Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [9] August 2014: 164-167
©2014 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Effects of Unilateral and bilateral Orchidectomy on serum levels of Carcinogenic Embryonic antigen in Male rats

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ABSTRACT
There are few studies indicating the relationship between sex steroid hormones and tumor markers. The aim of this study was to determine the orchidectomy effects on serum levels of carcinoembryonic antigen (CEA) in male rats. Male Wistar rats were *divided randomly into control, sham-operation, unilateral- and bilateral-orchidectomised groups of 7 in each. Ten weeks after orchidectomy, following serum preparation, CEA, testosterone and dihydroepiandrosterone (DHEA)* levels were measured *by radioimmunoassay method. The results were statistically analyzed using ANOVA. Serum level of CEA was significantly increased (P < 0.01) and of testosterone and DHEA was significantly decreased in bilaterally orchidectomised rats compared with control animals (P < 0.01 , P < 0.05, respectively). In unilaterally orchidectomised animals, despite significant decreasing in serum testosterone level (P < 0.01), serum DHEA and CEA level was not significantly changed compared with control rats. Serum level of testosterone was also more reduced in bilaterally orchidectomised than unilaterally orchidectomised rats (P < 0.01). Our findings indicated that considerable reducing in serum level of testosterone and DHEA results in enhanced serum CEA level, indicating the probable protective effect of male sex steroid hormones, in particular testosterone and DHEA, against potentially tumorigenic conditions.*
Keywords *: Orchidectomy, CEA, Testosterone, DHEA, Rat.*

Received 13.05.2014 **Revised 29.06.2014** **Accepted 21.07.2014**

INTRODUCTION

Tumor markers are proteins released by cancerous cells or different parts of body, in response to a tumor and often are found in the blood or urine [1]. Carcinoembryonic antigen (CEA), a 200 kDa glycoprotein with %50 carbohydrate, is one of the most common tumor markers [2, 3] identified in 1965 for the first time [4]. This antigen is a *glycosylphosphatidylinositol* (*GPI*) anchor glycoprotein, which is connected to the cell membrane and has an important role in metastatic spread of colon cancer cells [5, 6]. CEA is also an oncofetal antigen which is produced during fetal growth [7, 8]. Studies have shown that there are similarities between tissue distribution and chemical properties of rat and human CEA [9]. CEA also acts as an adhesion molecule that mediates interaction between collagen and colon epithelial cells [10].

CEA is *normally present a*t only very low concentration in adult plasma but its concentration is increased in the presence of many conditions including epithelial cell malignancies [11], colorectal [12], gastric, pancreatic, lung, breast and cervical cancers and also in sweat gland adenoma and metabolic syndrome [13-15]. In addition, C*EA levels* are slightly *elevated* in patients with hepatic or renal disorders [16]. It has also been shown that factors such as aging [17], cigarette smoking [18] and alcohol drinking [19] can increase serum level of CEA. Study on CEA genetic in mice has shown that CEA gene expression is influenced by several factors including neoplasms [20].

On the other hand, some investigations have shown that gonadectomy and subsequent reduced serum levels of sex steroids can cause pathologic disorders in mice [21], by which may affect on serum levels of CEA. In addition, gonadectomy can upset collagen fibrillogenesis in both male and female mice [22], thereby may also influence CEA secretion leading to alteration in serum levels of CEA. Increased CEA

levels can indicate some non-cancer-related conditions, including inflammation. It has also been reported that reduced plasma concentration of androgens can induce inflammation [23]. In this respect, it is expectable that reduced plasma level of androgens has the potential to alter serum levels of CEA. We have previously shown that gonadectomy in male rats is associated with development of intestinal inflammation [24], and we now report the effects of unilateral and bilateral orchidectomy on serum levels of carcinogenic embryonic antigen in male rats.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighting 180 ± 10 gr were purchased from an original stock of Pasteur Institute (Tehran, Iran). They were housed in plexy glass solid bottom cages with wood shavings for bedding. The temperature was at 22 ± 20C and animals kept under a schedule of 12h light: 12h darkness (lights on at 08:00 a.m.). The animals had free access to the standard laboratory pellet feed (Pars Company, Tehran, Iran) and water *ad libitum.* Care was taken to examine the animals for general pathological symptoms.

Surgical procedure

Orchidectomy was performed on the basis of standard methods [25]. At first, rats were anesthetized using ketamine hydrochloride (100-120 mg/kg) and xylazine hydrochloride (24mg/kg) intramuscularly. The anaesthetized animal was laid on its back. The skin of the scrotum was cleaned by alcohol and a small incision - about 1 cm - made at the tip of scrutom. Then, a small incision - 5 mm - was made on the muscular sac of testis at its tip and by pushing from the back, the testis with the epididymis, the vas deferens and the spermatic blood vessels were protruded. A single ligature was used around the blood vessels and the vas deferens, and after cutting them, testis and epididymis were removed. Finally, the skin incision was sutured with nylon thread and disinfectant solution was used topically in order to prevent infection. Also, rats received antibiotic trimetosol 48% solution by intramuscular rout for 3 days. In unilaterally-orchidectomy, one testis (right or left as random) and in bilaterally-orchidectomy, two testes were removed. In sham operations, the incisions were immediately sutured and the gonadal system was not manipulated. The subjects were allowed 7–8 days to recover from surgery, during which they were kept alone in a cage. Groups were then reformed and the animals remained in groups till the end of the experiment. All animal experiments were carried out in accordance with the guidelines of Institutional Animals Ethics Committee [26].

Protocol of study

This work was conducted in Laboratory Complex of IAU – HB (Hamedan, Iran). Animals were randomly divided into control, sham-operated, unilateral- and bilateral-orchidectomised groups of 7 rats in each. 10 weeks after operation, blood samples were collected and serum levels of testosterone, dehydroepiandrosterone (*DHEA) and CEA were measured using* radioimmunoassay (RIA) method.

Serum collection

Blood samples were collected in appropriate tubes by cardiac puncture technique. After collection, the blood samples were left to clot at room temperature for 15 minutes and then centrifuged at 2500 r.p.m. for 15 minutes. The serum layer was then separated and aliquot into small test tubes and stored at -20^{o}C until hormone or CEA determination.

Statistical analysis

The *normal distribution of data was assessed through* Levine's *test.* Statistical significance was evaluated by *ANOVA* (*analysis of variance*) method followed by post hoc Tukey's test *using* the software *SPSS 19*. Data are given as mean ± standard deviation (SD). A level of $p<0.05$ was accepted as statistically significant.

RESULTS

Post-mortem dissection of gonadectomised animals did not show any sign of testes. On statistical approach, normal distribution of data was manifested. Our findings indicated that serum level of CEA, testosterone and DHEA was not significantly changed in Sham-operation animals compared with control group. Serum level of DHEA and CEA was not also significantly changed, and of testosterone was significantly decreased ($P < 0.01$) in unilaterally orchidectomised rats compared to control animals. Serum level of testosterone and DHEA was significantly decreased ($P<0.01$ and $P<0.05$, respectively), and of CEA was significantly increased in bilaterally orchidectomised rats ($P<0.01$) compared with control animals. Serum level testosterone was more reduced in bilaterally orchidectomised than unilaterally orchidectomised rats ($P< 0.01$) (Table).

DISCUSSION

Our findings indicated that orchidectomy was followed by significant reduced serum levels of testosterone 10 weeks after operation. Testosterone reduction was also higher in bilaterally orchidetomised than unilaterally orchidetomised rats. Other studies also have shown marked decrease in serum level of testosterone very soon after orchidectomy [27]. We reported that serum level of DHEA was reduced in bilaterally orchidetomised rats. In accordance with our report, other studies have demonstrated the decrease in serum level of DHEA following orchidectomy [28]. This may reflect the role of testes in producing circulatory DHEA, and this role is eliminated after surgical removal of testes [29].

Table 1. Serum level of CEA, testosterone and DHEA,10 weeks after operation in rats.

Groups	CEA (ng/ml) (X ± SEM)	P	Testosterone (ng/ml) (X ± SEM)	P	DHEA (ng/ml) (X ± SEM)	P
Control	0.11 ± 0.01		2.99 ± 0.23		0.32 ± 0.05	
Sham	0.13 ± 0.01	N.S	2.33 ± 0.32	N.S	0.22 ± 0.01	N.S
Uni-ORC	0.11 ± 0.01	N.S	1.43 ± 0.18	< 0.01	0.33 ± 0.05	N.S
Bi-ORCX	0.44 ± 0.04	< 0.01	0.02 ± 0.00	< 0.01	0.19 ± 0.03	< 0.05

Data represent the mean ± SEM of 7 rats in each group. Uni-ORCX and Bi-ORCX indicate unilaterally-orchidectomised and bilaterally-orchidectomised rats, respectively. P values are versus control group. NS indicates non-significant difference**.**

We also have shown that reduced serum testosterone and DHEA levels following orchidectomy was followed by enhanced serum level of CEA in bilaterally orchidetomised rats. In line with this finding, other studies also show that sex steroid hormones influence secretion of several tumor markers including CEA [30]. It has been shown that disorders leading to reduced level of androgens can enhance serum tumor marker levels [31]. It has also been demonstrated that tissue injuries, in particular digestive tract injuries, results in enhanced serum CEA level [32]. We have previously shown that gonadectomy in male rats is associated with development of intestinal inflammation [24], which may followed by changes in cell membrane lipid, to which CEA is anchored. CEA is anchored to cell membrane by a glycosylphosphatidylinositol moiety which can be cleaved with phosphatidylinositol-specific phospholipase C [33]. Studies show that reduced serum level of androgens following orchidectomy lead to many pathophysiological changes in various tissues [34, 35], particularly in digestive tract tissue [36], which may activate phospholypase C to cleave CEA from cell membrane and eventually to increase serum level of CEA; however, the exact mechanism remains to be elucidated.

In conclusion, our findings indicated that reduction in serum levels of androgens, in particular testosterone and DHEA, leads to enhanced serum level of the tumor marker CEA. It can be concluded that the normal serum level of androgens has a potentially protective role against tumorgenesis.

ACKNOWLEDGEMENTS

This work was supported by Islamic Azad University of Iran - Hamedan Branch. We should express our hearty thanks to members of Laboratory Complex of IAU – SR for their helps.

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CITATION OF THIS ARTICLE

Rahim A, Shima S, Behrooz K, Sara R, Sedigheh M. Effects of Unilateral and bilateral Orchidectomy on serum levels of Carcinogenic Embryonic antigen in Male rats. Bull. Env. Pharmacol. Life Sci., Vol 3 [9] August 2014: 164-167