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Research Paper

Strontium fructose 1,6-diphosphate alleviates early diabetic testopathy by suppressing abnormal testicular matrix metalloproteinase system in streptozocin-treated rats

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Abstract

Objectives Male hypogonadism is frequently associated with testopathy in patients with type 2 diabetes and in middle-aged males. We hypothesized that abnormal matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in testis have large roles to play in male hypogonadism. It has been found in diabetic rats that a novel compound, strontium fructose 1,6-diphosphate (FDP-Sr), with extra high energy supply, could reverse male hypogonadism by normalizing MMP-9 and TIMPs in the testis. We investigated whether FDP-Sr could be promising in treating diabetic testopathy.

Methods Adult male Sprague-Dawley rats were administered a single dose of streptozocin (65 mg/kg, i.p.) to induce diabetes. The diabetic rats were treated with FDP-Sr in three doses or testosterone propionate in the final four weeks during the eight-week study.

Key findings Serum testosterone, activity of marker enzymes, and mRNA of MMPs and TIMPs and protein of MMP-9 in the testis were detected. After eight weeks, the activity of acid phosphatase, lactate dehydrogenase, succinate dehydrogenase and γ-glutamyl transpeptidase in testis were significantly decreased ($P < 0.01$), accompanied by down-regulated mRNA and activity of MMP-2 and MMP-9 ($P < 0.01$) and upregulated mRNA of TIMP-1 and TIMP-2. Downregulated MMP-9 protein and degenerative changes in histology were predominant in diabetic testis.

Conclusions FDP-Sr or testosterone propionate significantly normalized expression and activity of the MMPs–TIMPs system to attenuate changes in serum testosterone, marker enzymes and histology in testis. Effects of FDP-Sr were dose-dependent and comparable with those of testosterone propionate. By supplying extra energy, FDP-Sr could be promising in treating diabetic testopathy by normalizing abnormal MMP-9 and its endogenous inhibitors in testes.

Keywords male hypogonadism; strontium fructose 1,6-diphosphate; testopathy; type 2 diabetes

Introduction

High prevalence of male hypogonadism (MHG) due to degenerative changes in the testis has been found in patients suffering from type 2 diabetes metabolic syndrome. The patients are usually middle-aged, and the condition results in a compromised quality of life.[1] MHG is commonly associated with a low serum testosterone and erectile dysfunction that results from degenerative testopathy attributed to insults of hyperglycaemia to the testis.[2] Sexual dysfunction and infertility are frequently accompanied with dysfunction of the Leydig and Sertoli cells in diabetic patients.[3,4] Testis dysfunction, referred to as testopathy, can be produced in diabetic animals.[5,6] Oxidative stress and germ cell apoptosis were implicated in this pathologic process, and mechanisms underlying male hypogonadism are complicated with multiple factors, which have not been clarified yet.[7,8] Testosterone-replacement therapy has been effectively applied to provide benefits to testis dysfunction and regular medical checks of the prostate gland is recommended.[1,9]

Matrix metalloproteinases (MMPs), a family of proteins with more than 20 members, exert broad effects on cell migration, proliferation, apoptosis and morphogenesis in varying organs. In testes, activity of MMPs and tissue inhibitors of metalloproteinases (TIMPs) is

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balanced in playing important roles in the course of spermatogenesis by regulating metabolism of the extracellular matrix.[10] Normal expression of MMPs and TIMPs is essential for maintenance of normal function and structure of the testis, and an enhanced testicular TIMP-1 level is actively involved in early postnatal testicular growth.[11] Follicle-stimulating hormone, the initiator of spermatogenesis, activates the Sertoli cells in conjunction with upregulation of MMP-2 production and TIMP-2 gene expression, which critically affect the cytoskeleton within these cells.[12] Normal expression and activity of the MMPs–TIMPs account for a series of transformations of the seminiferous tubules that are necessary for maturation of the germ cells. With these regular changes in epithelial cells of the tubules, germ cells can complete their process of maturation by travelling from the basal to the top layers, and then entering the centre of the lumen. Changes in expression of MMPs and TIMPs significantly affect cultured Sertoli cells, which undergo remodelling in multiple steps in the course of spermatogenesis.[13] In diabetes, morphological alterations and changes in the Sertoli and Leydig cells were reported in association with changes in the extracellular matrix.[14,15]

The high prevalence of MHG creates interest in investigating new medicines for alleviating abnormalities of the testis in diabetes. MHG may be the result of a shortage of energy production in the mitochondria. A new compound has been derived from fructose 1,6 diphosphate (FDP) and is referred to as FDP-Sr (strontium fructose 1,6-diphosphate, see Figure 1). It contains four high energy phosphorylated bonds equivalent to two ATP molecules, with which FDP benefits the myocardium in alleviating ischaemic lesions.[16] The benefits of the compound in alleviating MHG in adenine-treated rats has been reported previously.[17]

We hypothesized that the microskeleton of spermatogenic epithelium of the seminiferous tubule was crucial in maintaining normal testis function. Disturbance in testicular microstructure can be recognized as an early sign of testopathy in diabetic rats. Thus, in this study MMPs and TIMPs in the testis were targeted to explore if FDP-Sr could improve early diabetic testopathy by reversing the abnormal expression of the MMPs–TIMPs system in streptozocin-treated rats.

Materials and Methods

Animals

Male Sprague–Dawley rats (approximately 250 g) were housed at 25°C with a 12 : 12 h day–night cycle, and allowed free access to water. The study conformed to the Guideline of Animal Handling Regulations of the Science and Technology Bureau of Jiangsu Province and the Principles of Laboratory Animal Care published by the US National Institutes of Health.[18]

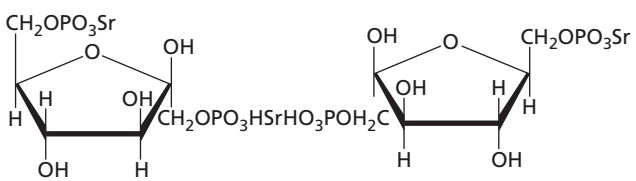

Figure 1 The chemical structure of strontium fructose 1,6-diphosphate.

Protocol

Streptozocin (Sigma, St Louis, MO, US) was administered as a single injection (65 mg/kg, i.p.) to induce diabetes. The fasting blood glucose level was measured on day 7, 14, 21 and 28, and rats with persistent hyperglycaemia over 16.7 mmol/l were considered as diabetic and selected for experimentation. From day 29, subsets of the diabetic rats ($n = 9$) were treated with either testosterone propionate (General Pharmaceutical Company, Shanghai, China; 3 mg/kg, s.c.) or FDP-Sr (at three doses of 50, 100, and 200 mg/kg, p.o.) once daily for four weeks. Rats in the control group were administered the same volume of distilled water. All rats were fed a restricted chow diet, 15 g per day on average, to maximize the survival rate in the untreated rats.

Animals were anaesthetized with urethane to enable blood samples to be taken via a cannula into the common carotid. Serum was separated by centrifugation (4000 rev/min, 4°C, 10 min) and stored at −20°C before use. Testes were harvested and immediately stored in liquid nitrogen after being rinsed with normal saline.

Enzyme activity in the testes

Testis tissue was homogenized in a nine-times volume of ice-cold normal saline, and the homogenates were centrifuged (4000 rev/min, 4°C, 10 min). Samples of supernatants were collected for measurements of acid phosphatase (ACP), lactate dehydrogenase (LDH), succinate-dehydrogenase (SDH), and γ-glutamyl transpeptidase (γ-GT) according to previously described method by using kits from Jianchen Bioengineering Company, Nanjing, China.[19,20]

Serum testosterone level

Serum testosterone level was detected by a radioimmunoassay method with kits provided by DPC Company (Tianjin, China). In brief, samples were incubated with [^{125}I]testosterone and testosterone antiserum at room temperature (21°C) for 24 h, and centrifuged (10 000 g, 4°C) for 15 min. Supernatants were removed and a gamma counter was used to detect the radioactivity of precipitates.

MMP-2, MMP-9, TIMP-1 and TIMP-2 mRNA expression in the testis

Reverse transcription polymerase chain reaction (RT-PCR) was performed according to Tang *et al*.[21] The primer of MMP-2 was sense: 5′-CCCAGAAAAGATTGACGC-3′, and antisense: 5′-CGACAGCATCCAGGTTAT-3′; the primer of MMP-9 was sense: 5′-CGTGGCCTAGTGACCTATG-3′, and antisense: 5′-GGATAGCTCGGTGGTGTCCT-3′; the primer of TIMP-1 was sense: 5′-GCTGCGGTTCTGGGATT-3′, and antisense: 5′-CCTCTGGCATCCTCTTGTT-3′; the primer of TIMP-2 was sense: 5′-GAAGAAAGGAGGTTGCAGT-3′, and antisense: 5′-TCCAGGAAGGGATGTCAAAG-3′; and the primer of GAPDH was sense: 5′-GCT GGG GCT CAC CTG AAG G-3′, and antisense: 5′-GGA TGA CCT TGC CCA CAG CC-3′. The PCR was conducted in 25 μl final volume with the following profile: predenaturation at 94°C for 5 min

before amplification, 40 s denaturation at 94°C; 40 s annealing (MMP-2 at 60°C; MMP-9 at 64°C; TIMP-1 at 60°C; TIMP-2 at 60°C; and GAPDH at 56°C) and extension at 72°C (MMP-2 for 60 s; MMP-9 for 60 s; TIMP-1 for 60 s; TIMP-2 for 60 s; and GAPDH for 40 s). The cycle number for MMP-2, MMP-9, TIMP-1, TIMP-2, and GAPDH was 33, 35, 30, 25, and 30, respectively. The last cycle was followed by a final extension step at 72°C for 10 min. The amplified products were separated by 2% agarose gel and stained with ethidium bromide. Density analysis was performed with a gel image analysis system (UVP, Cambridge, UK). All data were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Zymography assay of MMP-2 and MMP-9

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) zymography was performed according to Gerhards *et al.*[22] with some modifications. In brief, testis tissues were homogenized with the working buffer (50 mmol/l Tris-HCl, pH 7.6, 150 mmol/l NaCl, 5 mmol/l $CaCl_2$, 0.1% (v/v) Triton X-100, 10% (v/v) phenylmethyl sulfonylfluoride), and the homogenates were centrifuged at 12 000*g* for 5 min at 4°C. The supernatants were collected for assay. Protein content of the supernatants was detected by the Coomassie Brilliant Blue method. Samples of supernatants were mixed with loading buffer (400 mmol/l Tris-HCl pH 6.8, 5% SDS, 20% glycerol, and 0.03% bromophenol blue) and electrophoresed through an 8% SDS-PAGE gel containing 0.1% gelatin. After electrophoresis, the gel was washed twice with 2.5% Triton X-100 solution for 30 min each and soaked in incubation solution (Tris-HCl 50 mmol/l, $CaCl_2$ 10 mmol/l, NaCl 200 mmol/l, $ZnCl_2$ 2 mmol/l, pH 7.5) at 37°C for 18 h. The gel was stained with 0.5% Coomassie Brilliant Blue and destained with a solution containing 10% acetic acid and 30% methanol. Photo-density analysis was performed with a gel image analysis system (UVP).

MMP-9 protein expression

Protein expression of MMP-9 was measured by Western blot analysis according to a previous method.[23] Briefly, the testis tissues were homogenized with the lysate (20 mM hydroxyethyl piperazine ethanesulfonic acid, 25 mM NaCl, 2 mM ethylene glycol tetraacetate, 1 mM phenylmethyl sulfonylfluoride, and 0.1% Triton X-100), and the homogenates were mixed with loading buffer and run on SDS-PAGE (10%). The separated proteins were transferred to a nitrocellulose membrane at 200 mA for 1.5 h. The membrane was incubated with polyclonal rabbit anti-MMP-9 antibody, and horseradish peroxidase conjugated second antibody (Boster Bioengineering Company, Wuhan, China). Photo-density analysis was performed with a gel image analysis system (UVP) after staining by diaminobenzidine.

Histological assessment

The testis was fixed in 10% formalin and prepared for slicing and haematoxylin–eosin staining by routine procedures. The appearance and arrangement of the multilayered epithelial cells in the seminiferous tubule and inter-tubular space were evaluated and compared among groups.

Statistical analysis

Data were expressed as mean ± SD. Differences among groups were analysed by one-way analysis of variance, followed by Student–Newman–Keuls or Dunnett's test to compare the difference between two groups. $P < 0.05$ was considered statistically significant.

Results

Serum glucose and testosterone level

Eight weeks following the injection of rats with streptozocin, hyperglycaemia was predominant ($P < 0.01$) relative to the normal control and no blood glucose lowering effect was found with FDP-Sr. (Table 1). With sustained hyperglycaemia the serum testosterone level was low, as compared with the normal control. Testosterone propionate and FDP-Sr at doses of 100 or 200 mg/kg were effective in recovering the serum testosterone level, relative to the untreated diabetic rats (Table 1). In this experiment testosterone propionate, which was an exogenous supply, provided a testosterone level in serum comparable with that resulting from the high dose of FDP-Sr. FDP-Sr had no effect on blood glucose in rat early diabetic testopathy.

ACP, LDH, SDH, and γ-GT activity in testis

Biosynthesis of testosterone is dependent on normal activity of enzymes in the testes and the marker enzymes were assayed to study the testicular activity in diabetic rats. A dramatic decrease in activity of ACP, LDH, SDH and γ-GT was found, relative to the respective normal controls. The reduced enzymatic activity was recovered effectively by FDP-Sr treatment (100 or 200 mg/kg) and testosterone propionate ($P < 0.01$) as compared with the untreated diabetic rats (Table 2). No difference could be found between the efficacy of testosterone propionate and FDP-Sr 200 mg/kg-treated rats.

mRNA expression of MMPs–TIMPs in testis

We conducted gene expression of mRNA of MMP-2 and MMP-9 in the testis to find changes in diabetic rats. Interestingly, downregulation of testis MMP-2 and MMP-9 mRNA was significant in diabetic rats, relative to the respective normal controls (Figure 2). Concomitantly, an increased mRNA expression of TIMP-1 and TIMP-2 was found, relative to normal control. Thus, abnormal expression

Table 1 The effect of strontium fructose 1,6-diphosphate (FDP-Sr) on blood glucose and serum testosterone levels in rat early diabetic testopathy

Group	Dose	*n*	Glucose (mmol/l)	Testosterone (nmol/l)
Control	–	12	7.3 ± 2.3	11.7 ± 2.4
Diabetic	–	9	25.0 ± 5.3**	5.4 ± 0.7**
Testosterone	3	10	20.4 ± 4.9	9.8 ± 1.0‡
FDP-Sr	50	10	21.0 ± 6.1	5.7 ± 0.8
FDP-Sr	100	11	21.6 ± 5.4	6.2 ± 0.9†
FDP-Sr	200	11	23.5 ± 3.7	7.9 ± 1.2‡

**$P < 0.01$ compared with normal control. †$P < 0.05$, ‡$P < 0.001$ compared with the diabetic model.

Table 2 Effects of strontium fructose 1,6-diphosphate (FDP-Sr) on the activity of marker enzymes of the testis in diabetic rats

Group	Dose	*n*	γ-GT	LDH	SDH	ACP
Control	–	12	121 ± 12	2280 ± 526	8.1 ± 1.2	222 ± 21
Diabetic	–	9	65 ± 5.0**	1528 ± 280**	4.0 ± 0.5**	109 ± 26**
Testosterone	–	10	92 ± 12‡	2319 ± 366‡	5.1 ± 1.1†	153 ± 14†
FDP-Sr	50	10	73 ± 8	1700 ± 457	4.3 ± 0.9	124 ± 18
FDP-Sr	100	11	77 ± 13†	1942 ± 562†	5.0 ± 1.0‡	138 ± 24†
FDP-Sr	200	11	87 ± 9‡	2458 ± 285‡	5.4 ± 1.1‡	156 ± 17‡

Values are U/g protein except SDH which is U/ng protein. γ-GT, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase; SDH, succinate dehydrogenase; ACP, acid phosphatase. $^{**}P < 0.01$ compared with normal control. $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$ compared with diabetic model.

of the MMPs–TIMPs indicated maladaptive changes in the extracellular matrix contributing to changes in function and morphology in the diabetic testis. Testosterone propionate and FDP-Sr at doses of 100 and 200 mg/kg were effective in restoring these changes (Figure 2).

Zymography of MMP-2 and MMP-9

We measured activity of MMP-2 and MMP-9 in the testis using zymographic assay. As shown in Figure 3, the activity of latent MMP-2, active MMP-2 and MMP-9 was reduced significantly in diabetic testis, consistent with changes in mRNA expression. Changes in zymography were reversed significantly following treatment with either testosterone propionate or FDP-Sr at 100 and 200 mg/kg (Figure 3).

Protein expression of MMP-9

Consistent with results of mRNA expression and zymographic activity, protein expression of MMP-9 in diabetic testes was reduced dramatically relative to the normal control. Downregulation of protein expression of MMP-9 was completely reversed by administration of either testosterone propionate or FDP-Sr (100 or 200 mg/kg) (Figure 4). FDP-Sr at 200 mg/kg resulted in a greater elevation of the MMP-9 protein level as compared with 100 mg/kg FDP-Sr or testosterone propionate.

Histological examination

In the normal testis the multilayered epithelial cells were in good order and cell columns were closely connected to each other and tightly linked with the basement membrane. The centre of the lumen was rich in spermatozoa. The normal structure was seriously disrupted in diabetic testis, where layers of epithelial cells were less in association, with gaps between the cell columns. Gaps were also observed between the lowest lining of cells and the basement membrane. At the centre of the lumen far fewer spermatozoa were found, leaving a large space. These changes were improved greatly by the high and middle dose of FDP-Sr, but less with the low dose, and benefits were in a dose-dependent manner. Testosterone propionate provided significant improvement in the morphological changes (Figure 5).

Discussion

Hyperglycaemia of eight-weeks duration gave rise to profoundly decreased androgen production in association with compromised enzyme activity of SDH, LDH, ACP, and γ-GT. There were dramatic changes in the histology of the testis, indicating that early testopathy was predominant in streptozocin-treated rats. These enzymes serve as markers of testis function, playing marked roles in the process of spermatogenic epithelium maturation. SDH and LDH supply energy for spermatogenesis through catalysing glucose metabolism.[24,25] Activity of γ-GT closely correlates to reproduction and maturation of Sertoli cells, and ACP is actively involved in the biosynthesis of proteins that are essential for germ cell development.[26,27] A decrease in these enzymes leads to androgen deprivation, which is believed to result from an insufficiency in the energy and protein supply in mitochondria and endoplasmic reticulum for spermatogenesis in the germ cells, which occurs with diabetes.

Testicular oxidative stress has been found in diabetic rats and humans. Abnormality of MMPs and TIMPs is a consequence of testicular oxidative stress, which adversely affects the skeleton of the multi-layered epithelium and cytoskeleton of germ cells.[28,29] In this study we have proved that changes in expression of MMPs and TIMPs of the testis were reversed by either testosterone replacement therapy or FDP-Sr administration, resulting in restoration of the function of Leydig cells and Sertoli cells in the diabetic testis.

FDP-Sr and testosterone propionate did not affect the hyperglycaemia caused by streptozocin injection; however, benefits stemmed from blocking, at least in part, the downstream events. This may have been effective in counteracting the oxidative stress in the testis subsequent to insults from sustained hyperglycaemia. An improvement in the abnormality of the diabetic testis by FDP-Sr 200 mg/kg was significant, and the effectiveness was at least comparable with that achieved by administration of testosterone propionate. The exogenous supply of testosterone directly contributed to the serum testosterone levels, so that a higher serum level resulted. It improved diabetes-induced erectile dysfunction and responsiveness to sildenafil.[30] However, superiority of testosterone propionate over FDP-Sr cannot be concluded when considering the other data. Improvements of protein levels of MMP-9 and morphology in the diabetic testis were more impressive in the FDP-Sr 200 mg/kg group as compared with those in the testosterone propionate group. In general, the efficacy of FDP-Sr is comparable with the testosterone supplement in treating diabetic testopathy.

Function of MMP-2 and MMP-9 is counteracted and balanced by TIMPs. Thus, a harmonious interaction between

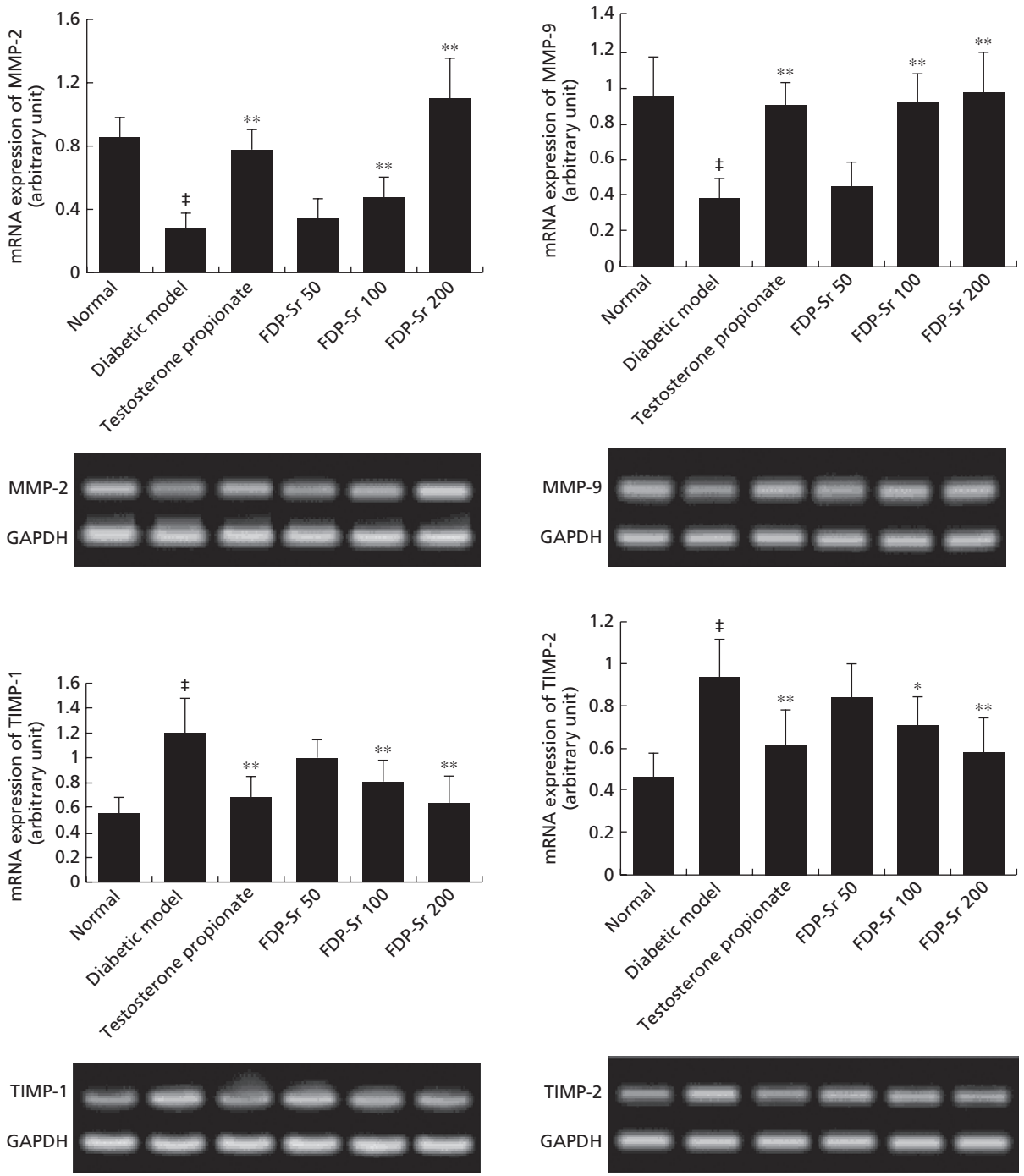

Figure 2 mRNA expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 in diabetic rat testes. In diabetic rat testes, mRNA expression of MMP-2 and MMP-9 was decreased, and that of TIMP-1 and TIMP-2 was increased relative to normal control, which could be restored significantly by either testosterone propionate or strontium fructose 1,6-diphosphate (FDP-Sr) administered at 200 or 100 mg/kg. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Values are mean ± SD, $n = 6$. $^{‡}P < 0.01$ compared with control. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with diabetic model.

MMPs and TIMPs is crucial for normal turnover of the extracellular matrix metabolism in both physiological and pathological conditions.[31] In the testis, the basement membrane is a modified extracellular matrix that contains various proteins such as laminin, type IV collagen, heparan sulphate proteoglycan and entactin. All of them are substrates of MMPs. There is substantial evidence that spermatogenic epithelium undergoes a progressive disruption and remodelling required for maturation of spermatogonia. In the process, activity of MMP-2 and MMP-9 is essential in a balanced manner for changes of the extracellular matrix.[32] Expression of MMP-2 and TIMP-2 in Sertoli cells is modulated

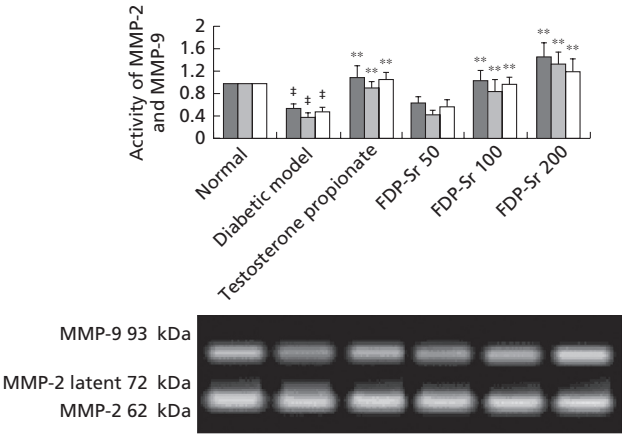

Figure 3 Activity of MMP-2 and MMP-9 in diabetic rat testis tissue. Secretion of active MMP-2 (dark grey), latent MMP-2 (mid-grey) and MMP-9 (white) was reduced significantly in testis tissue of diabetic rats. Testosterone propionate and strontium fructose 1,6-diphosphate (FDP-Sr; 200 or 100 mg/kg) normalized these abnormalities dramatically. Values are mean ± SD, $n = 6$. $^{‡}P < 0.01$ compared with normal control. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with diabetic model.

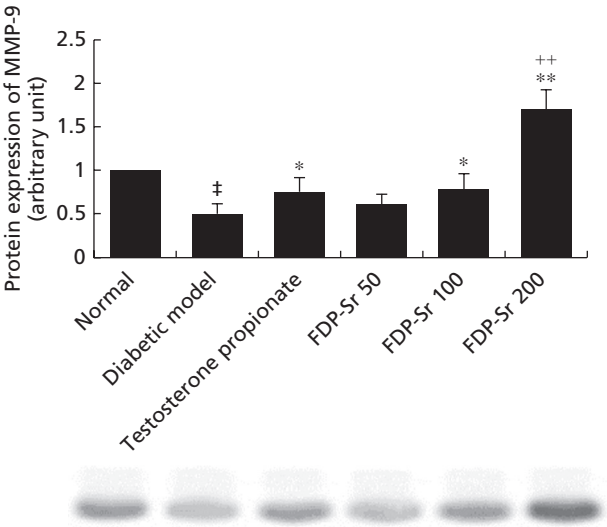

Figure 4 Protein expression of MMP-9 as measured by Western blot analysis. Western blot analysis showed downregulation of protein expression of MMP-9 significantly in diabetic testes, which was improved by testosterone propionate and strontium fructose 1,6-diphosphate (FDP-Sr) administered at 200 or 100 mg/kg. FDP-Sr 200 mg/kg was more effective than testosterone propionate. Values are mean ± SD, $n = 4$. $^{‡}P < 0.01$ compared with normal control. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with diabetic model. $^{++}P < 0.01$ compared with FDP-Sr 100 mg/kg and testasterone propionate.

by the effect of follicle-stimulating hormone.[12] Profound changes in expression of MMP-2 and MMP-9, and TIMP-1 and TIMP-2 are observed, resulting in dramatic changes by remodelling of the seminiferous tubule and decreasing density of the sperm at the centre of the lumen. Indeed, maintenance of normal testicular physiology requires modulation of Sertoli cells, affected by paracrine factors; among them a normal level of endothelin-1 (ET-1) is critical for spermatogenesis, and downregulation of the ET system may account for serious changes in testopathy caused by adenine.[17,33,34] In this process, paracrine factors, including reactive oxygen species (ROS) and ET-1, modulate the signalling pathway by altering expression and activity of MMP-2, MMP-9, TIMP-1 and TIMP-2 in the testis.

FDP-Sr, containing four high energy phosphorylated bonds in its moiety, shares pharmacological properties with FDP, which alleviates ischaemic lesions and shock, myocardial infarction and oxidative stress in the myocardium.[35,36] Damage in different types of cells can be

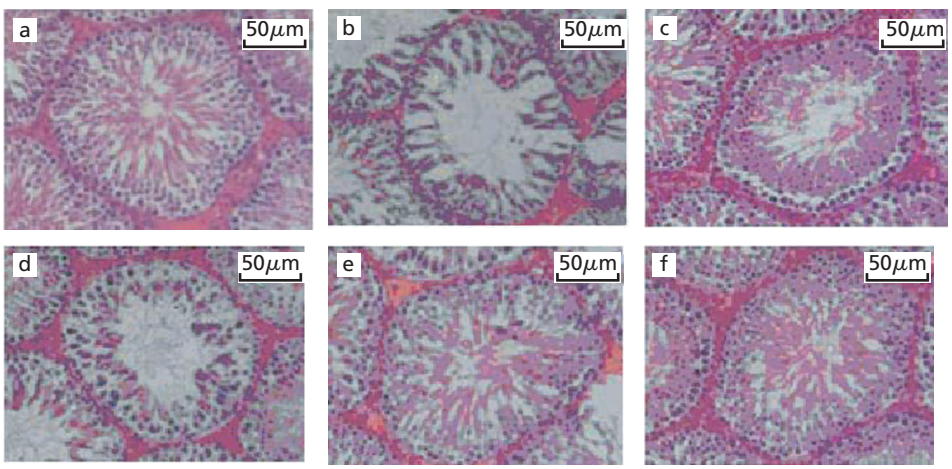

Figure 5 Histological examination of testis of normal and diabetic rats. The representative histological changes (200×) of testis are shown in diabetic testes. In diabetic testis seminiferous tubule we found decreased layers of columns of germ cells, a large gap between cell columns and a gap at the connection between germ cells and the basal membrane. There were far fewer spermatozoa, leaving a cavity at the centre of the lumen. These changes in the diabetic testis were significantly different from normal and were reversed by FDP-Sr and testosterone propionate. In alleviating morphological changes FDP-Sr at 200 mg/kg (f) was superior to testosterone propionate (c). a, Control; b, diabetic; c, testosterone propionate (s.c.); d, FDP-Sr 50 mg/kg (p.o.); e, FDP-Sr 100 mg/kg (p.o.); and f, FDP-Sr 200 mg/kg (p.o.).

caused by chronic exposure to elevated glucose and fatty acid concentrations in diabetes that is referred to as 'glucolipotoxicity', and insults in diabetes are always linked to an excess of ROS produced in mitochondria. Deficient energy supply due to glucolipotoxicity in mitochondria may be a causal factor contributing an important role in producing cellular damage in diabetes.[37] Then, NADPH oxidase-dependent generation of ROS in diabetes is enhanced and an excess of mitochondrial ROS generation impairs metabolism in testes and distorts parameters of signal transduction, resulting in maladaptive responses in the diabetic testis. Degenerative changes in mitochondria could be attributed to a defect in energy supply and in turn insults from ROS, and could also occur in other diseases such as Alzheimer's disease.[38] Abnormal mitochondria relating to oxidative stress can be found in diabetes and ageing, and correlate with a lack of energy production. These changes are likely to correlate with changes in endoplasmic reticulum that biosynthesizes unfolded proteins without normal activity, which serve as a basis of endoplasmic reticulum stress involved in diabetes.[39,40] Thus, a shortage of energy supply in the mitochondria in the diabetic testis could be effectively relieved by an extra supplement of high energy phosphorylated bonds, leading to recovery of testopathy in diabetic rats. It is conceivable that mitochondria may benefit from an extra ATP provided by FDP-Sr, which reduces ROS production, and thus a correction of the imbalance in MMPs and TIMPs in the testis could be achieved. In fact, FDP-Sr itself was effective in removing ROS in diabetic testis.[28]

FDP-Sr supplies extra high energy phosphorylated bonds and this favourably slows down the degenerative processes of the testis in the middle-aged and ageing population. The benefits can also be found in patients with metabolic syndrome and type 2 diabetes, by improving the quality of life, mental depression, decreased libido, erectile dysfunction and compromised cardiovascular activity. A potential hazard of testosterone replacement therapy is the possibility of serious adverse effects such as prostate cancer. The patients require monitoring, including regular digital rectal examination and prostate-specific antigen tests. In contrast to this, FDP-Sr is less toxic without the severe adverse effects.[41] Currently, the development of new drugs for the treatment of male hypogonadism is still mainly focused on the androgen system, activation of its receptors and the feedback system between the testes and pituitary.[42] Treatment with testosterone provides not only an exogenous supply to replace the missing hormone but also benefits from correcting maladaptive reactions due to deficiency in the male hormone.[43] Thus, it is still the main procedure in the treatment of male hypogonadism, as it has been for the past few decades.[44] However, the effectiveness of FDP-Sr in the testis was comparable with that of testosterone. FDP-Sr could offer an additional improvement to a patient's quality of life, and also has potential in treating osteoporosis, as hypogonadism may also contribute to changes in bone density.[45]

Conclusions

Diabetes is associated with abnormal expression of MMPs and TIMPs leading to testopathy. FDP-Sr significantly reversed the impaired testis in diabetes by supplying extra energy to affected mitochondria, therefore normalizing the MMP–TIMP system. Its effectiveness in the testis was comparable with that of testosterone. The supplement of energy to mitochondria by FDP-Sr in relation to an improvement in testopathy requires further investigation.

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Qi Zhang and Hao-Ran Liu contributed equally to this paper and both serve as co-first author.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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