The role of prolactin in prostatic inflammation

O papel da prolactina na inflamação prostática

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Abstract

Several reports have shown that prolactin (PRL) plays a role in prostatic growth, but few studies considered the role of PRL in the process of prostatic inflammation. Young (45 ± 5 days old) and adult (75 ± 5 days old) male Wistar rats were subcutaneously injected daily with domperidone (4.0 mg.kg-1) to maintain high serum PRL levels. The animals were treated for 15, 30, 45 or 60 days. Blood and prostate samples were collected at the end of each treatment for PRL dosage and histological analysis, respectively. Only young animals treated with DOMP for 15 and 30 days displayed inflammatory infiltrate in the prostate. These results confirm literature data in regards to PRL involvement in inducing prostate inflammation. Moreover, it was concluded that young animals are more susceptible then adults to the PRL action concerning prostate inflammation.

Keywords: Hyperprolactinemia. Domperidone. Prostate. Rats.

Resumo

A prolactina (PRL) influencia o crescimento prostático, entretanto poucos estudos investigaram o papel da PRL na inflamação prostática. Ratos Wistar jovens (45 ± 5 dias de idade) e adultos (75 ± 5 dias de idade) receberam injeções subcutâneas diárias de domperidona (4,0 mg.kg-1) para manter níveis séricos altos de PRL. Os animais foram tratados por 15, 30, 45 ou 60 dias. Amostras de sangue e próstata foram coletadas ao final dos tratamentos para dosagem de PRL e análise histológica, respectivamente. Apenas os animais jovens tratados com domperidona por 15 e 30 dias apresentaram infiltrado inflamatório na próstata. Esses resultados confirmaram a participação da PRL na indução da inflamação prostática. A conclusão obtida foi que animais jovens são mais susceptíveis à ação da PRL na inflamação da próstata que os adultos.

Palavras-chave: Hiperprolactinemia. Domperidona. Próstata. Ratos.

Introduction

Prolactin (PRL) modulates various aspects of male physiology (CRUZ-CASALLAS et al., 1999; SILVA et al., 2004). It has been established that PRL stimulates prostate growth and functioning in both young and adult rats (KEENAN; KLASE; THOMAS, 1981; NEGRO-VILAR; KRULICH; McCANN, 1973; PRINS; LEE, 1983). Moreover, inflammation in lateral prostate seems to be related to high serum PRL levels (STOKER; ROBINETTE; COOPER, 1999). High plasma PRL levels throughout newborn or prepubescent periods result in an increased incidence of prostatic inflammation in adult rats (STOKER et al., 1999). A causal relationship has been suggested between prostatic inflammation and benign prostatic hyperplasia in humans (GILLERAN et al., 2003). Coppenolle et al. (2001) reported hyperprolactinemia induced hyperplasia associated with inflammation in the rat lateral prostate lobe after treatments for 30 and 60 days with sulpiride.

The period prior to rat puberty, from the 15th to the 25th postnatal day, has been acknowledged as being critical to prostatic development. In this period, plasma PRL levels begin to rise, reaching the highest point on the 25th day of postnatal development (NEGRO-

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VILAR; KRULICH; McCANN, 1973). Prostates from immature rats display higher binding to PRL than that observed in adult animals (ARAGONA; FRIESEN, 1975).

In addition, various reports have emphasized that PRL plays a role in prostatic growth. Prolactin promotes a receptor mediated up-regulation of the L-aspartate transporter EAAC1, which is involved in mechanisms of hormonal regulation controlling the cellular environment (FRANKLIN et al., 2006). Prolactin also increases vitamin D receptor and p21 through up-regulation of short 1b prolactin receptor in human prostate cancer cells (WU et al., 2005).

This study was designed to investigate the age-related differences in the pharmacologically-induced increase in serum PRL levels as well as their influence on the spontaneous induction of prostatic inflammation. For this purpose, young and adult individuals were compared.

Material and Methods

Previous works have demonstrated that treatment with domperidone (DOMP, 4.0 $mg.kg^{-1}$) was able to increase PRL secretion and this effect lasted for about 72 hours (FELICIO; BRIDGES, 1992; NASELLO et al., 1995; NASELLO et al., 1997; SILVA et al., 2004). A total of 139 male Wistar rats from the colony of the FMVZ/USP were used: 68 young (45 ± 5 days old) and 71 adult rats (75 ± 5 days old) in order to evaluate the effects of DOMP-induced hyperprolactinemia on the induction of prostatic inflammation. The animals received free access to water and food and were maintained in a 12:12 hour light:dark cycle. They were separated into two major groups: experimental (treated with DOMP 4.0 $mg.kg^{-1}$, sc, daily) and control (saline/SAL). Animals were treated for 15, 30, 45 or 60 days. Animals were decapitated at the end of each treatment with a minimum of stress. Blood and prostate samples were collected for prolactin dosage by radioimmunoassay and histological analysis, respectively. The animals used in this study were maintained in accordance with the guidelines from the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

Blood was collected into a test tube and kept at room temperature until a clot was formed. If necessary, the blood was centrifuged for serum separation. Once serum was obtained, it was stored in duplicate in polyethylene tubes (eppendorfs) and kept at -80oC until hormonal dosage. PRL concentration was obtained by means of radioimmunoassay with double antibody, by using specific kits supplied by The National Institute of Diabetes and Digestive and Kidney Diseases (NIADDK, Baltimore, MD, USA). Antiserum anti-rat PRL-S9 for PRL was used and reference preparations were PRL-RP3. The lower limit of detection was 0.19 $ng.mL^{-1}$ and coefficients of variation intra and inter assay were 4.0% and 11.5%, respectively. PRL levels are expressed in $ng.mL^{-1}$.

After dissection, prostate samples were fixed into Bouin's solution for approximately 48 hours. Samples were transferred to alcohol 70% for routine paraffin embedding. The 5 μm sections were stained with hematoxylin and eosin (HE) for histological analysis.

Statistical analysis

Serum PRL concentrations were compared by Mann-Whitney U-test. Qualitative data specific to prostate histological alterations were analyzed by Exact test of Fisher. In both cases, $P < 0.05$ values, obtained using two-tailed comparisons, were considered statistically significant.

Results

Serum PRL levels increased significantly throughout the treatment in both young and adult animals treated with DOMP, except young animals treated for 45 days (Figure 1). Tables 1 and 2 show data from the prostates of young and adult animals, respectively. Young animals treated with DOMP displayed an intense multifocal inflammatory infiltrate in the lumen of prostatic acini, and, sometimes, an inflammatory

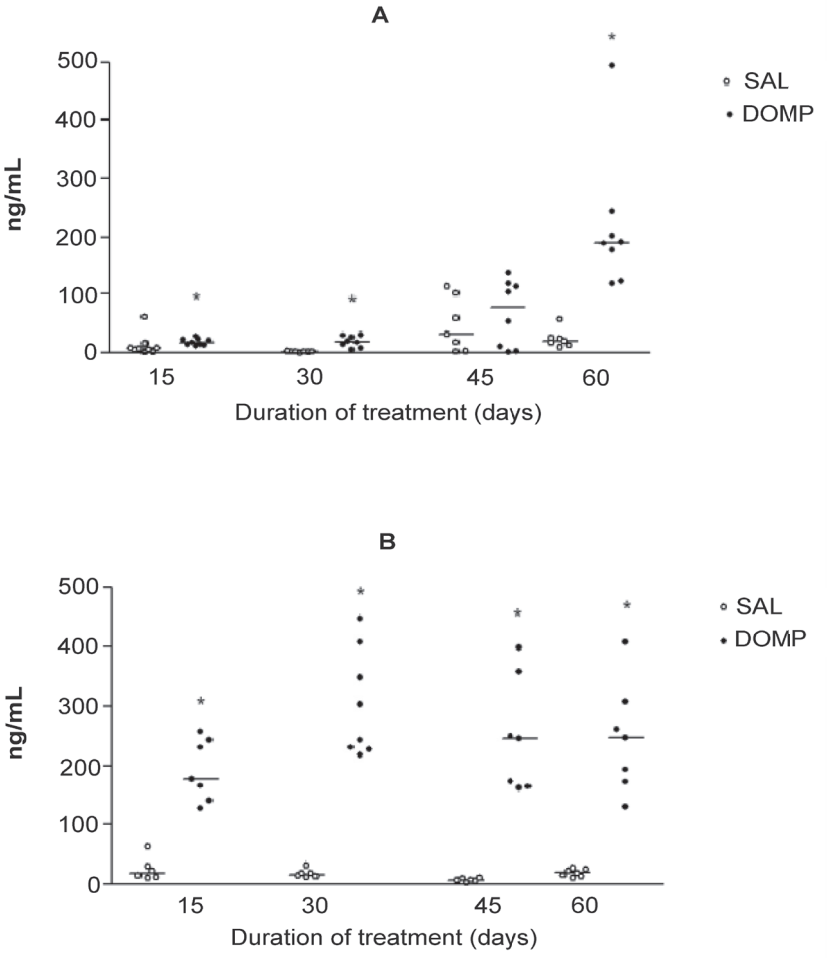

Figure 1 – Serum levels of PRL (ng/ml), in young (Figure 1A) and adult animals (Figure 1B), respectively, treated with DOMP and controls (SAL). Data are median and individual values.
* P < 0.05 compared to respective control groups

Table 1 - Effect of DOMP (4.0 mg.kg^{-1}) on the incidence of prostatitis in young rats. The number of animals with lesions over the total number of animals per group is presented

	Period of treatment (days)			
Groups	**15**	**30**	**45**	60
SAL	2/10	0/8	4 /8	0/7
DOMP	7/10	8/8	6/8	4/8
Degree of significance	P < 0.05	P < 0.0001	P = 0.30	P < 0.052

Statistical analyses Fisher Exact test

Table 2 - Effect of DOMP (4.0 mg.kg^{-1}) on adult animals presenting prostate changes such as prostatitis or cell desquamation. Numbers shown are of animals presenting prostate changes from total number of animals in group

	Period of treatment (days)			
Groups	**15**	**30**	**45**	**60**
SAL	1/7	1/8	2/6	2/8
DOMP	3/8	6/10	6/7	5/9
Degree of significance	P = 0.34	P = 0.07	P = 0.10	P = 0.22

Statistical analyses Fisher Exact test

infiltrate in connective tissue was noted (Table 1 and Figure 2). In respect to the type of cell found in the inflammatory infiltrate, polymorphonuclear (neutrophils) and mononuclear cells (lymphocytes) were found. The adult animals treated with DOMP showed no significant histological changes in any treatment day (Table 2).

Discussion

Consistent with literature data, animals treated with DOMP showed higher serum PRL levels than saline-treated rats. Nevertheless, DOMP-induced hyperprolactinemia seems to be more intense in adult than in young animals. These data show that when challenged with DOMP, young animals release PRL to a lesser extent than the adults.

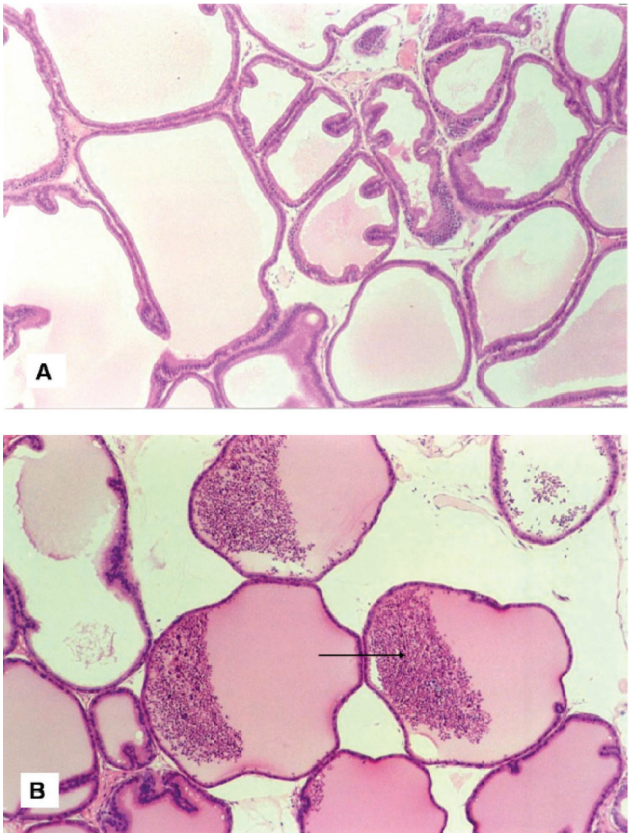

Figure 2 – Histological aspects of lateral prostate lobe of young animal of SAL (Figure 2A) and DOMP (Figure 2B) groups. Stained with hematoxylin and eosin (HE) magnification X100. Arrow in figure 2B shows leukocyte infiltrate

It has been demonstrated that an increase in serum PRL levels is a potent stimulus in inducing and maintaining inflammatory reaction in the lateral prostate in rats. Moreover, some studies have suggested that there is an association between prostate inflammation and prostate hyperplasia (KESSLER et al., 1998). Prostate inflammation has been commonly noted in samples of human prostate that have shown benign prostate hyperplasia (STOKER et al., 1999). Tangbanluekal and Robinette (1993) have observed that estradiol-induced hyperprolactinemia as well PRL itself can stimulate inflammation in the lateral prostate lobe in adult rats. They observed that the hyperprolactinemic animals showed leukocyte infiltrates in the lumen of prostate acini, neutrophils, and mononuclear leukocytes in the stroma. They also observed that the higher the PRL dose administered, the greater the inflammatory process. A similar result was found by Stoker et al. (1999). These authors have suggested that PRL plays a role in this process. Stoker et al. (1999) have demonstrated that 15-20 day old Wistar rats exposed to compounds stimulating PRL secretion such as 17β-estradiol, pimozide or bisphenol A presented inflammation in the lateral prostate lobe at 120 days of adulthood. Prostate inflammation has also been observed in Noble adult rats (11-12 weeks old) when treated with 17β-estradiol for 56 days (LANE et al., 1997). Transgenic mice expressing one specific prostatic gene linked to the PRL rat gene show inflammation, infiltrating the prostate (KINDBLOM et al., 2003). In this model, these authors have demonstrated that a local increase of PRL in the prostate is capable of inducing prostatic changes such as inflammatory cell accumulation, an increase in the stroma and prostate duct dilatation, which are basic features of benign prostatic hyperplasia in humans.

In the present work using DOMP to maintain high levels of serum PRL, we observed in the inflammatory infiltrate polymorphonuclear cells (neutrophils) and mononuclear cells (lymphocytes) in the prostate. We also observed that the polymorphonuclear cells

prevailed in the lumen of prostate acinus, while the mononuclear cells prevailed in the gland stroma. Regarding the degree of inflammation, the presence of inflammatory cells was more severe in the lumen of the prostate gland.

In a retrospective analysis, Suwa et al. (2001) reported a significant positive correlation between pituitary adenoma and inflammation dorsolateral prostate lobe in Fischer-344 rats. These authors have suggested that PRL, which has been produced in excess by pituitary adenoma, could be a predisposing factor in prostate dorsolateral lobe inflammation.

In spite of several works clearly demonstrating the role of PRL in prostate inflammation process, it is not clear how PRL induces such a response. It is known that lymphocytes have receptors for PRL (PELLEGRINI et al., 1992) and PRL is mitogenic for T-cells proliferation as well as an inducer for cytokines and antibody production (CLEVENGER; FREIER; KLINE, 1998; McMURRAY, 2001). Thus, these are possible mechanisms for PRL involvement in prostate inflammatory process.

Since we have noted no differences between serum PRL levels in young animals treated with DOMP for 15 and 30 days, there might be other inflammatory factors in young animals besides the increase in serum PRL levels. One such factor would be the extent of the period of treatment. Alternatively, the lack of finding a significant difference in prolactin at 45 days in the younger animals may have been due to the variation of prolactin levels in the control rats. On the other hand, there were no significant prostate changes in adult animals treated with DOMP for the same period in spite of the increased serum PRL levels observed in these animals. Since lymphocytes located in inflamed prostates have shown PRL-like transcripts, it has been suggested that a PRL-like substance produced locally could contribute to maintaining serum PRL-induced lateral prostate inflammation in rats. Therefore, a locally prostate produced PRL may play a role in inflammatory cell infiltrate (ROBINETTE; TREMPUS; ONKS, 1993). Alternatively, the possibility that DOMP itself has direct inflammatory effects on the prostate cannot be discharged. The possibility that DOMP stimulates local production of prolactin in the prostate before it increases pituitary prolactin production and which may give rise to the inflammation seen early in the younger rats has to be investigated in future studies. If that would be the case, it would still be in accordance with the general concept of a higher sensitivity of younger as compared to adult animals. Consistently, studies with DOMP have failed to reveal effects on prostate of adult animals (BAILEY; HERBERT, 1982).

Härkönen (2003) has proposed a model in which either pituitary or locally produced PRL could be bound the PRL receptors located in prostate epithelium and, hence, to initiate signal paths that will induce specific gene targeting expression. Once the gene product is stimulated, it would be distributed to various places in epithelial cells, lumen or prostate stroma where they could attract or have an effect on inflammatory cells. PRL also could interact with prolactin receptors located in inflammatory cells. The possible influence of each of these factors on the present results will be evaluated in future experiments.

Our results confirm literature data in regards to the role of PRL in inducing prostate inflammation. Moreover, we can conclude that young animals are more susceptible to the PRL action in respect to prostate inflammation.

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