at a density of $10^6$ cells·mL$^{-1}$ (2 mL/well) in medium containing human transferrin 5 mg·L$^{-1}$, insulin 2 mg·L$^{-1}$, epidermal growth factor 10 μg·L$^{-1}$, vitamin A and E 200 μg·L$^{-1}$, hydrocortisone 10 nmol·L$^{-1}$, sodium selenite 2 μg·L$^{-1}$, and cytosine arabinoside 3 mg·L$^{-1}$ to avoid fibroblast growing. Depending on the experiment 1, 10 or 100 nmol·L$^{-1}$ testosterone was added to the culture medium. In some experiments 1 μmol·L$^{-1}$ flutamide, an antiandrogen, was also added. Later on, media were collected every 48 hours, centrifuged at 3000 ×*g* and stored at –25 °C until assays. The percentage of epithelial cells was evaluated by immunocytochemical assay for cytokeratin (Immunotech, Marseille, France), showing more than 70 % of cells stained positive. The composition of these cells was carefully evaluated in parallel cultures showing no significant changes, either between cultures from different epididymal regions, or during the culture period.

### 2.4 Glutathione peroxidase assay

GPx activity was measured in 250 μL of conditioned media in a mixture containing 50 mmol·L$^{-1}$ Tris, 0.1 mmol·L$^{-1}$ EDTA, 0.35 mmol·L$^{-1}$ GSH, 0.2 mmol·L$^{-1}$ $H_2O_2$, pH 7.6 in a final volume of 2 mL. Plain culture medium was used as the control. Reaction was started with the addition of $H_2O_2$ and the mixture was incubated at 32 ºC for 40 min. After the reaction was stopped, the GSH consumption was measured. Enzyme activity was expressed as μmol of substrate·min$^{-1}$ per $10^6$ cells, considering the number of cells at the end of each experiment. For control purpose, lactate dehydrogenase (LDH) was measured according to a previously described method [14] in order to disregard the possibility that GPx in the conditioned media were due to cellular lyses instead of actual secretion. Also, GPx activity was evaluated in the conditioned media from epididymal stromal cell cultures as a negative control for enzyme secretion.

### 2.5 Glutathione assay

GSH was measured after enzyme reaction using 5,5-dithiobis (2-nitrobenzoic) acid. The optical density was recorded at 412 nm according to the Ball method [15].

### 2.6 Statistical analysis

Data were analyzed using the Kruskal–Wallis nonparametric ANOVA test followed by Dunn's test. $P < 0.05$ was considered significant.

## 3 Results

### 3.1 Glutathione peroxidase activity in conditioned media from cell cultures of caput, corpus and cauda epididymidis

GPx activity secreted into the culture medium was measured every 48 hours and it was found to be higher in cell cultures from corpus and cauda than that in caput epididymidis. Data showed that the enzyme activity between day 4 and day 6 was the highest (Figure 1). Enzyme activity was maintained for at least 1 week and

declined slowly thereafter in cultures from all three epididymal regions. A representative time-course curve for GPx activity is shown in the insert of Figure 1. LDH activity, used as control, was almost undetectable in all culture media (data not shown). Also, GPx activity in conditioned media from stromal cell cultures was not detectable by the method used (data not shown).

*3.2 Influence of testosterone on glutathione peroxidase activity in cultures from caput, corpus and cauda epididymidis.*

Testosterone increased the secreted activity of GPx in cell cultures from all three regions of human epididymis at all concentrations used (1–100 nmol·L$^{-1}$) (Figure 2). However, the highest increase was noted in caput cell cultures.

*3.3 Influence of flutamide on testosterone-stimulated glutathione peroxidase activity in cultures from caput, corpus and cauda epididymidis.*

Flutamide, an androgen antagonist, reverses completely the stimulated activity of GPx in all epididymal cell cultures indicating the specificity of the androgen effect (Figure 3).

## 4 Discussion

Glutathione-related enzymes might play an important role in the protection of maturing spermatozoa from electrophilic attack, especially ROS produced in the male reproductive tract [16, 17] and may reduce the fertilizing capacity [18]. In rats and other species, GPx3 and

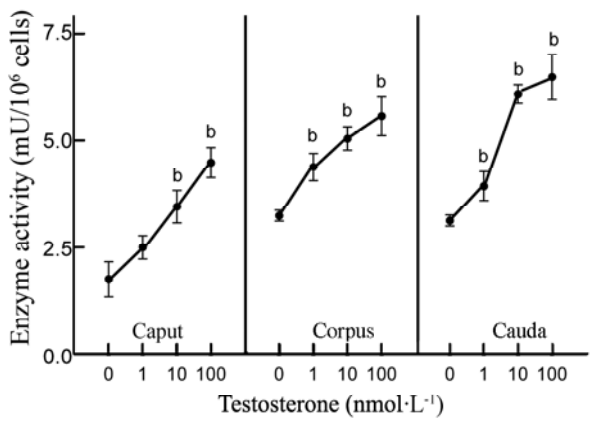


Figure 2. Influence of different concentrations of testosterone on glutathione peroxidase activity in cell cultures obtained from caput, corpus and cauda epididymides. Cells were cultured in absence or presence of 1, 10 or 100 nmol·L$^{-1}$ of testosterone. Values correspond to enzyme activity between days 4 and 6 of culture. Data are expressed as mean ± SD from six patients. $^{b}P < 0.05$, compared with the corresponding controls (0 nmol·L$^{-1}$ testosterone).

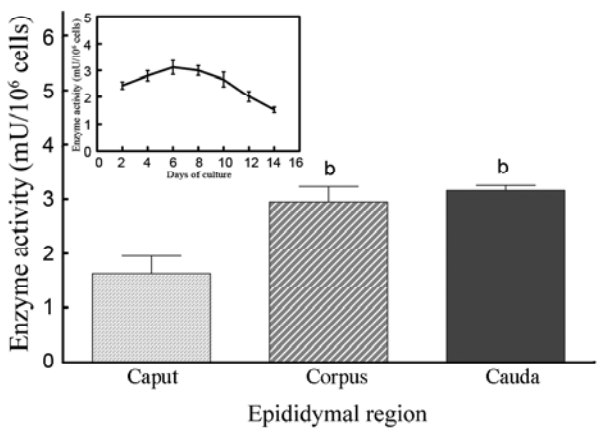


Figure 1. Glutathione peroxidase activity in conditioned media from cell cultures obtained from human caput, corpus and cauda epididymides. Media were collected every 48 hours. Values correspond to enzyme activity between days 4 and 6 of culture. Insert shows a representative curve of GPx activity time-course during 14 days of culture. Data are expressed as mean ± SD from six patients. $^{b}P < 0.05$, compared with the caput epididymides.

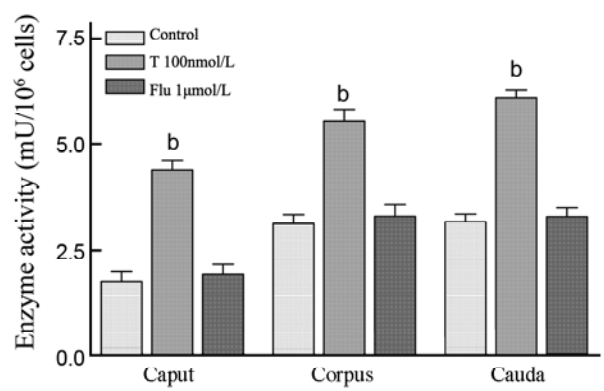


Figure 3. Influence of Flutamide on testosterone stimulated glutathione peroxidase activity in cell cultures obtained from caput, corpus and cauda epididymides. Cells were cultured in absence or presence of 100 nmol·L$^{-1}$ of testosterone alone or with 1 μmol·L$^{-1}$ of flutamide. Values correspond to enzyme activity between days 4 and 6 of culture. Data are expressed as mean ± SD from six patients. $^{b}P<0.05$, compared with the corresponding controls.

GPx5 isoforms (enzymes involved in ROS scavenging) have been identified in the epididymis [5–9]. However, there is only partial information about GPx expression in human epididymis. Only GPx3 isoform has been found in human seminal plasma and epididymis and has been suggested as a source of this enzyme activity [10]. GPx5 was shown to bind to the sperm acrosomic region during epididymal transit in other species [19], suggesting a protective role to oxidative damage. So far, no GPx5 activity has been found in human epididymis or seminal plasma. We have demonstrated that cultured cells obtained from different regions of human epididymis are able to secrete GPx activity (presumably GPx3 or another secretory isoform not yet identified). Regardless of the nature of the GPx isoform, an issue not addressed in the present work, the relevant point is that enzymatic GPx activity may also have a protective role against ROS in human epididymis. The enzyme activity is more abundantly secreted in the distal segments of the epididymis than in the caput. The opposite has been reported for the GPx5 in the rat and mouse, in which the gene is expressed mainly in the initial segment and caput epididymis [6, 8]. We have found the same difference between rats and men in other GSH related enzymes [20], suggesting that the genetic regulatory mechanism, an influence of testicular factors (which are not present in cultured cells), may be different in both species. However, androgen control of human epididymal GPx activity seems to be similar to other species studied [7]. We have found a strong androgen dependence on GPx activity in all three segments of human epididymis, but the extent of testosterone stimulation was highest in caput cells. This may be explained by differences in androgen receptors and/or 5-α-reductase activity in the different epididymal segment. These possibilities are being currently studied in our *in vitro* system. However, the possible difference in the intracellular conversion rate of testosterone into DHT can be overridden by adding an excess of testosterone or by directly using DHT. We have used up to a 100-fold physiological concentration of testosterone, considering that testosterone and DHT bind to the same receptor but with different affinity, and therefore, the same effect of DHT can be obtained with higher concentrations of testosterone. On the other hand, in previous work using the same culture system, we have compared the effect of different concentrations of testosterone and DHT on the secretion of several enzyme activities regulated by androgen and no significant differences were found [12]. In addition, a recent report showed no GPx3 changes in caput, corpus and cauda epididymidis when using a potent inhibitor of 5-α-reductase [21]. Flutamide, a widely used antiandrogen, consistently inhibited the androgen-induced stimulation of GPx secretion in all epididymal regions showing that receptor availability is necessary for enzyme stimulation.

We conclude that GPx activity is secreted in cell cultures from the different functional segments of human epididymis showing a regional pattern of secretion and androgen dependence. Together with the available data on the differences in seminal plasma levels of GPx between fertile and infertile men, our results suggest that human epididymal cells are the main source of GPx activity and that this enzyme may be involved in the protection of maturing spermatozoa from ROS damage.

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