Biochemical and Biophysical Research Communications 493 (2017) 1581–1586

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hyperglycemic condition during puberty increases collagen fibers deposition in the prostatic stroma and reduces MMP-2 activity

Sérgio Alexandre Alcantara dos Santos [a], Elaine Manoela Porto Amorim [b], Larissa Mayume Ribeiro [a], Jaqueline Carvalho Rinaldi [c], Flávia Karina Delella [b], Luis Antonio Justulin [a], Sérgio Luis Felisbino [a, *]

[a] Department of Morphology, Institute of Biosciences, Sao Paulo State University (UNESP), Botucatu, Sao Paulo, Brazil
[b] Center of Biological and Health Sciences (CCBS), State University of West Paraná (UNIOESTE), Cascavel, Parana, Brazil
[c] Department of Morphological Sciences, Biological Sciences Center, State University of Maringa (UEM), Maringa, Parana, Brazil

ARTICLE INFO

Article history:
Received 26 September 2017
Accepted 4 October 2017
Available online 6 October 2017

Keywords:
Puberty
Collagen
Hyperglycemia
Diabetes
Prostate
Metalloproteinase

ABSTRACT

Puberty is an important period for the growth and maturation of the male reproductive system, and is also a critical window for endocrine or environmental interference. The physiological levels of circulating insulin and hyperglycemic control are important factors for a normal prostate growth. Hyperglycemia during puberty is reported to retard the growth of the prostate gland, with remarkable effects on the epithelial compartment. Here, we investigated the impact of hyperglycemia along with a simultaneous or late insulin replacement on the ventral prostate growth in rats during puberty, paying special attention to the deposition of collagen fibers and activities of gelatinase, matrix metalloproteinase-2 (MMP-2), and −9 (MMP-9). Hyperglycemia was induced by streptozotocin (STZ) administration in 40-day-old male Wistar rats. A subset of hyperglycemic rats underwent an early insulin replacement (three days after the STZ administration), and another subset underwent a late insulin replacement (twenty days after the STZ administration). Animals were euthanized at 60 and/or 80 days of age. The ventral prostatic lobe was processed for picrosirius red staining, type I and III collagen immunohistochemistry, and gelatin zymography. Hyperglycemic animals showed an increased area of collagen fibers in the prostate, which was composed both types of collagens. MMP-2 activity was significantly reduced in the hyperglycemic animals, while MMP-9 activity was very low and showed no alteration. The simultaneous and late insulin administration restored collagen content and MMP-2 activity. In conclusion, puberty is a critical window for prostate maturation and type-1 diabetes-induced hyperglycemia affects the ratio of the prostatic parenchymal and stromal growth, leading to fibrotic tissues by also MMP-2 down regulation.

1. Introduction

The prostate development is a complex process that involves multiple signal pathways, including endocrine, paracrine, and autocrine, besides the transcription factors [1,2]. The prostate development in rats starts at gestational day 17–18 under an influence of androgen [3]. In response to the fetal testicular androgens, the epithelial buds emerge from the wall of the urogenital sinus, grow into the surrounding urogenital sinus mesenchyme, in a process called “branching morphogenesis” [4]. After birth, branching morphogenesis is followed by intense cell proliferation and ductal canalization [5,6]. At puberty (approximately 6 weeks or 40 days old), under the influence of hormones, the prostate grows in size, begins its secretory activity, and acquires the physical and functional characteristics of an adult prostate around the age of 12 weeks [7].

For the prostate to develop normally, a regulated stromal remodeling is required to permit the growth of the epithelial structures within the stroma. The proteolytic activity of the matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, also known as gelatinases, is extremely important for the extracellular matrix remodeling during the prostate growth, development [8–10], gland atrophy [11], and regrowth [12].

MMPs belong to a family of zinc-dependent endopeptidases,

* Corresponding author. Department of Morphology, Institute of Biosciences, Sao Paulo State University (Unesp), Botucatu, SP 18618-689, Brazil.
E-mail address: felisbin@ibb.unesp.br (S.L. Felisbino).

https://doi.org/10.1016/j.bbrc.2017.10.022
0006-291X/

which are structurally related, active at neutral pH, and can catalyze the normal turnover and degradation of protein components of the extracellular matrix (ECM) [13,14]. MMPs exert an important role in the morphogenetic and normal physiological conditions, such as embryonic development, angiogenesis, cell proliferation, cell motility, tissue remodeling, and tissue repair [15,16]. Besides their physiological role, MMPs are also involved in various pathological processes, such as inflammation, fibrosis, vascular diseases, and cancer [17–20], wherein their activities are generally deregulated.

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from a defective insulin secretion, function, or both [21]. The prevalence of diabetes mellitus is increasing globally at an alarming rate owing to population growth, lack of activity, aging, obesity, lifestyle, and other factors, may be responsible for this increase. Data from the World Health Organization (WHO) showed that, about 422 million people worldwide have diabetes [22].

Studies have shown that abnormalities in the activity and expression of MMPs and a consequential change in the constitution of ECM macromolecules are involved in the pathogenesis of several diseases, including complications related to diabetes mellitus in different organs, both in humans and in experimental animals [23–27]. We had previously shown that streptozotocin (STZ)-induced diabetes in the rats diminishes, but does not abolish, prostate growth during puberty. At 80 days of age, the prostate of the diabetic animals weighed approximately 50% lesser than the age-matched control glands, and treatment of diabetic rats with insulin was able to rescue the prostate growth and its morphology [28].

Because of the importance of MMPs, particularly MMP-2 and MMP-9, in the understanding of events involved in the growth and remodeling of the prostate, we believe that the slower growth of the prostate gland in diabetic animals, at least in part, could be related to the altered activity of these enzymes and the consequent change in the quantity and quality of the ECM. Thus, the objective of this study was to investigate whether the chronic hyperglycemia resulting from STZ-induced diabetes and the insulin replacement thereafter interfere with the activities of MMP-2 and MMP-9 and collagen content in the ventral prostate stroma during puberty.

2. Materials and methods

2.1. Animals

Wistar male rats (n = 60) were supplied by the Multidisciplinary Center for Biological Investigation of the University of Campinas, CEMIB - UNICAMP. The animals were maintained under controlled temperature conditions (22–25 °C), relative humidity (55%), and a 12 h photoperiod (light period beginning at 7 a.m.). The animals had free access to water and chow. This study was approved by the Biosciences Institute/UNESP Ethics Committee for Animal Experimentation (Protocol number 42/07).

2.2. Diabetes induction in rats

The procedure for inducing diabetes has been described by Porto et al. [28]. Briefly, diabetes was induced in male rats (40-day-old) with STZ (Sigma Aldrich™, St. Louis, MO, USA). STZ was administered intravenously (i.v.) into the caudal vein in a single dose of 40 mg/kg dissolved in a citrate buffer (0.1 M, pH 4.5) (Steger 1990). The control rats received the same volume of citrate buffer (i.v.). The glucose concentrations in the blood were measured 3 days after the induction of diabetes. The rats with blood glucose levels higher than 250 mg/dL were included in the diabetic group. Three days (referred to as “simultaneous”) and 20 days (“late”) after the STZ injection, the diabetic rats received protamine zinc insulin, long-acting insulin (Novolin®), via subcutaneous injection at a dose of 3U/100 g of body weight/day for 17 and 20 consecutive days, respectively.

2.3. Experimental groups

The rats were divided into six groups (n = 10 per group): two control groups consisting of the animals euthanized at 60 (C60) and 80 days of age (C80), two diabetic groups consisting of the animals euthanized at 60 (D60) and 80 (D80) days of age, a diabetic group of animals with simultaneous replacement of insulin (DI60) euthanized at 60 days of age, and a diabetic group of animals with late insulin replacement euthanized at 80 days of age (DI80). At the end of each experiment, the animals were weighed, had their fasting blood glucose levels (12 h) examined, and were euthanized with an overdose of sodium pentobarbital (30 mg/kg of body weight) injected intraperitoneally. The ventral prostate (VP) glands were removed, weighed, fixed, and processed for histochemistry, or immediately frozen in liquid nitrogen for immunohistochemistry or protein extraction for gelatin zymography.

2.4. Histological processing

The ventral prostates were fixed by immersion in 4% paraformaldehyde/phosphate-buffered saline (PBS) solution for 24 h, and processed for paraffin embedding. Five-micrometer histological sections were stained to Picrosirius red staining [29] for the detection of total collagen fibers (both type I and type III collagen fibers were stained red) by stereological analysis [30]. Image-Pro Plus software version 4.5 for Windows was used to digitalize the images of each histological section. Analyses were carried out using a DMLB Leica Microscope and the images were obtained by a Leica DFC300FX digital camera connected to the microscope.

2.5. Stereological analysis

The relative frequency of the collagen fibers in the ventral prostates from different experimental groups were determined by an automatic detection of red color in 20 different microscopic fields (x 20) and from 5 different individual prostatic lobe sections. The collagen relative frequency was determined as a percentage of the red-stained areas per total microscopic field area.

2.6. Immunohistochemistry for type I and type III collagen

The frozen sections of the ventral prostate (7 μm) fixed in cold methanol for 20 min were collected on the silanized glass slides that were blocked first with 3% hydrogen peroxide in methanol for 15 min, followed by 3% bovine serum albumin (BSA) in PBS for 1 h at 25 °C. Next, the sections were incubated with the primary monoclonal antibodies for 2 h at 25 °C: Type I Collagen, 1:300 (Genetex, GTX 26308) and Type III Collagen, 1:300 (Abcam, ab 6310-100). The primary antibody was detected using a secondary peroxidase-conjugated antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Chromogen color development was accomplished with 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA). The slides were counterstained with hematoxylin. The negative control was performed by abolishing the primary antibody incubation step.

2.7. Protein extraction and zymography

The complete protocol for gelatin zymography was described by

Justulin-Jr et al. [12]. Briefly, the frozen samples of VP were mechanically homogenized in an extraction buffer by crushing them with the Polytron homogenizer. The samples was quantified by the Bradford method [31]. The extracted protein (28 μg) from 5 different VPs from each experimental group were subjected to electrophoresis, thereafter, the gels were incubated for 22 h in 50 mM Tris-HCl buffer, pH 8.4, containing 5 mM of $CaCl_2$ at 37 °C. Finally, the gels were stained with Coomassie Brilliant Blue R-250 (Sigma-Aldrich, St Louis, MO, USA). The gelatinolytic bands of MMP-2 and -9 activities were scanned and analyzed by densitometry. The bands of was analyzed by obtaining the integrated optical density (IOD) of the bands using the software ImageJ. The values were plotted in a histogram showing mean ± standard deviation of IOD for both MMPs, from all experimental groups.

2.8. Statistical analysis

Stereological values of the percentage area of the collagen fibers are expressed as mean ± SD for the numeric data and as median (Q_1-Q_3) for the data in percentage. For gelatinolytic bands of zymography, one-way analysis of variance (ANOVA) was performed to determine whether significant differences existed between the groups ($p < 0.05$) and the Tukey–Kramer multicomparison post-hoc test was employed. A *p*-value of <0.05 was considered significant. The statistical tests were performed using Instat (version 3.0; Graph-Pad, Inc., San Diego, CA, USA).

3. Results

3.1. General features of the experimental groups

All the diabetic rats utilized in this study showed blood glucose levels above 300 mg/dL, along the experimental period. The simultaneous and/or late insulin replacement was effective in reducing the blood glucose levels of the diabetic rats to values close to those of the control group animals (<120 mg/dL) (data already published: Porto et al. 2011).

3.2. Histological and stereological analysis of the collagen fibers

Picrosirius red staining analysis of the ventral prostate from the both ages of control group revealed only few and thin collagen fibers (stained red). The induction of diabetes by STZ markedly increased the area of collagen fibers in the ECM of the VP from insulin-deprived diabetic rats, mainly at age of 80 days. The simultaneous insulin replacement inhibited the accumulation of collagen in the interstitial stroma, while late insulin replacement significantly decreased the collagen deposition in the ECM (Fig. 1, Table 1).

3.3. Type I and type III collagen immunohistochemistry

The immunostaining of type I and type III collagens showed more immunoreactivity in the stroma of the VP from diabetic animals. In contrast, both the control and diabetic rats, treated with insulin (simultaneously or lately), showed moderate immunostaining of COL I and COL III with a similar intensity throughout the prostatic stroma (Figs. 2 and 3).

3.4. Determination of MMP-2 and MMP-9 activity by zymography

The typical clear bands of the gelatinolytic activity of MMP-9 (the proenzyme (92 kDa) and active form (81 kDa) and MMP-2 (proenzyme (72 kDa), intermediate (64 kDa), and active form (59 kDa) were visualized in the VP from all experimental groups at both the ages analyzed (60 and 80 days) (Fig. 4A). Densitometric analysis of MMP-2 and MMP-9 bands showed that all forms of MMP-2 were significant reduced in the rat ventral prostate from diabetic rats at both ages (Fig. 4B). In the animals treated with insulin, both simultaneously and lately, the activity of MMP-2 was restored (Fig. 4B). MMP-9 activity was very low and showed no statistically significant differences among the experimental groups (data not shown).

4. Discussion

Our study provides novel information about the effects of

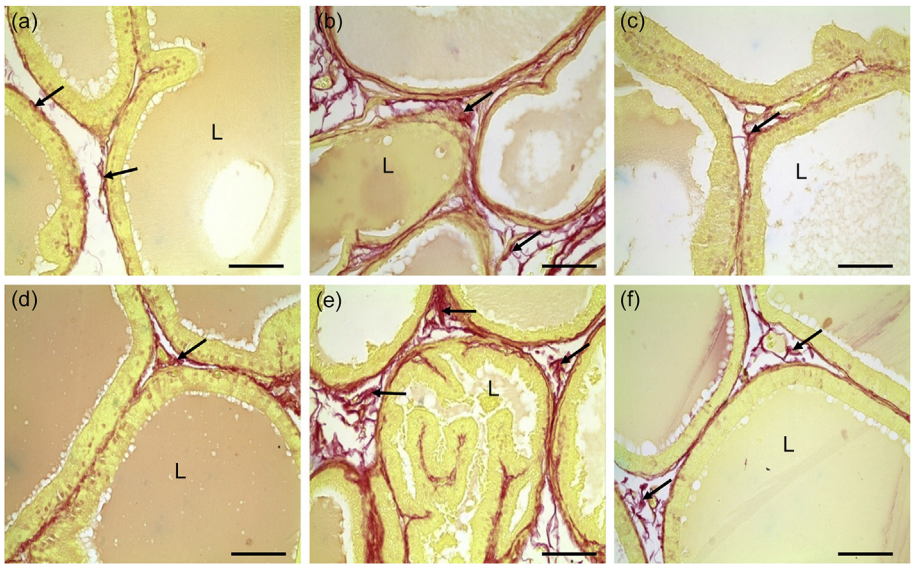

Fig. 1. Representative Picrosirius red stained sections of rat ventral prostates from control day 60 (a), diabetic day 60 (b), diabetic plus insulin treatment simultaneously day 60 (c), control day 80 (d), diabetic day 80 (e), and diabetic plus insulin treatment lately day 80 (f) experimental groups. Collagen fibers (arrows) are observed around acini, mainly in the interstitial space, and appeared thickened in diabetic animals. L, luminal space. Scale bars: a–f = 50 μm.

Table 1
Effect of streptozotocin-induced diabetes and insulin treatment on collagen fibers relative frequency (%) of the rat ventral prostate.

Parameter	DAB	C	D	DI
Collagen fibers Relative frequency (%)	60	4,55 (3,65–5,52)	5,29* (4,46–6,67)	3,36 (3,09–3,61)
	80	4,54 (3,87–6,69)	8,12** (6,51–10,02)	4,36 (2,98–6,96)

Values are expressed in median (Q_1- Q_3). Analyzed by Kruskall-Wallis. *$p < 0.05$ and **$p < 0.01$ when compared with age-matched control. DAB, days after birth; C, control; D, diabetic; DI, diabetic plus insulin.

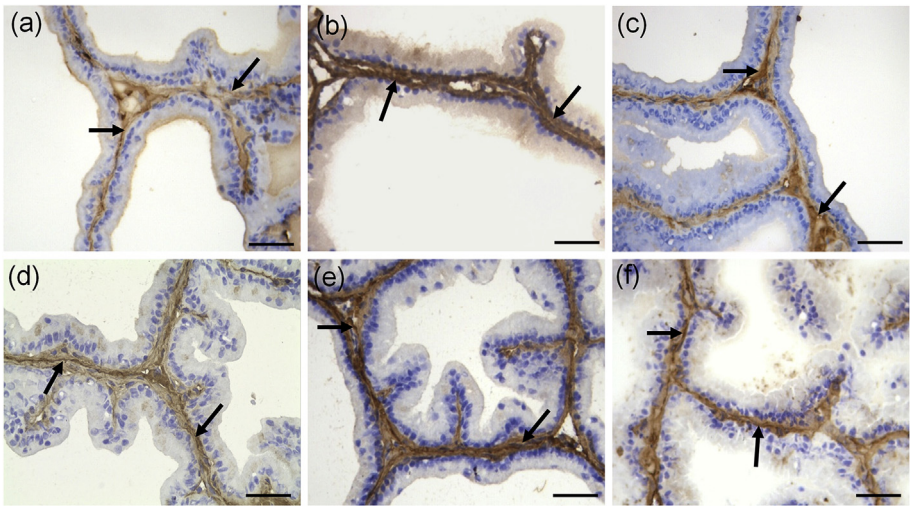

Fig. 2. Representative immunohistochemistry for type I collagen on rat ventral prostate sections from control day 60 (a), diabetic day 60 (b), diabetic plus insulin treatment simultaneously day 60 (c), control day 80 (d), diabetic day 80 (e), and diabetic plus insulin treatment lately day 80 (f) experimental groups. Positive reaction for type I collagen fibers was observed in the around prostatic acini (arrows), exhibiting an increased intensity in the prostate from diabetic rats. Scale bars: a-f = 50 μm.

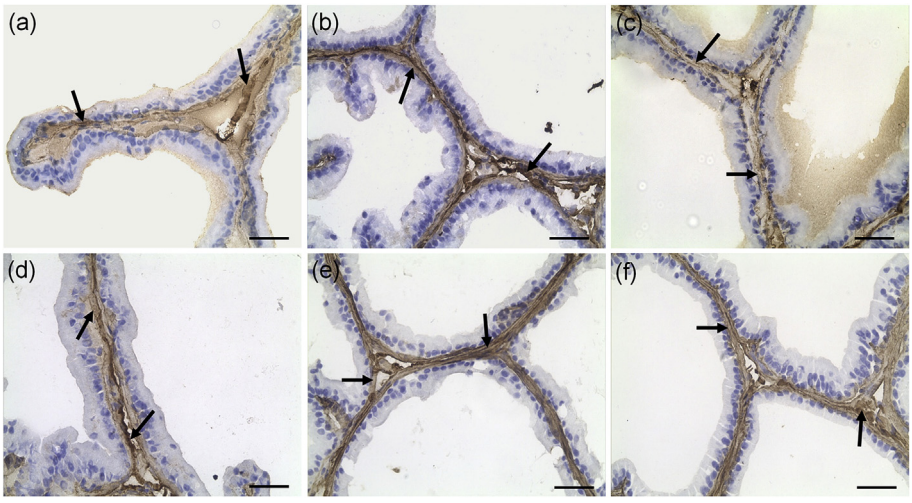

Fig. 3. Representative immunohistochemistry for type III collagen on rat ventral prostate sections from control day 60 (a), diabetic day 60 (b), diabetic plus insulin treatment simultaneously day 60 (c), control day 80 (d), diabetic day 80 (e), and diabetic plus insulin treatment lately day 80 (f) experimental groups. Positive reaction for type III collagen fibers was observed in the around prostatic acini (arrows), exhibiting an increased intensity in the prostate from diabetic rats. Scale bars: a-f = 50 μm.

chronic hyperglycemia during experimental diabetes on the activities of MMPs −2 and −9 during prostate growth in rats at puberty. Our findings demonstrated that the activity of MMP-2, but not MMP-9, decreased and promoted significant changes in the amount of collagen fibers in the ventral prostate stroma from diabetic animals. The density of collagen fibers was significantly increased in the ventral prostate from diabetic animals. The accumulation of collagen observed in the diabetic animals can have an important impact on the behavior of the basal and secretory epithelial cells.

Collagen produced and secreted by a variety of stromal cells, mainly fibroblasts, is the main fibrillar protein of the extracellular matrix in most organs and provides the major structural support necessary for the organization of cells making up the tissue [32]. Studies show that chronic hyperglycemia can lead to fibrosis in different organs, the pathological condition typically characterized by an accumulation of interstitial collagen, as observed in several

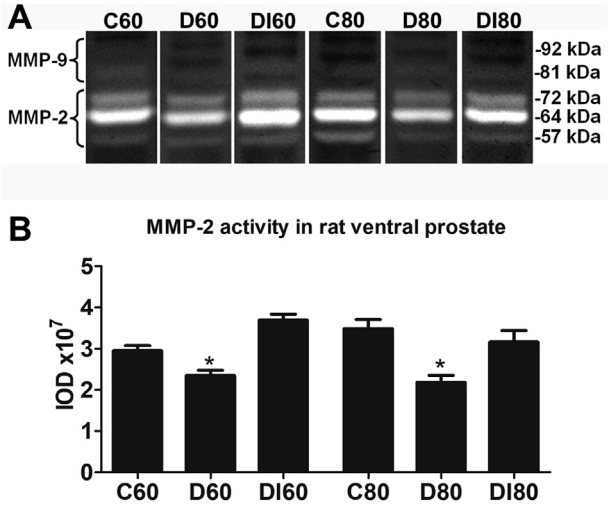

Fig. 4. (A) Representative zymography of the gelatinolytic activity observed in the rat ventral prostate extracts from control day 60 (C60), diabetic day 60 (D60), diabetic plus insulin treatment simultaneously day 60 (DI60), control day 80 (C80), diabetic day 80 (D80), and diabetic plus insulin treatment lately day 80 (DI80) experimental groups. Note high activity of three bands of MMP2 (latent, intermediate and active forms; 72kDa/67kDa/59 kDa, respectively), while very low activity of the two bands of MMP-9 (latent and active forms; 92kDa/81 kDa, respectively) were observed. B) Densitometric values of all bands of MMP-2 were summed and expressed in IOD. MMP-2 activity in the ventral prostates from diabetic groups was significant different from age-matched CT groups. MMP-9 activity showed no alteration (data not showed). * mean significantly different from age-matched CT group with $p < 0.05$.

diabetic complications, such as the micro and macro vascular diseases, retinopathy, nephropathy, and cardiomyopathy [27,33–35]. The above studies support the hypothesis that chronic hyperglycemia, if not controlled, can cause an imbalance in the regulatory factors that control the synthesis and/or degradation of the matrix molecules in various tissues, which may finally result in the development of fibrosis and impaired function of various organs. Thus, the reduced ECM degradation contributed to the increased collagen deposition/accumulation in the VP of diabetic animals in our study.

In this study, the glycemic control achieved by insulin treatment was effective in preventing and/or reversing the alterations in MMP-2 activity and hence the collagen deposition in the VP of diabetic rats. The simultaneous replacement with insulin in the pubertal animals blocked the alterations caused by hyperglycemia in the prostatic stroma. However, the most noteworthy observation was that the changes generated in the rat prostate for a period of 20 days after the induced hyperglycemia were reversed with the 20 days of delayed treatment with insulin.

The mechanism by which hyperglycemia interferes with the synthesis and/or degradation of the ECM molecules is not fully understood, but diabetes is characterized by deep biochemical and metabolic pathway changes, leading to changes in the biochemical properties of the collagen molecule and decreased activity and/or secretion of enzymes that regulate the extracellular matrix [34].

Diabetes is associated with a significant increase in the concentrations of glucose in the body fluids. Glucose and other sugars covalently bind to the macromolecules in the body such as collagen, through a process of non-enzymatic glycosylation [36]. These glycosylation form “advanced glycation end-products” (AGEs), the AGE-modified collagen has been observed in animal and human diabetics [37–39]. Studies suggest that the AGE increases the number of collagen cross-links, thereby making it more resistant to proteolytic degradation by specific enzymes [40].

MMP-2 and MMP-9 (or gelatinases) are responsible for the final degradation of fibrillar collagen after the initial cleavage by collagenases [41]. Our data demonstrate that chronic hyperglycemia in the ventral prostate reduced the activity of MMP-2. We did not observe significant differences in the activity of MMP-9 among experimental groups. The reduction in the activity of MMP-2 in the diabetic animals, therefore, appears to have a direct relationship with the accumulation of collagen in the extracellular matrix observed in these animals. Other authors have also reported prostatic alterations in diabetic animals, such as increase in extracellular matrix elements (collagen and chondroitin sulphate) associated with increased inflammation and these authors consider these changes possible precursors of prostatic diseases [42–44].

Together, our results showed hyperglycemia diminished MMP-2 activity, inducing an increased accumulation of collagen fibers in the prostatic stroma, which take places in the deleterious effects of diabetes on prostatic growth. Exogenous insulin administration is effective in preventing and restoring the adverse effects of hyperglycemia on the prostatic pubertal growth, showing how prostatic tissue is markedly dynamic against such metabolic and hormonal imbalance at this age.

Disclosure statement

All authors report no conflicts of interest.

Funding

This paper was supported by FAPESP (Sao Paulo Research Foundation, grant numbers 2006/60115-2 and 06/60116-9); and CNPq (National Council of Scientific and Technological Development, grant number 305391/2014-3).

References

[1] P.C. Marker, A.A. Donjacour, R. Dahiya, G.R. Cunha, Hormonal, cellular, and molecular control of prostatic development, Dev. Biol. 253 (2003) 165–174.
[2] G.S. Prins, O. Putz, Molecular signaling pathways that regulate prostate gland development, Differentiation 76 (2008) 641–659, https://doi.org/10.1111/j.1432-0436.2008.00277.x.
[3] M. Welsh, P.T.K. Saunders, M. Fisken, H.M. Scott, G.R. Hutchison, L.B. Smith, R.M. Sharpe, Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism, J. Clin. Invest. 118 (2008) 1479–1490, https://doi.org/10.1172/JCI34241.
[4] B.G. Timms, L.E. Hofkamp, Prostate development and growth in benign prostatic hyperplasia, Differentiation 82 (2011) 173–183, https://doi.org/10.1016/j.diff.2011.08.002.
[5] S.W. Hayward, G.R. Cunha, The prostate: development and physiology, Radiol. Clin. North Am. 38 (2000) 1–14.
[6] A. Bruni-Cardoso, H.F. Carvalho, Dynamics of the epithelium during canalization of the rat ventral prostate, Anat. Rec. Adv. Integr. Anat. Evol. Biol. 290 (2007) 1223–1232, https://doi.org/10.1002/ar.20591.
[7] P.S.L. Vilamaior, S.R. Taboga, H.F. Carvalho, Postnatal growth of the ventral prostate in Wistar rats: a stereological and morphometrical study, Anat. Rec. Part A Discov. Mol. Cell. Evol. Biol. 288A (2006) 885–892, https://doi.org/10.1002/ar.a.20363.
[8] A. Bruni-Cardoso, R. Rosa-Ribeiro, V.D.B. Pascoal, A.A. De Thomaz, C.L. Cesar, H.F. Carvalho, MMP-2 regulates rat ventral prostate development in vitro, Dev. Dyn. 239 (2010) 737–746, https://doi.org/10.1002/dvdy.22222.
[9] A. Bruni-Cardoso, C.C. Lynch, R. Rosa-Ribeiro, L.M. Matrisian, H.F. Carvalho, MMP-2 contributes to the development of the mouse ventral prostate by impacting epithelial growth and morphogenesis, Dev. Dyn. 239 (2010) 2386–2392, https://doi.org/10.1002/dvdy.22382.
[10] A. Bruni-Cardoso, P.S.L. Vilamaior, S.R. Taboga, H.F. Carvalho, Localized matrix metalloproteinase (MMP)-2 and MMP-9 activity in the rat ventral prostate during the first week of postnatal development, Histochem. Cell Biol. 129 (2008) 805–815, https://doi.org/10.1007/s00418-008-0407-x.
[11] F.K. Delella, L.A. Justulin Jr., S.L. Felisbino, Finasteride treatment alters MMP-2 and -9 gene expression and activity in the rat ventral prostate, Int. J. Androl. 33 (2010) e114–e122, https://doi.org/10.1111/j.1365-2605.2009.00970.x.
[12] L.A. Justulin, H.H.M. Della-Coleta, S.R. Taboga, S.L. Felisbino, Matrix metalloproteinase (MMP)-2 and MMP-9 activity and localization during ventral prostate atrophy and regrowth, Int. J. Androl. 33 (2010) 696–708, https://doi.org/10.1111/j.1365-2605.2009.01016.x.

[13] T.H. Vu, Z. Werb, Matrix metalloproteinases: effectors of development and normal physiology, Genes Dev. 14 (2000) 2123–2133.
[14] L.J. McCawley, L.M. Matrisian, Matrix metalloproteinases: they're not just for matrix anymore!, Curr. Opin. Cell Biol. 13 (2001) 534–540.
[15] C. Amălinei, I.D. Căruntu, R.A. Bălan, Biology of metalloproteinases, Rom. J. Morphol. Embryol. 48 (2007) 323–334.
[16] A. Knapinska, G.B. Fields, Chemical biology for understanding matrix metalloproteinase function, ChemBioChem. 13 (2012) 2002–2020, https://doi.org/10.1002/cbic.201200298.
[17] C.J. Malemud, Matrix metalloproteinases (MMPs) in health and disease: an overview, Front. Biosci. 11 (2006) 1696–1701.
[18] A. Page-McCaw, A.J. Ewald, Z. Werb, Matrix metalloproteinases and the regulation of tissue remodelling, Nat. Rev. Mol. Cell Biol. 8 (2007) 221–233, https://doi.org/10.1038/nrm2125.
[19] F. Artacho-Cordón, S. Ríos-Arrabal, P.C. Lara, A. Artacho-Cordón, I. Calvente, M.I. Núñez, Matrix metalloproteinases: potential therapy to prevent the development of second malignancies after breast radiotherapy, Surg. Oncol. 21 (2012) e143–e151, https://doi.org/10.1016/j.suronc.2012.06.001.
[20] J.-P. Tsai, J.-H. Liou, W.-T. Kao, S.-C. Wang, J.-D. Lian, H.-R. Chang, Increased expression of intranuclear matrix metalloproteinase 9 in atrophic renal tubules is associated with renal fibrosis, PLoS One 7 (2012) e48164, https://doi.org/10.1371/journal.pone.0048164.
[21] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care 37 (2014) S81–S90, https://doi.org/10.2337/dc14-S081.
[22] WHO | Diabetes, WHO. (2017).
[23] K.C. Lewandowski, E. Banach, M. Bieńkiewicz, A. Lewiński, Matrix metalloproteinases in type 2 diabetes and non-diabetic controls: effects of short-term and chronic hyperglycaemia, Arch. Med. Sci. 2 (2011) 294–303, https://doi.org/10.5114/aoms.2011.22081.
[24] D.C. Hardy, J.H. Ross, C.A. Schuyler, R.S. Leite, E.H. Slate, Y. Huang, Matrix metalloproteinase-8 expression in periodontal tissues surgically removed from diabetic and non-diabetic patients with periodontal disease, J. Clin. Periodontol. 39 (2012) 249–255, https://doi.org/10.1111/j.1600-051X.2011.01788.x.
[25] R. Kumari, L.B. Willing, S.D. Patel, K.A. Baskerville, I.A. Simpson, Increased cerebral matrix metalloprotease-9 activity is associated with compromised recovery in the diabetic db/db mouse following a stroke, J. Neurochem. 119 (2011) 1029–1040, https://doi.org/10.1111/j.1471-4159.2011.07487.x.
[26] Y. Searls, I.V. Smirnova, L. VanHoose, B. Fegley, R. Loganathan, L. Stehno-Bittel, Time-dependent alterations in rat macrovessels with type 1 diabetes, Exp. Diabetes Res. 2012 (2012) 1–11, https://doi.org/10.1155/2012/278620.
[27] C. Tsioufis, I. Bafakis, A. Kasiakogias, C. Stefanadis, The role of matrix metalloproteinases in diabetes mellitus, Curr. Top. Med. Chem. 12 (2012) 1159–1165.
[28] E.M. Porto, S.A.D.A. Dos Santos, L.M. Ribeiro, L.M. Lacorte, J.C. Rinaldi, L.A. Justulin, S.L. Felisbino, Lobe variation effects of experimental diabetes and insulin replacement on rat prostate, Microsc. Res. Tech. 74 (2011) 1040–1048, https://doi.org/10.1002/jemt.20991.
[29] L.C. Junqueira, G. Bignolas, R.R. Brentani, Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections, Histochem. J. 11 (1979) 447–455.
[30] J.C. Rinaldi, L.A. Justulin, L.M. Lacorte, C. Sarobo, P.A. Boer, W.R. Scarano, S.L. Felisbino, Implications of intrauterine protein malnutrition on prostate growth, maturation and aging, Life Sci. 92 (2013) 763–774, https://doi.org/10.1016/j.lfs.2013.02.007.
[31] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding, Anal. Biochem. 72 (1976) 248–254, https://doi.org/10.1016/0003-2697(76)90527-3.
[32] I. Stamenkovic, Extracellular matrix remodelling: the role of matrix metalloproteinases, J. Pathol. 200 (2003) 448–464, https://doi.org/10.1002/path.1400.
[33] K.M. Thrailkill, R. Clay Bunn, J.L. Fowlkes, Matrix metalloproteinases: their potential role in the pathogenesis of diabetic nephropathy, Endocrine 35 (2009) 1–10, https://doi.org/10.1007/s12020-008-9114-6.
[34] C.R. Ban, S.M. Twigg, Fibrosis in diabetes complications: pathogenic mechanisms and circulating and urinary markers, Vasc. Health Risk Manag. 4 (2008) 575–596.
[35] Q.Q. Li, S.-Z.S.-Z. Sun, Y.Y. Wang, Y.-J.Y.-J. Tian, M.-H.M.-H. Liu, The roles of MMP-2/TIMP-2 in extracellular matrix remodelling in the hearts of STZ-induced diabetic rats, Acta Cardiol. 62 (2007) 485–491, https://doi.org/10.2143/AC.62.5.2023412.
[36] B.K. Kilhovd, T.J. Berg, K.I. Birkeland, P. Thorsby, K.F. Hanssen, Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease, Diabetes Care 22 (1999) 1543–1548.
[37] M. Aldrovani, A.M. Aparecida Guaraldo, B. de Campos Vidal, Fluorescence, birefringence and confocal microscopy of the abdominal aorta from nonobese diabetic (NOD) mice, Acta Histochem. 109 (2007) 248–254, https://doi.org/10.1016/j.acthis.2007.01.003.
[38] K. Koga, S. Yamagishi, T. Okamoto, Y. Inagaki, S. Amano, M. Takeuchi, Z. Makita, Serum levels of glucose-derived advanced glycation end products are associated with the severity of diabetic retinopathy in type 2 diabetic patients without renal dysfunction, Int. J. Clin. Pharmacol. Res. 22 (2002) 13–17.
[39] D.S. Fosmark, P.A. Torjesen, B.K. Kilhovd, T.J. Berg, L. Sandvik, K.F. Hanssen, C.-D. Agardh, E. Agardh, Increased serum levels of the specific advanced glycation end product methylglyoxal-derived hydroimidazolone are associated with retinopathy in patients with type 2 diabetes mellitus, Metabolism 55 (2006) 232–236, https://doi.org/10.1016/j.metabol.2005.08.017.
[40] E.W. Howard, R. Benton, J. Ahern-Moore, J.J. Tomasek, Cellular contraction of collagen lattices is inhibited by nonenzymatic glycation, Exp. Cell Res. 228 (1996) 132–137, https://doi.org/10.1006/excr.1996.0308.
[41] P. Vihinen, R. Ala-aho, V.-M. Kähäri, Matrix metalloproteinases as therapeutic targets in cancer, Curr. Cancer Drug Targets 5 (2005) 203–220.
[42] D.L. Ribeiro, S.R. Taboga, R.M. Góes, Diabetes induces stromal remodelling and increase in chondroitin sulphate proteoglycans of the rat ventral prostate, Int. J. Exp. Pathol. 90 (2009) 400–411, https://doi.org/10.1111/j.1365-2613.2009.00657.x.
[43] D.L. Ribeiro, E.J. Caldeira, E.M. Cândido, A.J. Manzato, S.R. Taboga, V.H. Cagnon, Prostatic stromal microenvironment and experimental diabetes, Eur. J. Histochem 50 (2006) 51–60.
[44] S. Lee, G. Yang, W. Mulligan, J. Gipp, W. Bushman, Ventral prostate fibrosis in the akita mouse is associated with macrophage and fibrocyte infiltration, J. Diabetes Res. 2014 (2014) 1–7, https://doi.org/10.1155/2014/939053.