Research and Reviews: Orthopedics

The Effect of Matrix Metalloproteinase Inhibition on the Shoulder Joint in Diabetic Rats

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Research Article

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ABSTRACT

Objective: The aim of this study was to reveal the effect of doxycycline related inhibition of the matrix metalloproteinase (MMPs) on the shoulder joint of the diabetic rats.

Material and methods: 36 adult Sprague-Dawley male rats were used. To introduce a diabetes, 35 mg/kg intra-peritoneally streptozotocin were applied 24 rats. Control group rats (n=12) received 35 mg/kg an intraperitoneal injection of serum physiologic. 72 h later, doxycycline was applied to diabetic group rats (n=12) and control groups rats (n=6) via orogastric tube 130 mg/kg per day during the two weeks. Diabetic control group rats (n=12) and sham group rats (n=6) received 130 mg/kg serum physiologic via orogastric tube. Rats were sacrificed at three weeks. Consequently histologic analysis was done.

Results: The mean blood glucoses level 89.8 ± 10.6 on the first day of the study, the mean blood glucoses level 414.7 ± 69.4 72 h after streptozotocin administration (t test; $p<0.01$). Histological evaluations exposed preserved joint space and were not identified inflammation, increased vascularity and cartilage degeneration with the Haematoxylin-eosin stained. Fibrosis evaluated with Masson's trichrome stain and type I and type III collagen proportion evaluated with Picrosirius red-stain; no differences were found (Chi-square test; $p>0.01$).

Conclusion: Streptozotocin administration was determined to be an effective experimental model in rats to induce diabetes. Sacrification time not sufficient to reveal the changes in shoulder joint capsule due to the diabetes and the effect of doxycycline. Further studies including larger groups are needed to define the mechanism of the MMPs inhibitors in the shoulder joint in chronic diabetic rats and in the generation of new clinical applications in orthopedics.

Level of evidence: Level 1, experimental study.

INTRODUCTION

Diabetes mellitus is one of the most common and devastating diseases. Unfortunately, diabetes is accompanied by a numerous systemic complications including almost every tissue in the body. Diabetes mellitus is known to cause a wide-range of musculoskeletal disorders, including joint stiffness, tendon contracture and synovitis. During the diabetic process, glycosylation of the protein construction results in variations in the structure of collagen [1]. The shoulder joint is one of the commonly affected sites. Adhesive capsulitis is a well-documented shoulder disease in diabetic patients. Both clinical and basic studies have revealed that diabetes is related with shoulder joint stiffness. It is considered that these complication consequences alter the amount and function of the structural macromolecules of the extracellular matrix in the connective tissue [2,3].

Matrix metalloproteinases (MMPs) are zinc-dependent proteinases that provide the reparative and degenerative processes and maintain homeostasis of the extracellular matrix in the connective tissue [4]. They are expressed in the normal tissue and

regulate the turnover of the connective tissue matrix. The overexpression of MMPs leads to the increased breakdown of connective tissue and a possible enhancement in connective tissue pathologies [5].

Tetracycline group antibiotics (for example, doxycycline) have been documented to inhibit MMPs activity by a mechanism that is independent of their antimicrobial activity. It has also been suggested that tetracycline would limit matrix degeneration by decreasing the MMPs [6]. MMPs inhibition may improve the structure of collagen. It has been shown in an experimental study that doxycycline can improve the connective tissue healing by inhibiting MMP enzymes [7].

The aim of this study was to reveal the effect of doxycycline-related inhibition of the MMPs on the shoulder joint of the diabetic rats. We hypothesized that MMPs inhibition improve the connective tissue healing therefore improve the structure of collagen of the shoulder joint in diabetic rats. MMPs inhibition via doxycycline minimalizes the negative effect of the diabetes in the shoulder joint.

MATERIALS AND METHODS

Study design

This study was approved by the Başkent University Ethical Committee for Experimental Research on Animals and supported by Başkent University Research Fund.

Thirty six adult male Sprague-Dawley rats with a mean age of 12 months and mean body weight of 383 ± 42 g were included into this study. Rats were acclimatized to the laboratory conditions for 1 week and were allowed to feed on standard rat diet and tap water *ad libitum*. The room temperature and humidity were maintained at 20-24°C and at 50-60%, respectively. The light:dark cycle was fixed at 12:12 h.

In order to induce diabetes, 35 mg/kg streptozotocin (Sigma, St Louis, MO) was injected intraperitoneally to 24 rats. Induction of diabetes was verified by measuring blood glucose levels in the rats before and after streptozotocin injections. Rats with a blood glucose level ≥ 250 mg/dl were accepted as diabetic and were included in the diabetic group. Blood glucose levels were checked for 3 days in all rats to confirm the maintenance of the euglycemic and hyperglycemic state. Sham group (n=12) received intraperitoneal saline injections of the same volume with their counterparts.

72 h later, doxycycline treatment (130 mg/kg) was applied through the oral route to the diabetic group (n=12) and the control rats (n=6) via an orogastric cannule for two weeks. Diabetic control group (n=12) and sham group rats (n=6) received the same volume of saline through the same route for the same period.

Finally, all rats were sacrificed with intraperitoneal thiopental injection (200 mg/kg) at the end of the third week. Consequently histological analysis was performed. The study design is demonstrated in **(Figures 1A and 1B)**.

Histological evaluation

For histology, whole shoulders were left entirely intact. Right shoulder joints were carefully dissected. Tissue samples were fixed in 10% neutral buffered formalin and then dehydrated with alcohol. The fixed tissue was managed, inserted in paraffin, and longitudinally sectioned at 7 µm. Consequently, tissue sections were stained by haematoxylin-eosin according to standard protocols for the assessment of joint space, capsular thickness of the axillary recess, inflammation, vascularity, cartilage degeneration. To investigate fibrosis, Masson's trichrome stain was used. Type I and type III collagen proportion was evaluated by Picrosirius red-stain.

For each specimen two slides were prepared. Slides were morphologically examined by the same pathologist under light microscope (Olympus Bx50) and were read blind.

Statistical analysis

Data were evaluated using SPSS 17.0 software (SPSS Inc., Chicago, IL). Descriptive statistics were calculated including frequency, mean and standard deviation, minimum and maximum values. The *t*-test was used to ascertain the differences blood glucose level. Histological findings were analyzed by chi-square test. Statistical level of significance was set at 0.01.

RESULTS

The mean blood glucose levels were 89.8 ± 10.6 mg/dl on the first day of the study in the rats while the mean blood glucose levels were 414.7 ± 69.4 mg/dl; 72 h after streptozotocin administration in the diabetic group rats ($p<0.01$) **(Table 1).**

The glenohumeral joint cavity could be observed in the serial histological sections. No macroscopic difference was noted in the diabetic and control rats. However we could not observe any hyperplasia in the capsule at the 3rd week, nor could detect any joint adhesion **(Figure 2).**

No inflammation, vascularity and cartilage degeneration was identified in the shoulder joint with the Haematoxylin-eosin stain in control group (control doxycycline and sham groups). Light microscopic evaluation with Haematoxylin-eosin stain did not reveal any difference between the diabetic groups (diabetic and diabetic+doxycycline-treated rats; $p>0.01$). **Figure 3** demonstrates the histological findings from the diabetic+doxycycline-treated rats.

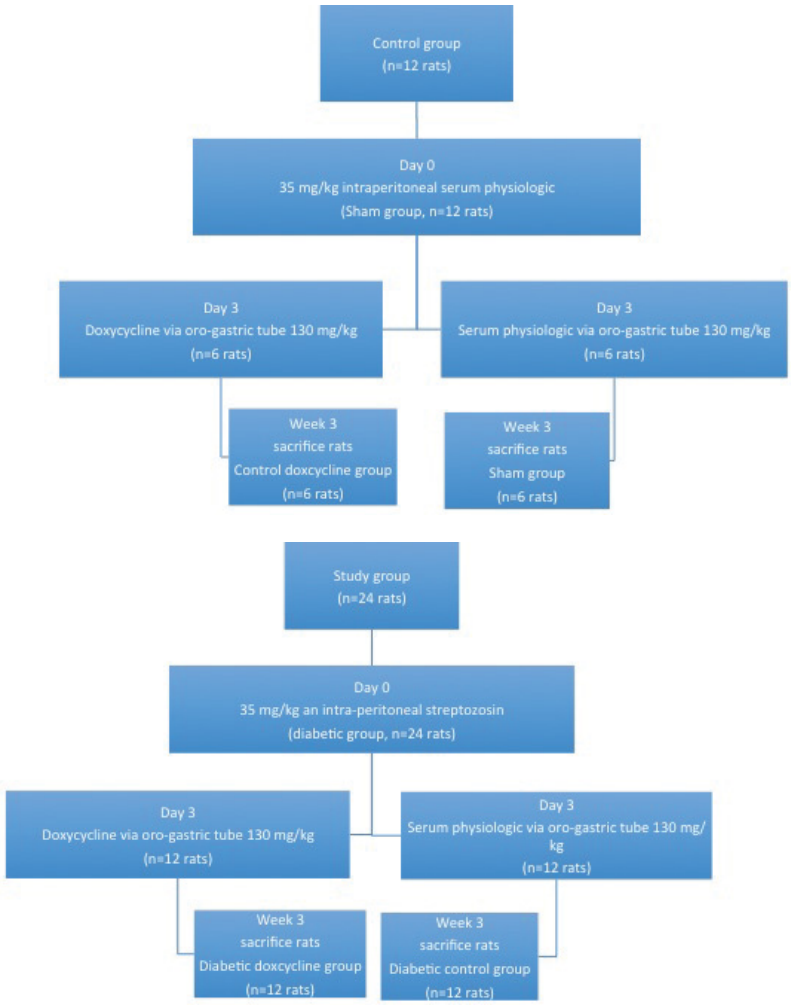

Figure 1: A and B: Study design in diabetic and control groups, respectively.

Table 1. Blood glucose levels.

Before streptozotocin (all rats)	72 h after streptozotocin (diabetic groups rats)	P value
89.8 ± 10.6 (73-111)	414.7 ± 69.4 (308-582)	*P<0.01
Blood glucose level mean ± standard deviation (minimum-maximum) mg/dl, *t test		

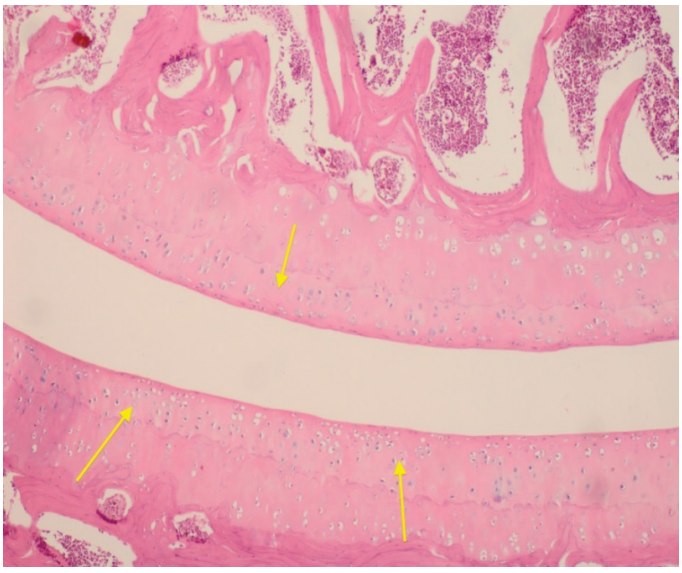

Figure 2. Sample microscopic view from a diabetic rat. No adhesion and capsular thickness. Yellow arrow indicates the joint line (Haematoxylin-eosin stained x100).

Fibrosis was evaluated with Masson's trichrome stain **(Figure 4).** Distribution of collagen fiber types I and III was evaluated with Picrosirius red-stain **(Figure 5).** No differences were found between the groups (p>0.01).

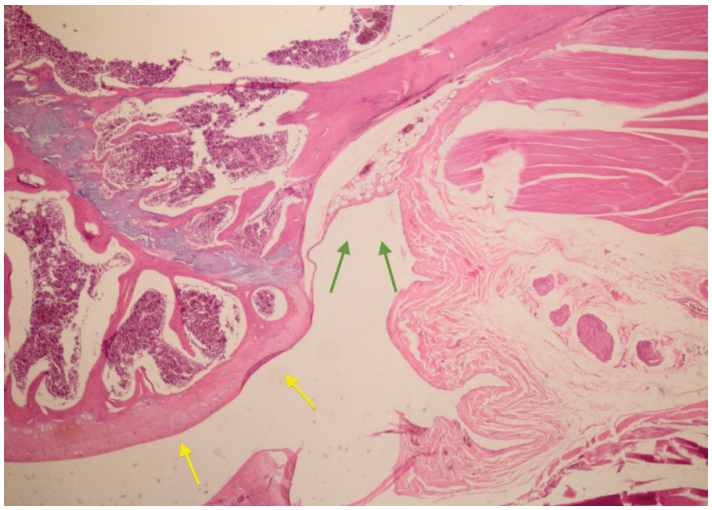

Figure 3. Sample microscopic view from a diabetic rat. Yellow arrows indicate the chondral surface of the humerus head, green arrows indicate the axillary recess (Haematoxylin-eosin stained x40).

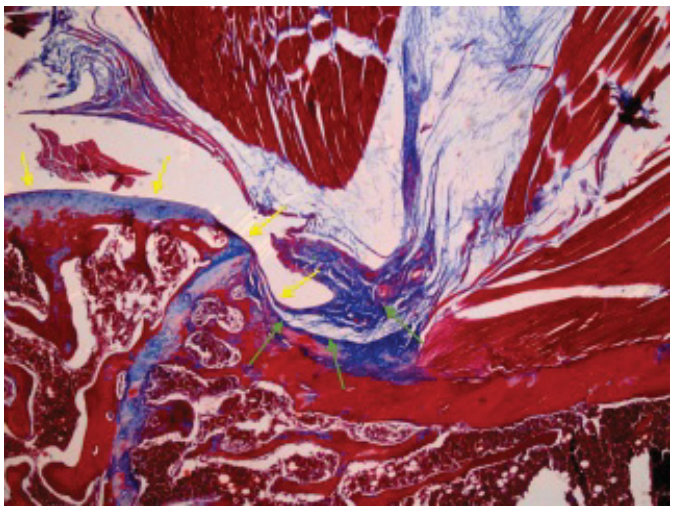

Figure 4. Sample microscopic view from a diabetic rat. Disorganized collagen fiber orientation appears blue color; yellow arrows indicate the chondral surface and green arrows indicate the axillar recess (Masson-Trichrome stained x100).

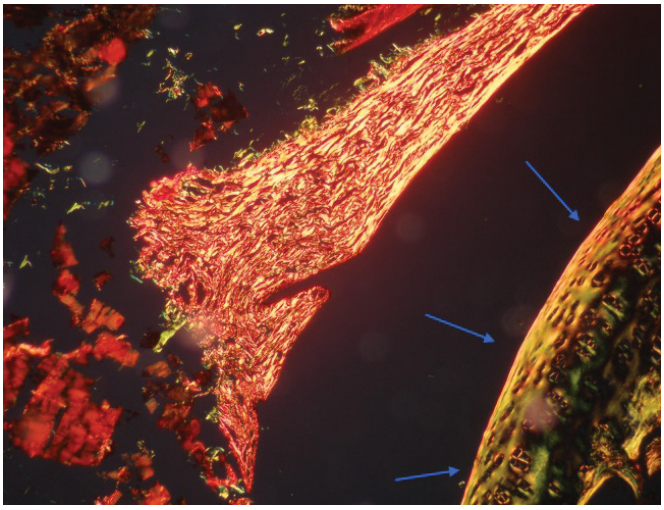

Figure 5. Picrosirius red-stained section of the shoulder joint from a diabetic rat. Type I collagen appears in orange while type III collagen appears in green; blue arrows indicate the joint surface (Picrosirius red-stained x200).

DISCUSSION

It has been reported that diabetic patients are more likely to have painful and stiff shoulder than do healthy people [8]. However, the reason for this condition has not been established yet [9]. Alteration in the collagen structure due to the glycosylated collagen proteins may cause changes in the extracellular matrix in diabetic patients. Accumulation of the final products due to the

advanced glycosylation may lead to cell damage and alterations in the extracellular matrix components. These mechanisms may underlie the connective tissue-related complications in the diabetics [10].

Decreased collagen content and defects in collagen cross-linking have been observed in experimental studies in streptozotocin-induced diabetic rats [11,12]. Bedi et al. have reported that collagen fiber organization is significantly reduced in diabetic rats [13]. These findings may have important clinical implications for the diabetic patients. Thomas et al. have demonstrated in diabetic rats that 8 weeks of hyperglycemia leads to a decreased external rotation but no other mechanical changes in the uninjured shoulder joint. In addition, they have also shown that tumor necrosis factor alpha increases in the superior region of the capsule, and concluded that hyperglycemia does not diminish the mechanical functions of the shoulder while inducing a chronic inflammatory response [14]. Rodeo et al. have reported that patients with adhesive capsulitis have an increased expression of the inflammatory markers within the joint capsule [15].

We observed that intraperitoneal injection of streptozotocin is an appropriate experimental model to induce diabetes in Sprague-Dawley rats. All of the rats assigned into the diabetic groups had elevated levels of blood glucose 3 days after the injection. Diabetic rats were sacrificed on the 3rd week. However, histopathological findings of the present study elicited that 3 weeks of hyperglycemic status was not enough to induce changes in the connective tissue structure. It is well known that average laboratory rats live for approximately 3 years. In adult rats, every month of the life span approximately equals to 2.5 human years [16]. Although the rat shoulder has been demonstrated to be the most anatomically similar to humans shoulder [17], there is not any animal model resembling human diseases perfectly. Thus, in the present study streptozotocin-induced diabetes model probably failed to produce the chronic effects of this longstanding disease in the shoulder joint exactly.

Extracellular matrix exhibits a dynamic organization, it is constantly being remodeled. MMPs are crucial for the development and maintenance of the healthy tissue because of their ability to remodel the extracellular matrix [18]. The balance between MMPs and their inhibitors is important to maintain healthy connective tissue [4,19]. Loss of this balance resulting from the enhanced activity of the MMPs has been associated with various pathological conditions including orthopedic joint diseases, a condition in which the collagen in the cartilage, bone and tendons surrounding the joints is progressively and irreversibly degraded [20-23].

Doxycycline, a tetracycline, inhibits MMPs activity by a mechanism that is independent of its antimicrobial activity. Doxycycline-mediated inhibition of the MMP-13 favorably influences tendon-to-bone healing. Up-regulated MMP-13 expression reflects increased degradation of matrix proteins. MMP-13 plays an important role in the regulation of tendon extracellular matrix degradation and tissue remodeling. *In vivo* and *in vitro* studies have demonstrated that tetracycline group antibiotics reduce the cartilage and tendon degeneration that is associated with increased MMP activity [2,21,24,25].

Researchers defined that MMP inhibitors may reduce pathologic tissue degradation and favorably influence healing after rotator cuff repair [7,26]. Bedi et al. investigated the effect of MMP inhibition after rotator cuff repair in a rat model and reported that MMPs are most active at the immediate postoperative period. They demonstrated that MMP activity returned to basal levels and was less susceptible to inhibition by doxycycline at the 4 weeks postoperatively [21].

The objective of the present study was to determine the effect of doxycycline in the shoulder joint in a diabetic rat model. However, we could not demonstrate any histopathological difference in the shoulder joints of the doxycycline-treated diabetic rats. Our study had several limitations. Our main limitation, it seems that the length of diabetes (3 weeks) is clearly not enough to induce any negative effects on the shoulder joint. This time should be longer to show any negative effect of the shoulder joint. Another limitation, we applied doxycycline only for 2 weeks. Time-dependent activity of the MMP inhibitors could be studied in larger groups.

CONCLUSION

In conclusion, despite the considerable correlative evidence relating extracellular matrix glycation to diabetes, little is known of how extracellular modification. Biological modulation of the activity of endogenous MMPs to basal levels may reduce pathological tissue degeneration in the diabetic shoulder joint in humans. Further studies with larger groups are needed to define the mechanism(s) of MMP inhibitors in shoulder joint pathologies due to chronic diabetes.

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