

Evaluation of Prolidase Activity, Oxidative Stress, and Antioxidant Enzyme Levels in Testicular and Penile Tissues after Human Chorionic Gonadotropin Treatment in Rats by Predicting Infertility and Erectile Dysfunction

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Significance of the Study

- This study investigated the effect of human chorionic gonadotropin on prolidase activity, oxidative stress, and antioxidant enzyme levels in testicular and penile tissue in a rat model. We observed that human chorionic gonadotropin negatively affected testicular and penile tissue in rats. Studying the changes in these parameters could provide information on infertility and erectile dysfunction.

Keywords

Antioxidant · Human chorionic gonadotropin · Oxidative stress · Prolidase

Abstract

Objectives: Prolidase plays a vital role in collagen turnover, matrix remodeling, and cell growth. We aimed to evaluate the association between treatment with chorionic gonadotropin and infertility and erectile dysfunction by investigating tissue prolidase activity, oxidative stress, and levels of antioxidant enzymes. **Materials and Methods:** The 16 male Wistar albino rats used in this study were randomly divided into 2 groups: rats treated with human chorionic gonadotropin (hCG) and control rats ($n = 8$ in each group). The rats in

the hCG group were subcutaneously injected with 50 IU hCG daily for 15 days, while the rats in the control group were subcutaneously injected isotonic saline. All of the rats were sacrificed by a lethal overdose of sodium pentobarbital at the first month after hCG administration. Prolidase activity and levels of malonyl aldehyde, glutathione reductase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were estimated in the testicular and penile tissue. The testicles and penis were transversely dissected and placed in formalin. **Results:** Levels of prolidase and malonyl aldehyde in the testicular and penile tissues were significantly higher in the hCG group than in the control group ($p < 0.001$), while levels of glutathione reductase, SOD, GSH-Px, and CAT were significantly lower in the hCG group than in the control group ($p < 0.001$). **Conclusions:** In this

study, we observed that treatment with hCG increased proli-
dase activity and oxidative stress and decreased the anti-
oxidant capacity of penile and testicular tissues; therefore,
this may affect fertility and erectile function.

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Introduction

Human chorionic gonadotropin (hCG) is a polypep-
tide hormone produced by the human placenta, with an
 α subunit that is almost identical to the corresponding
 α subunits of follicle-stimulating hormone, thyroid-stim-
ulating hormone, and luteinizing hormone, and a mecha-
nism of action similar to that of luteinizing hormone.
hCG induces superovulation in females and is used to in-
crease pregnancy rates by enhancing the number of oo-
cytes and embryos, while it is used for the treatment of
hypogonadotropic hypogonadism and cryptorchidism in
males [1]. Although the use of hCG has beneficial effects
like provocation of maturation of the seminiferous epi-
thelium, facilitation of testicular descent and growth, im-
provement of sperm quality, and regaining of fertility by
hypogonadotropic hypogonadism, it may also increase
intravascular leucocytes and free oxygen radicals depend-
ing on the time of application and the dose, which causes
deterioration of spermatogenesis and may lead to infertil-
ity [2].

It has been reported that hormonal treatment given for
testicular descent may harm germ cells by causing in-
creased apoptotic germ cell death through testicular oxi-
dative stress coupled with the production of reactive oxy-
gen species (ROS) [3, 4]. ROS are chemical species con-
taining 2 unpaired electrons. They are well known for
both their deleterious and beneficial effects. In healthy
individuals, ROS are continuously formed at low concen-
trations as a result of internal reactions and external fac-
tors, and some ROS have important physiological func-
tions [5]. Antioxidant defense systems which consist of
glutathione reductase, superoxide dismutase (SOD), glu-
tathione peroxidase (GSH-Px), and catalase (CAT) can
provide protection from the harmful effects of ROS. In-
sufficient defense systems may result in severe metabolic
malfunctions and oxidative damage to DNA [6].

Prolidase is a member of the matrix metalloproteinase
family. It plays a vital role in collagen turnover, matrix
remodeling, and cell growth. Increased levels of prolidase
seem to be associated with increased levels of nitric oxide
and oxidative stress along with decreased levels of anti-

oxidant [7]. Also studies on vasculogenic erectile dys-
function have detected higher serum levels of prolidase
[8, 9]. In this study we aimed to examine the effect of hCG
on prolidase activity, oxidative stress, and levels of anti-
oxidant enzyme in the testicles and penile tissue in a rat
model.

Materials and Methods

The study protocol was approved by the Animal Research
Committee of Yuzuncu Yil University, Van, Turkey (THD-2016-
5116). Six-week-old male Wistar albino rats weighing 250 ± 50 g
were maintained under standard conditions of a constant tem-
perature of $20\text{--}22^\circ\text{C}$ and a 12-h day/night cycle with food and
water provided ad libitum. The rats were randomized to the hCG
and control groups ($n = 8$ each). The rats in the hCG group were
given daily subcutaneous injections of 50 IU of hCG (Pregnyl, Or-
ganon) for 15 days as previously described [10, 11]. Rats in the
control group received subcutaneous isotonic saline. All of the rats
were sacrificed using a lethal overdose of sodium pentobarbital
(100 mg/kg, administered intraperitoneally) to obtain the normal
descending testicular and penile tissue at the first month after ad-
ministration of hCG.

Biochemical Analysis

Measurement of Thiobarbituric Acid Reactive Substances

To measure the levels of thiobarbituric acid reactive substances
(TBARS), 50 mg of tissue samples were obtained and homogenized
in 0.15 mol/L KCl. The homogenate was centrifuged at 1,600 g, and
the levels of TBARS in the supernatants were determined by the
thiobarbituric acid reaction according to the method of Yagi [12].
The method is based on measurement of the absorbance of the
pink color produced by the interaction of thiobarbituric acid with
TBARS at 530 nm. Values were expressed as milligrams per deci-
liter.

Measurement of SOD and GSH-Px

The tissues were homogenized in physiological saline (1 g in 5
mL) using a homogenizer (B. Braun Melsungen AG, Germany)
and centrifuged at 4,000 g for 20 min (Heraous Labofur 200, Ger-
many). GSH-Px activity was estimated by measuring the changes
in the absorbance of nicotinamide adenine dinucleotide phosphate
at 340 nm and by measuring the decrease in absorbance at 240 nm.
SOD activity was measured using the method based on nitroblue
tetrazolium reduction rate. One unit of SOD activity was expressed
as the amount of enzyme that causes 50% inhibition in the nitro-
blue tetrazolium reduction rate [13].

Measurement of CAT Enzyme Activity

Biochemical analysis of tissue CAT activity was performed ac-
cording to the method described by Sedlak and Lindsay [14]. Su-
pernatants (0.1 mL) were added to a quartz cuvette containing 2.95
mL of 19 mmol/L hydrogen peroxide solution prepared in 0.05 M
potassium phosphate buffer (pH 7.00). The change in absorbance
was monitored at 240 nm for 5 min using a spectrophotometer
(Shimadzu UV-1201; Japan).

Table 1. Penile tissue prolidase activity, oxidative stress, and levels of antioxidant enzymes

	Human chorionic gonadotropin		Control		<i>p</i>
	mean ± SD	range	mean ± SD	range	
Prolidase, IU/L	2.21±0.23	1.95–2.51	1.08±0.09	0.99–125	0.001
TBARS, nmol/mL	110.01±0.78	108.91–110.88	70.50±2.01	67.40–72.85	0.001
GSH-Rx, mmol/g Hb	6.63±0.71	5.67–7.85	13.46±1.03	12.17–14.83	0.001
CAT, u/mL	46.52±3.74	42.07–52.07	67.20±3.24	62.04–70.56	0.001
SOD, IU/mL	5.59±0.61	4.87–6.55	9.95±0.65	9.03–10.91	0.001
GSH-Px, IU/mL	57.86±5.50	49.40–63.01	96.68±3.83	90.15–100.66	0.001

CAT, catalase; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSH-Rx, glutathione reductase; Hb, hemoglobin.

Table 2. Testicular tissue prolidase activity, oxidative stress, and levels of antioxidant enzymes

	Human chorionic gonadotropin		Control		<i>p</i>
	mean ± SD	range	mean ± SD	range	
Prolidase, IU/L	1.89±0.35	1.35–2.56	0.90±0.08	0.80–0.99	0.001
TBARS, nmol/mL	113.10±4.95	107.45–119.43	81.23±5.69	72.12–90.00	0.001
GSH-Rx, mmol/g Hb	11.83±2.96	7.25–15.27	21.18±1.93	19.21–25.02	0.001
CAT, u/mL	57.10±8.18	44.00–66.29	79.32±8.64	70.40–97.12	0.001
SOD, IU/mL	9.63±1.44	7.44–11.53	11.52±1.90	8.75–14.20	0.001
GSH-Px, IU/mL	57.60±4.44	50.35–63.01	91.45±10.51	79.03–107.19	0.001

CAT, catalase; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSH-Rx, glutathione reductase; Hb, hemoglobin.

Measurement of Prolidase Enzyme Activity

For measurement of the prolidase enzyme activity, a ninhydrin reaction described by Chinard [15] was used. One milliliter of glacial acetic acid and 1 mL of modified Chinard solution were added to 0.5 mL of clear supernatant of the tissue homogenate. The mixture was incubated at 90 °C for 20 min and then cooled on ice. Then, the absorbance of the samples was measured at 515 nm using a sample without the substrate as the control. The measured proline concentrations were calculated against a standard proline curve of 5 mg/dL L-proline. One enzyme activity unit was defined as the amount of proline (in µmol/L) formed in the reaction step per minute [16].

Measurement of GSH-Rx Enzyme Activity

As described previously, 1 g of tissue sample was homogenized in 5 mL of physiological saline using a homogenizer and then centrifuged at 4,000 g for 20 min. GSH-Rx activity was measured by observing the changes in the absorbance of nicotinamide adenine dinucleotide phosphate at 340 nm and by measuring the decrease in absorbance at 240 nm.

Statistical Analysis

Descriptive statistics for the studied characteristics were presented as medians, means, SD, and ranges. Group comparisons were performed using the Mann-Whitney *U* test. The SPSS (version 13) statistical program was used for all statistical computations.

Results

While the prolidase and TBARS levels in penile tissue were significantly higher in the hCG group than in the control group, levels of glutathione reductase, SOD, GSH-Px, and CAT were significantly lower in the hCG group than in the control group ($p < 0.05$; Table 1).

Levels of prolidase and TBARS were significantly higher in the testicular tissue of the hCG group than in the control group. The levels of glutathione reductase, GSH-Px, SOD, and CAT were significantly lower in the

testicular tissues of the hCG group than in those of the control group ($p < 0.05$; Table 2). TBARS, a sign of oxidative stress, is negatively correlated with levels of antioxidant enzymes.

Discussion

Prolidase is a cytosolic enzyme necessary for specific splitting of imidodipeptides with proline or hydroxyproline at their C-terminals, and it has a major role in collagen turnover and cell growth [9, 17]. The relationship between collagen and proli-dase activity was observed in a study on fibrotic processes, where an increase in proli-dase activity was found to be accompanied by an increase in deposition of tissue collagen [17]. The negative effect of free radicals is mediated by degradative agents such as proteolytic enzymes, and the final step of collagen degradation is mediated by proli-dase [18].

Prolidase is a specific peptidase that has an important function in human tissues and cells. Prolidase activity has been shown in plasma erythrocytes, leucocytes, and fibroblasts and in organs like the kidney, the brain, the heart, the liver, the small intestines, the stomach, the lung, the spleen, the thymus, and the uterus [19]. While a higher proli-dase activity is observed in patients with osteoporosis, osteoarthritis, hypertension, coronary artery disease, and erectile dysfunction [8, 9, 17, 18, 20, 21], a lower level is observed in renal insufficiency because the main source of proli-dase is the kidney [19]. The severity of the antioxidant stress is directly correlated with the inhibition of collagen production in which proli-dase is the main enzyme [19]. Tissue proli-dase activity may be an indicator of tissue fibrosis [9]. Fibrosis in the testicular and penile tissues may affect the fertility and erectile capacity. Thus, tissue proli-dase activity may be an indicator of fertility and erectile capacity. Savas et al. [9] reported that serum proli-dase activity is closely associated with vasculogenic erectile dysfunction and its severity, and thus an increase in serum proli-dase may be an independent predictive factor for erectile dysfunction.

The use of hCG is reported to have noxious effects which can lead to male infertility due to impaired spermatogenesis because of an increase in free oxygen radicals [2, 22]. To the best of our knowledge, no other studies in the literature have studied the effects of hCG treatment on proli-dase activity, oxidative stress, and antioxidant enzyme levels in the testes and penile tissue. In our study, we found that proli-dase activity in the penile and testicular tissues was significantly higher in the hCG

group than in the control group. The results do not directly or necessarily apply to treatment in humans.

The body's defense cells may also be important mediators of oxidative stress by the release of important enzymes related to immune reactions. Antioxidant enzymes are responsible for inhibiting oxidative stress. SOD and CAT serve as the primary line of defense in the detoxification of free radicals. Finally, one of the major toxic effects of excessive ROS is damage to the cell membrane via lipid peroxidation, which can be monitored by measuring the levels of TBARS. If the detoxification and neutralization of ROS are insufficient or if excess production of ROS occurs, this will lead to oxidative stress, which represents an imbalance between the level of free radicals and antioxidant defenses [23].

Because developmental parameters in rats are similar to those in humans, histologic evaluation of the rat testis is considered useful. Kaya et al. [24] reported that hCG decreased the number of testicular germinal cells. Another study showed that the effect of hCG is dose dependent and reversible [11]. The negative effect of hCG on Leydig cells is mainly related to oxidative stress and apoptosis [22]. The amount of collagen in penile tissue was shown to be higher in the hCG-treated rats, while the diameters of cavernosal sinus lumens and the diameter of the penis were lower than in the hCG group [25]. In these studies, the effect of hCG on testicular and penile tissue was studied histopathologically, while we studied the effects of hCG by analyzing biochemical parameters.

Treatment with hCG raises testicular levels of hydrogen peroxide along with an increase in lipid peroxidation and a concomitant decrease in enzymatic antioxidant activities like SOD, CAT, and glutathione-transferase. Gautam et al. [2] reported an increase in apoptosis in testicular germ cells and associated this with high oxidative stress and H_2O_2 which is a sign of low antioxidant activity. Oz-bek et al. [26] found a significant increase in the number of giant cells and degenerated and desquamated cells in a testicular ischemia/reperfusion injury model. Additionally, levels of TBARS were significantly higher, while levels of SOD, CAT, GPX, and reduced glutathione (GSH) were significantly lower. As shown in previous studies, both apoptosis and oxidative stress induce smooth muscle cell and endothelial cell damage in the corpus cavernosum [27]. Oxidative stress increases the tissue levels of TBARS, which is the end product of lipid peroxidation. Atilgan et al. [28] reported that the collagen density in cavernosal tissue increased after oxidative injury induced by bilateral cavernous nerve injury, which may lead to the development of fibrosis in cavernosal tissues. They also used tissue

levels of TBARS as an indicator of oxidative stress. Yu et al. [29] studied SOD activity and levels of TBARS as a measure of oxidative stress in the corpus cavernosum. SOD is a major cellular defense against the superoxide and TBARS is a reactive aldehyde and one of the many reactive electrophile species that can cause the toxic stress in cells. Yu et al. [29] found that levels of SOD decreased but levels of TBARS increased in diabetes-induced erectile dysfunction. In our study, based on the relatively high TBARS levels and the low levels of antioxidants in the hCG-treated group, we concluded that hCG can negatively impact penile and testicular tissue.

One limitation of this study is that the collagen content was not estimated for fibrosis. The other is that the conver-

sion of the therapeutic human dose of hCG into an experimental dose in rats has not been standardized, although many studies in the literature have applied 50 IU/kg.

Conclusion

In this study, tissue prolidase and levels of TBARS were higher and levels of SOD, GSH-Px, and CAT were lower in hCG-treated rats than in control rats. hCG negatively affected testicular and penile tissues. These observations may contribute to a better understanding of infertility and erectile dysfunction.

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