doi:10.4149/endo_2011_01_29

Prostatic assessment in rats after bilateral orchidectomy and calcitonin treatment

[1]Al-Shahat AR, [2]Shaikh MA, [3]Elmansy RA, [2]Shehzad K, [4]Kaimkhani ZA

[1]Department of Anatomy, Faculty of Medicine, Cairo University, Egypt; [2]Department of Anatomy and Histology, College of Medicine, Al-Qasim University, Al-Qasim, Saudi Arabia; [3]Department of Anatomy Faculty of Medicine, AinShams University, Cairo, Egypt; [4]Department of Anatomy, King Saud University, Riyadh, Saudi Arabia
e mail: shaikhm_63@hotmail.com

Objective. Bilateral orchidectomy is widely used as a treatment in patients with metastatic prostatic cancer, but post-orchidectomy osteoporosis is a common sequel which is commonly treated by postoperative calcitonin injection. Since the increase in the invasiveness of malignant prostatic cells has been attributed to the use of calcitonin, this study was aimed to elucidate the effect of calcitonin on the structure of the prostate after orchidectomy in rats used as mammalian model.

Methods. A total of 84 adult male albino rats were divided into three groups: Group 1 (12 control rats); Group 2 (36 rats subjected to bilateral orchidectomy); Group 3 (36 rats subjected to bilateral orchidectomy and injected subcutaneously with calcitonin (5 μg/kg) every other day. Six animals of Group 2 and 3 were sacrificed two, four, eight, sixteen and twenty four weeks after orchidectomy. The prostates were removed and processed for morphometric measurements by using the image analyzer computer system.

Results. The present study demonstrated a decrease in the height and apoptosis of the epithelial lining of the prostatic acini. There was also an increase in the interacinar fibromuscular stroma. However, calcitonin administration following orchidectomy limited these changes.

Conclusion. Bilateral orchidectomy produced time related atrophic changes in the prostate, while a simultaneous administration of calcitonin inhibits the development of these atrophic changes.

Key words: prostate, prostatic carcinoma, calcitonin, prostatic acini, osteoporosis, prostatic atrophy

Prostatic cancer is considered the second most common cause of cancer mortality in males (Debruyne 2002). Treatment by radical prostatectomy and radiotherapy is useful in the early stages of the disease (Cheng et al. 1995). After the occurrence of metastases, patients are usually treated by orchidectomy (Zalcberg et al. 1996; Boccon et al. 1997; Eisenberger et al. 1998; Mahler et al. 1998).

Orchidectomy results in a rapid and significant reduction of blood flow to the mature rat ventral prostate (Shabsigh et al. 1998; Burchardt et al. 2000); prostatic atrophy, one week after orchidectomy and complete epithelial involution by the third week (Wahlgvist et al. 1999). This reduction coincided with the appearance of striking degenerative changes within the vascular system of the prostate (Burchardt et al. 2000). Some authors recorded the flattening of cell surface, reduction of the size and number of microvilli, some blurring of the cell borders, cessation of the secretory activity and diminution of the gland volume. Eisenberger et al. (1998) found that orchidectomy affected all bones negatively with marked osteoporosis and latter resulted in pathological fractures. Most studies have shown that calcitonine administration improved bone density and

Corresponding author: Prof. Dr. Masood Ahmed Shaikh, Department of Anatomy and Histology, College of Medicine, POBOX #6666, POSTAL CODE-51452 , Al Qasim University, Al-Qasim, Saudi Arabia; Phone: 00966-6-3800050-ext-2503, Cell: 00966-540886257, Fax: 00966-6-3802275; e.mail: shaikhm_63@hotmail.com

prevented this osteoporosis by inhibiting osteoclastic activity.

Ritchie et al. (1997) proposed that calcitonin might play an integral role in the regulation of normal prostatic cell growth and metastases. Increased invasiveness of malignant human prostatic cells as a result of elevation of intracellular cyclic adenosine monophosphate after parentral administration of calcitonin has been reported by Wu et al. (1996). On the other hand, Nagakawa et al. (1998) did not find any significant effect of calcitonin administration on the growth of the normal or prostatic tumor cells.

The aim of the present study is to bring some more concrete evidence about the effects and use of calcitonin on the prostate. Such attempt may be helpful to access the safety of calcitonin administration in prevention and treatment of post-orchidectomy osteoporosis in patients with advanced prostatic carcinoma.

Materials and Methods

A total of 84 adult male albino rats, weighing between 200-250 g were used in this study. The animals were fed *ad libitum* and allowed free water supply. They were housed in cages, six rats per cage, under strict care and hygiene to keep the animals in normal and healthy conditions.

The animals were divided into three groups. Group 1 (control) consisted of 12 rats receiving sterile saline. Group 2 consisted of 36 rats subjected to bilateral orchidectomy (OE) under light ether anesthesia when being fixed on a wax plate in supine position. The skin of each animal was shaved and sterilized with 70 % ethanol. A low midline trans-scrotal skin incision was made. The spermatic cords were dissected and the testicular vessels were ligated using Vicryl 3/0, synthetic, absorbable, polyglycan sutures and then testicles were excised. Aqueous penicillin powder was sprayed in the operation field to minimize the risk of postoperative infection. The incision was closed by interrupted 0/5 silk sutures and gentamycin cream was applied locally as a wound dressing to reduce the risk of postoperative wound infection. Group 3 consisted of 36 rats subjected to bilateral orchidectomy and receiving subcutaneous injection of calcitonin (5 μg/kg b.w.; Miaca1cic ampoules; 100 μg/ml; Swiss Pharma Company, Cairo, Egypt) immediately after the operation and then every other day until they were sacrificed (Deftos and Granin 1998).

Six animals of Groups 2 and 3 each, and two animals from Group 1 were picked up randomly and sacrificed by deep ether inhalation after two, four, eight, sixteen and twenty four weeks. Suprapubic incision in the anterior abdominal wall was made to expose the prostate (Deftos and Granin, 1998) which was then removed and fixed in 10 % formol saline (Culling 1974). Paraffin sections of 5 μm thickness were cut and stained with haematoxylin-eosin and Periodic Acid Schiff (PAS) (Cormack 1993).

Morphometric study. The morphometric measurements were obtained by using the image analyzer computer system. This image analyzer consisted of software of Leica Quin 500 based on windows, connected to a microscope provided with Panasonic video camera and a monitor screen. The height of the lining epithelial cells of prostatic acini was measured. The estimations were done in ten fields from each section and the mean values were obtained.

Statistical evaluation. The data was analyzed statistically using ANOVA test. The values were given as the mean and the difference was considered as non-significant at $p>0.05$, while the difference showing $p<0.05$ was considered significant, that of $p<0.01$ highly significant and that of $p<0.001$ very highly significant.

Results

Light microscopic observations. Group 1 (controls). In this group acini of variable size and shape were present, some of them showing cuboidal epitelium, while several others had columnar and pseudo stratified columnar epithelium. Acinar lumen was filled with acidophilic secretions and the acini were separated by fibro muscular stroma (Fig.1). The PAS reaction was strongly positive in the basal laminae underneath the epithelium and in the apical or luminal border of the cells and also the prostatic secretion showed a strong PAS positivity (Fig.2)

Group 2 (subjected to bilateral OE). One week after OE the prostate showed a decrease in the height of the epithelial lining of the prostatic acini. Besides of the secretory material also desquamated epithelial cells were present in the acini lumen and the wall of some acini showed few adherent flat epithelial cells (Fig. 3). Apical blebing of the epithelial lining cells was also observed, some of blebs being also present in the lumen of acini (Fig.5). Inflammatory cells were seen in the acini and fibro muscular stroma (Fig.3). In PAS stained sections, an intense reaction was seen in the thickened basal

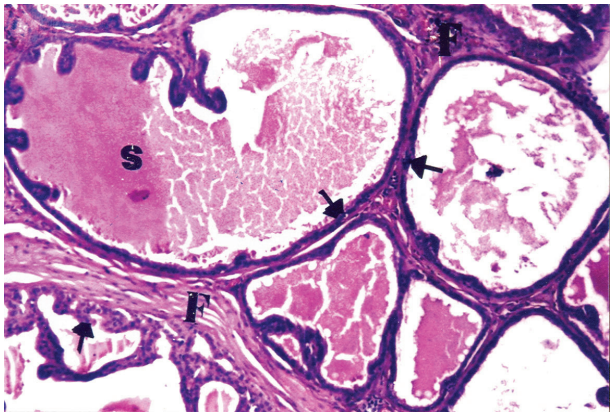

Fig. 1. Control rat: prostatic acini of variable size and shape, separated by fibromuscular stroma (F); epithelial lining of variable height (arrows); acidophilic secretion (S); hematoxyline and eosin, x 200.

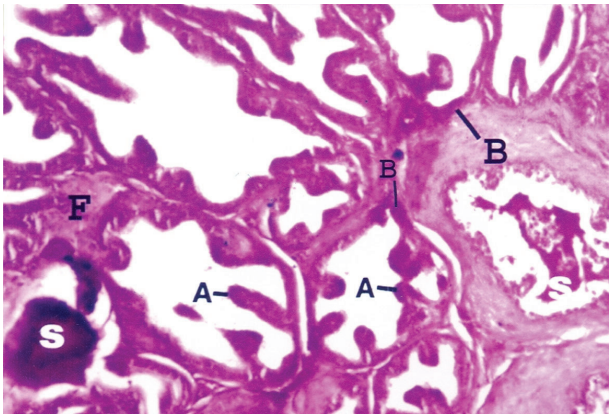

Fig. 2. Control rat: fibromuscular stroma (F). Strongly positive PAS reaction in apical border (A), basal lamina (B) and prostatic secretion (S); PAS; x 100.

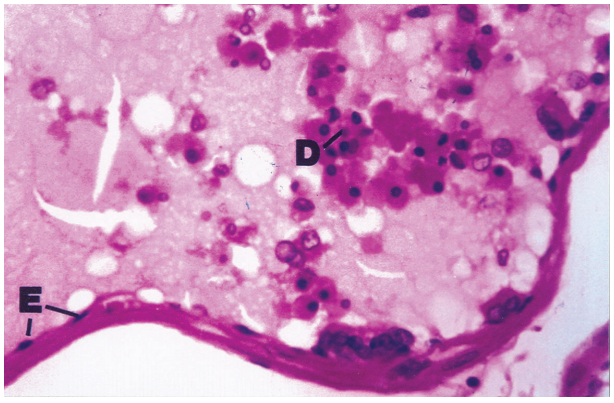

Fig. 3. Group 2 (after one week): the wall of prostatic acinus with few adherent flat epithelial cells (E); desquamated epithelial cells in the luimen (D); hematoxyline and eosine.

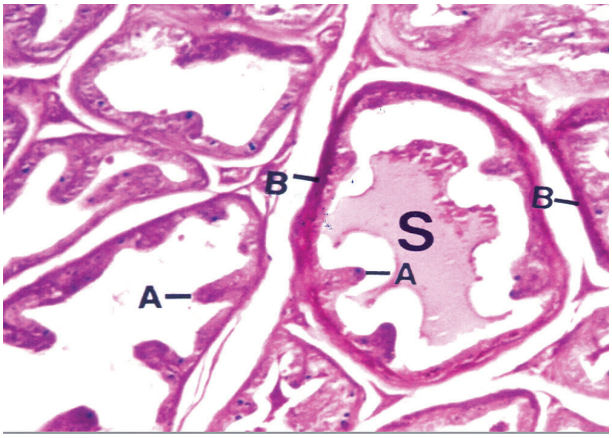

Fig. 4. Group 2 (after one week): decreased PAS reaction in apical parts of epithelial cells (A) and intense reaction in basal lamina (B) of prostatic acini; PAS, x 100. epithelial cells; PAS; x 100.

lamina of acini and a weak reaction was seen in the apical parts of the acini lining cells (Fig.4).

Two weeks after OE increased number of desquamated cells and inflammatory cells was found in their lumen and fibromuscular stroma (Fig. 6). The PAS stained sections showed intense reaction in the thickened basal lamina of acini and weak reaction in the apical part of the lining epithelial cells and in the secretory material in the lumen.

Four weeks after OE a decreased height of the epithelial lining cells was observed and also few desquamated epithelial cells with increased fibro muscular stroma in between the acini were found (Fig.7). PAS staining showed an intense reaction in the thickened basal lamina, but a weak reaction in the apical parts of the epithelial lining of the acini.

Eight weeks after OE almost all acini were lined by flattened epithelial cells, their size and secretion amount being still more decreased than that found after one or two weeks. Similarly, still more desquamated epithelia in the lumen of acini and more increased fibro muscular stroma in between the acini appeared (Fig.8). In contrast to previous weeks, PAS staining showed a weak reaction in thickened basal lamina of acini and in the apical parts of epithelial lining.

Sixteen and twenty four weeks after OE a marked decrease in the height of the epithelial lining continued and it finally became extremely flattened, also an extensive damage and increased fibro muscular stroma and inflammatory infiltrate being noted (Fig.10). There was a markedly weak PAS reaction in the epithelial lining cells and their secretory material (Fig.9).

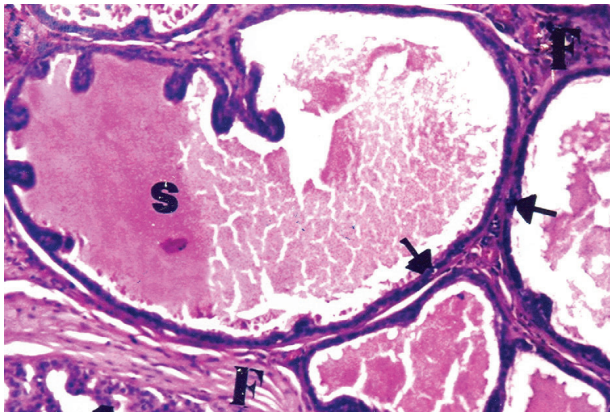

Fig. 5. Group 2 (after one week): apical blebbing (arrows) attached to the epithelial lining of a prostatic acinus, some of them being released in the lumen (arrow head); hematoxyline and eosine, x 1000.

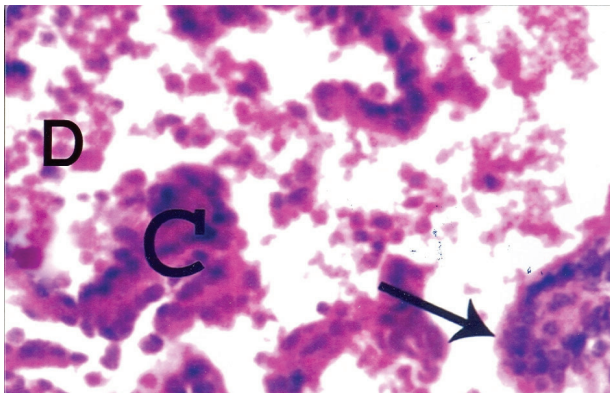

Fig. 6. Group 2 (after two weeks): normal epithelial lining of prostatic acini (arrow) with increased desquamated epithelial cells and inflammatory cells (C) in their lumen; few inflammatory cells in the fibromuscular stroma (I) between the acini; hematoxyline and eosine, x 1000).

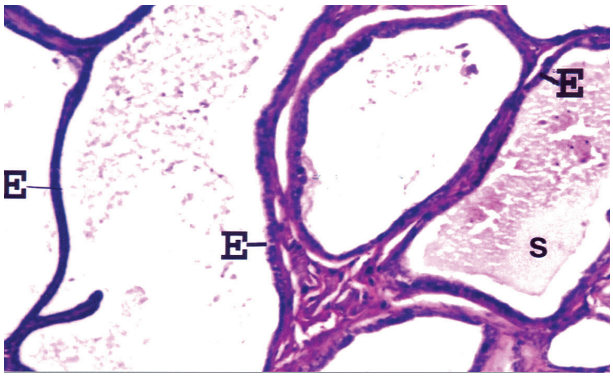

Fig. 7. Group 2 (after one month): decreased height of epithelial lining cells of prostatic acini (E); weak reaction of secretory material in the lumen; hematoxyline and eosine, x 1000.

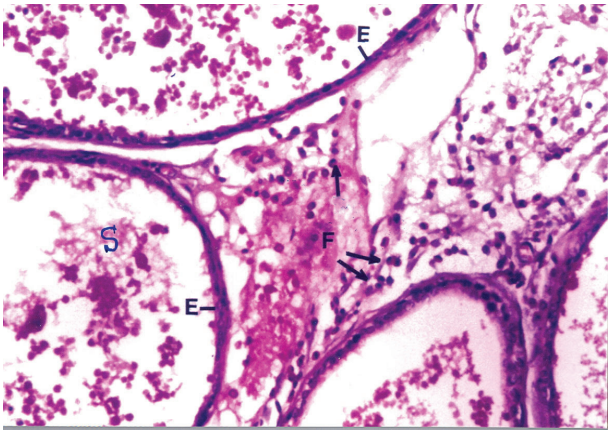

Fig. 8. Group 2 (after two months): variable size of prostatic acini with decreased height of lining epithelial cells of these acini to become flattened simple squamous cells (E); decreased amount of secretory material (S) and increase fibromuscular stroma (F) which showed marked inflammatory cells infiltration; hematoxyline and eosine, x 1000.

Group 3 (subjected to bilateral OE and receiving calcitonin). One week after OE, the variability in the size and shape of acini, a decreased height of the epithelial lining cells of acini and infiltrating inflammatory cells in fibro muscular stroma were found. The acini showed secretory material and desquamated epithelial cells in their lumen. There was intense PAS reaction in the basal lamina of acini lining with slightly weak reaction in the apical parts. A weak reaction of secretory material in the lumen of acini was also seen.

Two weeks after OE a mild decrease in the size of acini with increased surrounding fibro muscular stroma and infiltrating inflammatory cells were found.

Four weeks after OE the acini of variable size were widely separated with increased fibro muscular stroma. The height of the epithelial lining of acini was moderately decreased and low cuboidal shape of the cells was observed, while the fibro muscular stroma increased and appeared infiltrated by inflammatory cells.

Eight weeks after OE the acini still showed a variability of size, being widely separated by the fibro muscular stroma. PAS reaction in apical parts of epithelial lining of acini showed a marked decrease as compared to their thickened basal laminae.

Sixteen and twenty four weeks after OE there were no obvious recordable differences in the microscopic features. However, both groups showed markedly decreased reaction in the apical parts of the epithelial lining of the prostatic acini as compared to their thickened basal laminae (Figs. 11, 12). There was also a variability in the size

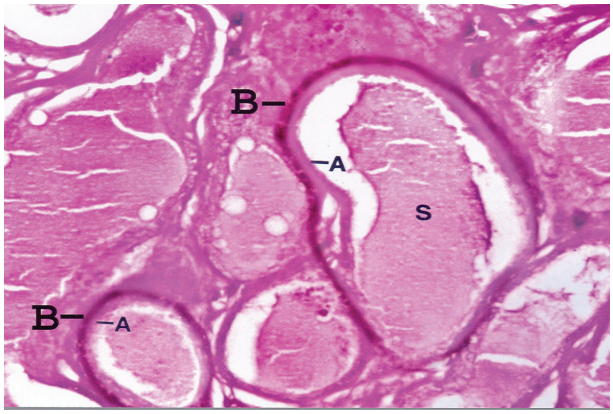

Fig. 9. Group 2 (after four months): decreased PAS reaction in apical border (A) and basal lamina (B) of epithelial cells lining the prostatic acini; secretory material (S) also shows a weak PAS reaction; PAS, x 200.

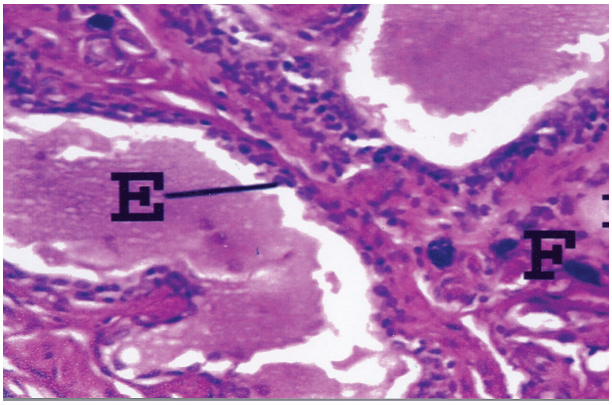

Fig. 10. Group 2 (after six months): decreased height of epithelial lining cells which became transformed in flattened simple squamous cells (E); marked inflammatory cell infiltration (arrow) within the increased fibromuscular stroma (F) in between the prostatic acini; hematoxyline and eosine, x 1000.

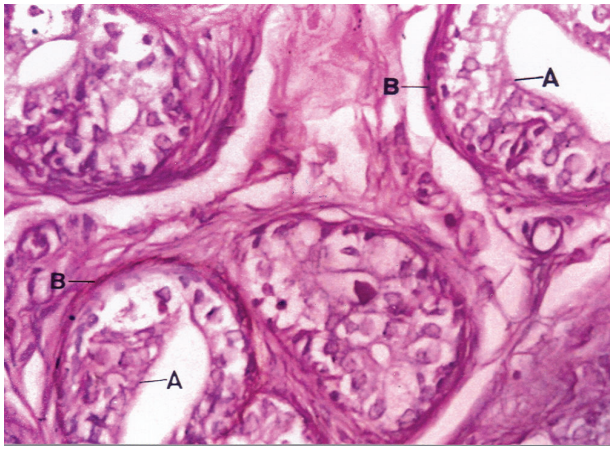

Fig. 11. Group 3 (two months after): markedly decreased reaction in the apical parts (A) of the epithelial lining of the prostatic acini as compared to their thickened basal laminae (B); PAS, x 200.

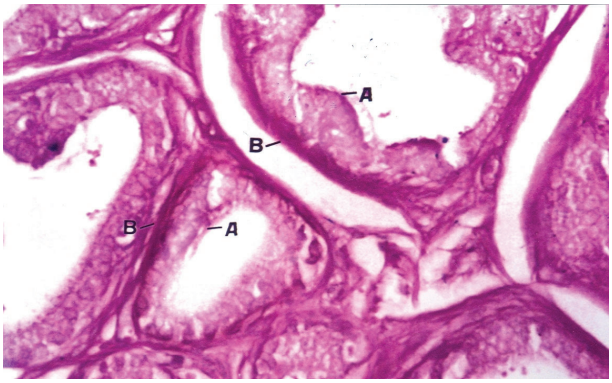

Fig. 12. Group 3 (after six months): variability in the size of prostatic acini which are widely separated by fibromuscular stroma; also markedly decreased PAS reaction in apical parts (A) of epithelial lining of the prostatic acini (E) as compared to their thickened basal laminae (B); PAS; X 400.

of acini which were widely separated by fibro muscular stroma and showed an inflammatory infiltrate.

Morphometric assesment (Table 1). The differences in the mean value of epithelial height between controls and orchidectomized rats (Group 2) as well as orchidectomized plus calcitonin treated rats are shown in Table 1. It appeared that the mean height of epithelial cells in pairs of control rats incresaed from 12.1 μm at the beginning of experiment up to 15.7 μm after 2 months and to 19.6 μm after six months. Although, at the beginning of experiment, the mean epithelial height in six anamals of Group 2 (11.8 μm) and Group 3 (11.3

Table 1

Mean values (μm) of the epithelial height (EH) of prostatic acini in individual groups of rats

Duration	Epithelial heigth (μm)		
	Control	Group 2	Group 3
1 week	12.1	11.8±1.19*	11.3
2 weeks	12.2	11.7	11.6
1 month	14.6	10.13	12.32
2 months	15.7	9.3	11.4
4 months	16.9	7	9.6
6 months	19.6	5.7±1.20*	9.6

*- $p<0.05$ between two labeled means

μm) has been about the same as in two control animals (12.1 μm), it strikingly decreased (to 5.7 μm) mainly in the orchidectomized Group 2, while such decrease in orchidectomized and calcitonin treated Group 3 (to 9.6 μm) appeared somewhat less striking which apparently shows beneficial effect of calcitonin treatment on prostatic epithelial cells.

Discussion

Prostatic carcinoma is among most frequent malignancies in males above the age of 65 years (Bishop 1996). Most of such patients are usually presented in advanced stage with distal metastases (Spry et al. 1996). Sandblom et al. (1997) mentioned that the initial treatment for advanced prostatic carcinoma is bilateral OE which results in atrophy of the primary tumor and also of all its metastases with increasing chances of survival. At the same time, OE is complicated by marked osteoporosis which may result in pathological fractures (Shabsigh et al. 1998). However, subcutaneous calcitonin injections improved the bone density by inhibiting the osteoclastic activity and were opted by many clinicians as a treatment for post OE osteoporosis (Moyad 2002). In contrast, however, calcitonin administration has also been associated with increased invasiveness of malignant prostatic cells.

The present study showed a reduction in the height of epithelial lining of prostatic acini after OE in rats and this effect was found time dependent since more severe flattening was observed in subgroups at several weeks after orchidecomy. Our findings are in agreement with those by Stattin et al. (1998) who reported a regressive morphology and decreased cellular activity three months after castration in rats and Burchardt et al. (2000) who proposed a supranuclear atrophy of the acini cells as a cause of this regression.

The epithelial lining of the prostate reflected the level of testosterone and any alteration in its level through cellular atrophy and loss of secretory activity (Preston et al. 2002). These trophic changes were observed with any factor which either removed or antagonized the effect of testosterone on the accessory sex organs such as antiandrogens (Cheng et al. 1995).

One of the histological findings of the present study was the appearance of apoptotic cells in the epithelial lining of the prostatic acini after OE. Malkowicz et al. (2001) suggested that the activated protease enzymes were responsible for these apoptotic changes. Shabsigh et al. (1998) reported a decline in prostatic blood flow in the early period after castration. Debruyne (1989, 2002) proposed a counterbalancing effect of androgens between the agonistic cellular proliferative and antagonistic cellular death activity. Since androgens might influence DNA synthesis and induce the synthesis of substances with mitogenic effect on the prostate. Castration results in a striking reduction in androgens level which resulted in a loss of the mitogenic effect (Cheng et al. 1995). Reduction in blood flow, loss of mitogenic influence of androgens, loss of counterbalancing effect of androgens and an increase in the activity of proteolytic enzymes in the epithelial lining cells may be contributory factors towards the apoptotic activity, atrophic changes and a reduction in prostatic size in a short period of time seen after bilateral OE in the present study.

Our present observations showed a weak reaction in the apical portion of acini lining cells in PAS stained sections in animals of Group 2 (Figs. 2, 4 and 9). This may be explained by a decrease in amount of the secretory products resulting from decreased activity of the lining cells secondary to the decrease of testosterone after OE. Another significant finding of this study is the presence of blebs (Fig. 5) in the lining epithelial cells which has been proposed as an early sign of injury (Ross and Small 2002) which is further supported by the finding of inflammatory cells in fibromuscular stroma in the present study.

Moreover, increased amount of fibromuscular stroma has been found in animals of Group 2 which seems to be associated with atrophy of the prostatic acini as suggested by Debruyne (1989, 2002) who observed a doubling in the amount of fibromuscular tissue and attributed this to a diminution of testicular hormones. Group 3 animals injected by calcitonin every other day after bilateral OE have shown delayed and limited atrophic changes in epithelial lining cells of the prostatic acini, but most significant changes being present in the animals at sixteen and twenty four weeks after OE. From this possibly follows that the administration of calcitonin may prevent the effects of androgens loss after OE only for a limited period, but after some time that effect of androgens loss appeared overwhelmed by the effects of calcitonin as shown by the above mentioned animals of Group 3 at sixteen and twenty four weeks after OE.

Thus, it was concluded that OE showed a definite regressive effect on the epithelial lining of prostatic acini. However, this regressive effect was less marked when OE was followed by calcitonin injection.

References

Bishop MC: Experience with low-dose oestrogen in the treatment of advanced prostate cancer. Br J Urol 78, 921–927, 1996.

Boccon-Gibod LA, Foumier G, Bottet P, Marechal J, Guiter J, Rischman P, Hubert J, Soret J, Margin P, Mallo C, Fraysse C: Flutamide versus orchidectomy in the treatment of metastatic prostate carcinoma. Eur Urol 32, 391-396, 1997.

Burchardt M, Burchardt T, Chen M, Hayek 0, Knight C, Sizabsigh A, Taille A, Buttyan R: Vascular endothelial growth factor A-expression in the rat ventral prostate gland and the early effects of castration. Prostate 43, 184-194, 2000. doi:10.1002/(SICI)1097-0045(20000515)43:3<184::AID-PROS4>3.0.CO;2-6

Cheng C, Koong HN, Foo KT: Radical prostatectomy for prostate cancer-an experience of fiftheen cases in Singapore. Ann Acad Med Singapore 24, 557-561, 1995.

Cormack DH: Essential Histology: The Male Reproductive System, 7th ed., pp 377-398. Lippincott-Raven Publishers, Philadelphia 1993.

Culling CFA: Handbook of histopathological and histochemical techniques. 3rd ed. Butterworth, London 1974.

Debruyne F: Luteinizing hormone-releasing hormone analogues alone in the treatment of advanced disease. Horm Res 32, (Suppl. 1), 62-65, 1989.

Debruyne F: Hormonal therapy of prostate cancer. Semin Urol Oncol 20, 4-9, 2002. doi:10.1053/suro.2002.35051

Deftos LJ, Granin A: Parathyroid hormone related protein and calcitonine gene products in neuroendocrine prostate cancer. Prostate 8, 23-31, 1998. doi:10.1002/(SICI)1097-0045(1998)8+<23::AID-PROS5>3.0.CO;2-H

Eisenberger MA, Blumenstein BA, Crawford ED, Miller GD, Mcleod DG, Loehrer PJ, Wilding GB, Sears KE, Culkin DJ, Thompson LM, Bueschen AJ, Lowe BA: Bilateral orchidectomy with or without flutamide for metastatic prostate cancer. N Engl J Med 339, 1036 -1042, 1998. doi:10.1056/NEJM199810083391504

Mahler C, Verhelst I, Denis L: Clinical pharmacokinetics of the antiandrogens and their efficacy in prostate cancer. Clin Pharmacokinet 34, 405-417, 1998. doi:10.2165/00003088-199834050-00005

Malkowicz SB: The role of diethylstilbestrol and orchidectomy in the treatment of prostate cancer. Urology 58, 108-113, 2001. doi:10.1016/S0090-4295(01)01252-3

Moyad MA: Complementary therapies for reducing the risk of osteoporosis in patients receiving luteinizing hormone-releasing hormone treatment and orchidectomy for prostate cancer. Urology 59, 34-40, 2002. doi:10.1016/S0090-4295(01)01174-8

Nagakawa 0, Ogasawara M, Fujii H, Murakami K, Murata L, Fuse H, Saiki L: Effect of prostatic neuropeptides on invasion and migration of PC-3 prostate cancer cells. Cancer Lett 133, 27– 33, 1998.

Preston DM, Torrens JL, Hording PE, Howard RS, Duncan WE, McLeod DG: Androgen deprivation in men with prostate cancer is associated with an increased rate of bone loss. Prostate Cancer Prostatic Dis 5, 304-310, 2002. doi:10.1038/sj.pcan.4500599

Ritchie CK, Thomas KG, Andrews LR, Tindall DJ, Fitzpatrick LA: Effects of the calciotrophic peptides calcitonine and parathyroid hormone on prostate cancer growth and chemotaxis. Prostate 30, 183-187, 1997. doi:10.1002/(SICI)1097-0045(19970215)30:3<183::AID-PROS6>3.0.CO;2-N

Ross RW, Small EJ: Osteoporosis in men treated with androgen deprivation therapy for prostate cancer. J Urol 167, 1952-1958, 2002. doi:10.1016/S0022-5347(05)65060-4

Sandblom, G, Dufmats M, Astrand M, Nordenskjold K, Varenhorst E: Decreasing number of orchidectomies in prostatic cancer: Results of 9-year registration of the disease are reviewed. J Pharmacol Exp Ther 94, 3966-3971, 1997.

Shabsigh A, Tanji N, Agati K, Burchardt M, Rubin M, Goluboff E, Heitjan D, Kiss A, Buttyan R: Early effects of castration on the vascular system of the rat ventral prostate gland. Endocrinology 140, 1920-1926, 1999. doi:10.1210/en.140.4.1920

Shabsigh AE, Chang DT, Heitjan DF, Kiss AT, Olsson CA, Puchner PJ, Buttyan RS: Rapid reduction in blood flow to the rat ventral prostate gland after castration. Prostate 36, 201-206, 1998. doi:10.1002/(SICI)1097-0045(19980801)36:3<201::AID-PROS9>3.0.CO;2-J

Spry NA, Meffan PM, Christie DR, Morum PE: Orchidectomy prior to defnitive radiotherapy for localized prostatic cancer. Int J Radiol Oncol BioI Phys 34, 1045-1053, 1996. doi:10.1016/0360-3016(95)02385-2

Stattin P, Westin P, Damber J, Bergh A: Short-term cellular effects induced by castration therapy in relation to clinical outcome in prostate cancer. Br J Cancer 77, 670-675, 1998.

Wahlgvist R, Dahl E, Tvete K: Effects of castration upon the morphology of the accessory sex organs of the male rat - a scanning electron microscopy study. Scanning Microsc 10, 1155-1162, 1999.

Wu G, Burzon DT, di Sant'Agnese PA, Schoen S, Deftos LJ, Gershagen S, Cockett AT: Calcitonine receptor mRNA expression in the human prostate. Urology 47, 367-381, 1996.

Zalcberg JR, Raghaven DE, Marshall VR, Thompson PJ: Bilateral orchidectomy and flutamide versus orchidectomy alone in newly diagnosed patients with metastatic carcinoma of the prostate. Br J Urol 77, 865-869, 1996.

Book Review

STEM CELL THERAPY FOR DIABETES

Edited by Shimon Efrat (Tel Aviv University, Israel)

Published by Humana Press (Totowa, New Jersey, 07512, USA) - 2010
Hard cover, 289 pp., US $ 159.905

As the Editor wrote in his Preface, "regenerative medicine is an old human dream, and for the first time in human history its realization in within reach. Diabetes ranks high on the priority list of diseases that can benefit from regenerative medicine intervention" ….."stem cells hold a promise for providing an abundant source of cells for cell therapy for diabetes. The generation of human embryonic stem cell lines created expectations for an imminent unlimited supply of all cell types needed in regenerative medicine."

The Editor contacted 39 experts from three continents who contributed to this unique work by 13 chapters divide in three sections reviewing the three main approaches for the generation of sufficient numbers of insulin-producing cells for restoration of an adequate beta-cell mass: beta-cell expansion, stem cell differentiation, and nuclear reprogramming.

The first section opens with pancreas and islet development which is a highly complex process in which two distinct cell types musty derive from one simple epithelium. It should be emphasized that this chapter includes nearly 300 up to date references. Next chapter describes he procedures of islet cell transplantation, clinical protocols and outcomes including immunosuppressive therapy and this section is concluded by chapters on cell cycle regulation in human pancreatic cells, islet regeneration and beta-cell expansion in vitro.

The second section is entitle "Beta cells from non-beta cells" and contains five chapters which consider alternative cell sources for deriving insulin-producing cells and opens with the overview of the makeup of normal beta-cells. Next chapter describe the generation of beta-cells from acinar cells, from pancreatic duct cells and also of adult cells reprogramming by using nonpancreatic cell sources and also of embryonic stem cells.

Finally, the third section is devoted to tissue engineering and immune protection. It include a chapter on functional tissue reconstruction with the use of biologic scaffolds and, moreover, a chapter on immunoisolation in cell transplantation and the last one on the prevention of islet graft rejection and recipient tolerization.

Thus monograph contains numerous instructive figures, schemes and microphotos which help to understand sophisticated biological and technical processes representing the contemporaneous armament being used in this newly and rapidly development field of diabetology.