Original Article
Morphological changes and expression of MMPs and TIMPs in rabbit degenerated lateral meniscus after PCL-transection

Pengfei Lei1*, Rongxin Sun2*, Kanghua Li1, Yihe Hu1, Zhan Liao1
1Department of Orthopedics, Xiangya Hospital, Central South University, Changsha 410008, Hunan, China; 2Department of Orthopedics, The Sixth Affiliated Hospital of Xinjiang Medical University, China. *Equal contributors.
Received June 19, 2015; Accepted October 9, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: Introduction: Whether the expression level of MMP-1, MMP-13 and TIMP-1 has association with the degeneration of lateral meniscus after posterior cruciate ligament (PCL) fracture is poorly understood. The aim of this study was to investigate the influence of PCL fracture on lateral meniscus, including morphological changes, histological changes and roles of matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-13 (MMP-13) and tissue inhibitor of metalloproteinase-1 (TIMP-1) expression level in the secondary injury. Materials and Methods: Sixty male rabbits were used as PCL transection models and randomized into the PCL-transection side, which underwent PCL transection surgery, and the control side, which underwent PCL exposure without transection. On 4, 8, 12, 16 and 24 weeks after PCL-transection, 12 rabbits were randomly killed for H&E staining to determine the histological changes of lateral meniscus. Immunohistochemical staining was undertaken to evaluate the expression level of MMP-1, MMP-13 and TIMP-1 in lateral meniscus. The results were statistically analyzed using SPSS 15.0. Results: The lateral meniscus of PCL-transection side presented abnormal morphology. Histological evaluation score of meniscal degeneration in PCL-transection group was higher than that in the control group with statistical difference (P < 0.05). The expression levels of MMP-1, MMP-13 and TIMP-1 were significantly elevated in meniscus of the PCL-transection group with statistical difference (P < 0.05). MMP-1 expression displayed an increasing trend firstly then kept stable after PCL transection; MMP-13 and TIMP-1 expression displayed high level firstly then decreased in advanced stage after PCL transection. Conclusions: PCL transection may induce a coordinated response of degeneration of lateral meniscus in a time-dependent manner. The high expression level of MMP-1, MMP-13 and TIMP-1 would contribute to the degeneration of lateral meniscus after PCL transection.

Keywords: PCL-transection, lateral meniscus, MMP-1, MMP-13, TIMP-1

Introduction

Posterior cruciate ligament (PCL) is the strongest ligament in the knee and its total tear results in posterior translation of the tibia as well as increased strain on the medial femoral condyle and posterolateral structures [1]. The PCL of human knee plays an important role in controlling and stabilizing knee joint but has a poor healing after injury. It is reported that an isolated PCL injury accounts for approximately 17% of all knee injuries [2, 3]. When PCL is transected, menisci have to compensate to maintain normal function of the knee, which is likely lead to menisci injury [4]. Moreover, PCL deficiency may result in knee instability, pain and even progressive degeneration of other intra-articular tissues like cartilage and meniscus, which are known risk factors for osteoarthritis development [5].

However, whether PCL injury would induce molecule changes in meniscal tissues is poorly understood. Tissue remodeling occurs continuously in both normal and injured tissues. In this process, old or damaged structures are degraded and replaced with newly synthesized molecules [6]. The balance between the degradation and biosynthesis of this process is controlled by the activities of matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) [7]. Previous studies have established close relationship between connective tissue damage/degeneration and
Research on meniscus after PCL-fracture

Table 1. Scoring system of lateral meniscus histology

<table>
<thead>
<tr>
<th>Histological characteristics</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface layer</td>
<td></td>
</tr>
<tr>
<td>Smooth</td>
<td>0</td>
</tr>
<tr>
<td>Rough</td>
<td>1</td>
</tr>
<tr>
<td>Local dent</td>
<td>2</td>
</tr>
<tr>
<td>Fissure or breakage</td>
<td>3</td>
</tr>
<tr>
<td>Cartilage cells</td>
<td></td>
</tr>
<tr>
<td>Oval or fusiform, orderly arrangement, big nucleus</td>
<td>0</td>
</tr>
<tr>
<td>Oval or fusiform, less orderly arrangement with diffusivity, big nucleus</td>
<td>1</td>
</tr>
<tr>
<td>Irregular shape, disorderly arrangement, big nucleus</td>
<td>2</td>
</tr>
<tr>
<td>Disorderly arrangement, less cells, vacuolus cell</td>
<td>3</td>
</tr>
<tr>
<td>Rare cells</td>
<td>4</td>
</tr>
<tr>
<td>Collagen fibers</td>
<td></td>
</tr>
<tr>
<td>Thick, orderly arranged and compact</td>
<td>0</td>
</tr>
<tr>
<td>Thick, orderly arranged and loose</td>
<td>1</td>
</tr>
<tr>
<td>Unevenly thick, orderly arranged and loose</td>
<td>2</td>
</tr>
<tr>
<td>Unevenly thick, disorderly arranged and loose</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Morphological characteristics of lateral meniscus

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>PCL-rapture group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All time points</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Structural integrity</td>
<td>Integrated</td>
<td>Integrated</td>
</tr>
<tr>
<td>Surface</td>
<td>Smooth</td>
<td>Smooth</td>
</tr>
<tr>
<td>Color</td>
<td>Bright white</td>
<td>Gray-white</td>
</tr>
<tr>
<td>Elasticity</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>

the expression of MMPs and TIMPs [8-10]. This research about the expression of MMPs and TIMPs in meniscus tissues induced by PCL transaction may improve our understanding of meniscus degeneration induced by PCL deficiency and development of osteoarthritis [11].

However, it is poorly understood whether the expression level of MMP-1, MMP-13 and TIMP-1 has association with lateral meniscus degeneration after PCL transection. In this study, we focused on the meniscus tissue structure changes of rabbit knee joint and the expression level of MMP-1, MMP-13 and TIMP-1 and the association between them. It may contribute to better understand pathological changes in the lateral meniscus following transection.

Methods

Animals

A total of 60 adult male rabbits (body weight, range 2.6 ± 0.4 kg) from the Animal Center of Central South University were used in the present study. All animal experiments were carried out in accordance with animal welfare guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) of Central South University (Approval No. 20130028). Animals were caged with free access to food and water under a 12-h light-dark cycle, relative humidity of 55 ± 5% and temperature of 21 ± 3°C. The knee joints of each rabbit were randomized into PCL-transection side which subjected to PCL transection and the other side subjected to PCL exposure without transaction [29]. Every twelve rabbits were killed on 4, 8, 12, 16 and 24 weeks after PCL transection for the experiments described.

PCL transection model

Rabbits were anesthetized with 3% pentobarbital (0.03 mg/kg) and placed on a sterilized tray. Preoperatively, the drawer test was performed to examine knee stability. A medial incision was made to expose the patella which was dislocated to access the PCL for transection. Push and dislocate the kneecap, and then transect the PCL with scissors. After PCL transection was confirmed by the drawer test and hemostasis, articular cavity was washed with 3% aque hydrogen dioxide and normal saline alternative-ly. The incision was closed. As for the control knee, the surgery was performed without PCL.
Research on meniscus after PCL-fracture

transection. The weight and injury condition of all rabbits were recorded every day. Rabbits were administered with penicillin (800000 units, 1 time/day) through intramuscular injection for 7 days.

Morphological change

Rabbits were killed using air embolism method. The knees were exposed to observe the meniscal morphology including surface flatness, color, flexibility and intactness.

Histological examination

After the rabbit was killed, lateral meniscus was cut off with the surrounding soft tissue removed. The lateral meniscus was cleaned with normal saline and then was fixed in 4% paraformaldehyde for 24 h. Then it was processed with routine histological methods and embedded in paraffin blocks. Sections of 3 μm thickness were used for H&E staining and immunohistochemical staining. The specimens were treated with dimethyl benzene dewaxing, gradient ethanol dehydration, hematoxylin dyeing (5 min), tap water rinsing (1 min), 1% hydrochloric acid ethanol differentiation (30 s), tap water soaking (15 min), 0.5% eosin staining (3 min) and washing with distilled water. After ethanol and xylene dehydration, the piece was sealed for observation. The histological changes in the meniscus tissue sections were evaluated using light microscopy. The damage showed in the H&E staining images was evaluated with the scoring system [12] (Table 1).

For immunohistochemical assay, the sections were dewaxed according to the above method following with $\text{H}_2\text{O}_2$ (3%) incubation at room temperature for 10 min. After washed with PBS and incubation for 15 min using goat serum, it was incubated with rabbit polyclonal antibody MMP1/MMP13/TIMP-1 at 4°C overnight. They were rinsed and incubated with rabbit IgG at 37°C for 15 min. The MMP-1, MMP13 and TIMP-1 expression intensity in paraffin-embedded tissue sections was determined using Motic Images System. The specimen was detected
with light microscopy for the correction cell number. The results were expressed as positive cell rate (PCR, PCR = positive-staining cell count/total cell count × 100%).

All histomorphological and immunohistochemical assessments were evaluated by examining six non-overlapping meniscus sections and a minimum of 10 fields per side of each animal.

**Statistical analysis**

Statistical analysis was done using SPSS 15.0. The results were expressed as mean ± SD. Paired data were evaluated by paired t-test. If the mean of the sample met the homogeneity of variance, pairwise comparison was performed using SNK-q test (Student-Newman-Keuls test); if the mean of the sample didn’t meet the homogeneity of variance, Dunnett’s-T3 test was performed. Nonparametric test was performed with Nemeyi rank-sum test and Wilcoxon rank-sum test. P < 0.05 was considered to be statistical difference.

**Results**

**Morphological degeneration of meniscus after PCL-tensection**

The morphology of meniscus in the PCL-tensection group showed degenerative characteristics while it was normal in the control group (Table 2). It indicated that PCL-tensection induced progressive degeneration of meniscus.

**Histological abnormalities of meniscus after PCL-tensection**

Compared with control group, the histological characteristics of meniscus showed some ab-
normities and deterioration in a time-dependent manner in the PCL transection group (Figure 1A-F).

The histological scores of meniscus were 0-1 (within the normal range) in the control group (Table 3). The histological scores in the PCL transection group were above 2.5 with statistical difference (P < 0.05) except for the comparison between the score on 4 weeks after PCL transection and the score on 8 weeks after PCL transection. There were statistical significance between the histological scores of control group and that of the PCL transection group on all time points (Table 4). It suggested that PCL transection induced the progressive degeneration of meniscus tissue.

**Increased expression of MMP-1, MMP-13 and TIMP-1 in meniscus**

MMP-1 didn’t express in control group, while there was an increasing trend of MMP-1 expression in cytoplasm and matrix in a time-dependent manner in the PCL transection group (Figure 2A-F; Table 3). MMP-1 expression level showed an increasing trend (P < 0.05) and then remained a relative level (P > 0.05) after PCL transection. The comparison between the MMP-1 expression level in the control group and that in the PCL transection group showed statistical significance at all time-points (Table 4).

There was a small amount of MMP-13 expression in cytoplasm of control group. In the PCL transection group there was an increasing trend of MMP-13 expression level in cytoplasm and matrix with time firstly and then a low expression level in advanced stage after PCL transection (Figure 3A-F; Table 3). The expression level of MMP-13 in the PCL transection group was higher than that in the control group with statistical significance (Table 4).

As for TIMP-1 expression in the meniscus, it showed a very low level in the control group; it displayed an increasing trend in the early stage and reached the peak on 12 weeks after PCL transection and then decreased in late stage in the PCL transection group (Figure 4A-F; Table 3). The comparison of TIMP-1 expression level between the control group and the PCL transection group showed statistical significance (Table 4).

**Discussion**

PCL injury may induce the degeneration of meniscus which further may result in osteoar-
Research on meniscus after PCL-fracture

Figure 3. MMP-13 expression in the PCL-rupture side and the control side. A. In the control side, a very small number of MMP-13 positive-staining cells were observed. B. On 4 weeks after PCL-rupture, a small number of MMP-13 positive-staining cells were observed. C. On 8 weeks after PCL-rupture, a small number of MMP-13 positive-staining cells were observed. D. On 12 weeks after PCL-rupture, more MMP-13 positive-staining cells were observed with strongly-staining image. E. On 16 weeks after PCL-rupture, strongly-staining image was observed. F. On 24 weeks after PCL-rupture, MMP-13 positive-staining cells decreased.

Figure 4. TIMP-1 expression in lateral meniscus of PCL-rupture side and the control side. A. In the control side, a very small number of TIMP-1 positive-staining cells were observed. B. On 4 weeks after PCL-rupture, a small number of TIMP-1 positive-staining cells were observed. C. On 8 weeks after PCL-rupture, a small number of TIMP-1 positive-staining cells were observed in weakly positive-staining image. D. On 12 weeks after PCL-rupture, more TIMP-1 positive-staining cells were observed. E. On 16 weeks after PCL-rupture, the image showed weak-staining. F. On 24 weeks after PCL-rupture, the image showed weak-staining and a few positive-staining cells.

Osteoarthritis. MMPs and TIMPs have close association with connective tissue degeneration [13]. With a rabbit PCL transection model, we found the morphological degeneration of lateral me-
Research on meniscus after PCL-fracture

Meniscus with time. MMP-1 expression levels were significantly elevated in a time-dependent manner in the early and middle stage after PCL injury and remained a relative stable high expression level in the late stage. MMP-13 and TIMP-1 showed high expression level firstly then low expression in advanced stage after PCL rupture.

In the present study, the observation time points were determined according to the previous researches [14, 15]. Wang et al. constructed rabbit PCL-transection model and found that on 12 weeks after PCL transection there were transections in cartilage, osteophyte formation and medial meniscus tear; on 26 weeks after PCL transection, cartilage degeneration and osteophyte were obvious [16]. In the present study, we used 4, 8, 12, 16, 24 weeks after PCL transection as the observation time for lateral meniscus tissue scoring and the immunohistochemical experiments. The morphological results demonstrated that the meniscus tissue degenerated with an increasing trend with time, which is in accordance with the result of Wang et al. study [16]. Therefore, we proposed that the degeneration of meniscus fibrous cartilage probably synchronized the degeneration of joint cartilage after PCL transection.

In the present study, MMP-1, MMP13 and TIMP-1 were used as molecular markers for meniscus degeneration. Tissue remodeling occurs continuously in both normal and injured tissues. In this process, old or damaged structures are degraded and replaced with newly synthesized molecules [6]. The balance between the degradation and biosynthesis is controlled by MMPs and TIMPs [7]. MMP-1 can initiate the degradation of interstitial collagen, with the substrate collagen type I, II and III. Meniscus consists of 98% collagen type I and less than 2% collagen type II [4]. Previous studies have established close relationship between connective tissue damage/degeneration and the expression of MMPs [8-10]. TIMPs, as tissue inhibitors of metalloproteinases, include TIMP-1, TIMP-2, TIMP-3 and TIMP-4 and play an important role in osteoarthritis and meniscus injury after anterior cruciate ligament (ACL) transection. Among TIMPs, TIMP-1 is the most critical one as the inhibitor [17]. MMP-1, MMP13 and TIMP-1 were studied in the osteoarthritis pathology but, as far as we know, there is not any report about the association between the expression level of MMP-1, MMP13 and TIMP-1 and meniscus injury after PCL transection. Therefore, in this study we focused on MMP-1, MMP13 and TIMP-1 to determine the early molecule response underlying the tissue damage and degeneration induced by PCL transection, which is helpful for understanding the PCL transection-induced knee degeneration as well as development of osteoarthritis.

The morphological change of meniscus was obvious on 12 weeks after PCL-transection and the histological score showed an increasing trend, which is in accordance with the previous study [16]. The present result showed that the expression level of MMP-1, MMP13 and TIMP-1 in the PCL transection group was higher than that in the control group in all time points, indicating that the degeneration of lateral meniscus possibly has close relationship with the higher expression level of MMP-1, MMP13 and TIMP-1. In the PCL transection group, the expression of MMP-1 and MMP13 obviously increased, which is in accordance with the initiating role of MMP-1 and meniscus morphological change [18]. In ACL transection, the expression of MMP-1 and MMP-13 was obvious in the early stage (on 4, 8 weeks after ACL transection) [15] while in the present study the expression of MMP-1 and MMP13 was unobvious until 12 weeks after PCL transection. On 24 weeks after PCL transection, the expression of MMP-13 decreased, while the expression of MMP-1 still remained the high level and was in accordance with the result of Fernandes’ study [19]. The different expression level and trend are possibly due to the different regulation pathways, ECM degradation, and chondrocyte apoptosis. It’s worth noting that the decreased expression level of MMP-13 did not mean the end of meniscus injury or apoptosis. Maybe MMP-13 plays an important role in the middle stage of degeneration rather than in the whole stage.

In ACL transection model, the expression of TIMP-1 obviously increased 4 weeks after transection [15]. In the present study the expression of TIMP-1 obvious increased and reached the peak value on 12 weeks after PCL transection and decreased on 16 weeks after PCL transection. This possibly is due to the compensatory repair characteristic of TIMP-1. Specifically, TIMP-1 binds with MMP-1 and MMP-13 in the ratio of 1:1. With the deterioration of
Research on meniscus after PCL-fracture

Conclusions

PCL rupture may induce a coordinated response of degeneration of lateral meniscus in a time-dependent manner. The high expression level of MMP-1, MMP-13 and TIMP-1 would contribute to the degeneration of lateral meniscus after PCL rupture.

Acknowledgements

The research was funded by Open-End Fund for the Valuable and Precision Instruments of Central South University (CSUZC201404046) and Hunan Provincial Innovation Foundation for Postgraduates (CX2014B111).

Disclosure of conflict of interest

None.

Abbreviations

PCL, Posterior cruciate ligament; MMPs, Matrix metalloproteinases; TIMPs, Tissue inhibitors of metalloproteinases; MMP-1, Matrix metalloproteinase-1; MMP-13, Matrix metalloproteinase-13; TIMP-1, Tissue inhibitor of metalloproteinase-1; ACL, Anterior cruciate ligament.

Address correspondence to: Drs. Yihe Hu and Zhan Liao, Department of Orthopedics, Xiangya Hospital of Central South University, 87 Xiangya Road, Changsha 410008, Hunan, P. R. China. Tel: +86-0731-89753706; Fax: +86-0731-89753006; E-mail: csuhuyihe@163.com (YHH); csuliaozhan@163.com (ZL)

References


Research on meniscus after PCL-fracture


