The purpose of this study was to investigate the preventive effect of mannose-6-phosphate on flexor tendon adhesion formation. From a total of 84 adult New Zealand White rabbits, 36 were randomly divided into 2 groups, the normal saline group and the mannose-6-phosphate group, after anastomosis of the flexor tendons. Tendons were harvested at 4 weeks, and biomechanics testing was conducted. The other 48 rabbits were randomly divided into 2 groups, the normal saline group and the mannose-6-phosphate group, after anastomosis of the flexor tendons, and tendons were harvested at 7, 14, 28, and 56 days and analyzed by in situ hybridization to determine the mRNA expression of transforming growth factor (TGF)-B1 and collagen I.

The results of biomechanics testing indicated that mannose-6-phosphate can effectively prevent flexor tendon adhesion formation after anastomosis. The in situ hybridization examination revealed that TGF-B1 and collagen I mRNA expression in the mannose-6-phosphate group was lower than that in the normal saline group at each time point. Mannose-6-phosphate can effectively inhibit the function of TGF-B1 and prevent adhesion formation after flexor tendon injury.

Figure: Histological inspection of the tendon 4 weeks postoperatively revealing fibroblasts and regular collagen fibrils (arrow) in the experimental digit (A) and fibroblasts and irregular collagen fibrils (arrow) in the control digit (B) (hematoxylin-eosin stain ×40).
Flexor tendon healing is complicated by adhesions to the surrounding fibroosseous sheath. Adhesions between the tendon and sheath impair the gliding mechanism of the tendons and result in poor finger range of motion (ROM). Clinical attempts to modulate adhesion formation have proven unsuccessful.1-5 Transforming growth factor (TGF)-β is a cytokine with numerous biologic activities related to wound healing, including fibroblast and macrophage recruitment, stimulation of collagen production, downregulation of proteinase activity, and increases in metalloproteinase inhibitor activity.6,7 Transforming growth factor-β accelerated the wound-healing process in several models.8 It has become widely appreciated that TGF-β is a key cytokine in the pathogenesis of fibrosis and scar formation, resulting from excessive disordered collagen deposition.9,10

Excessive production of TGF-β has been linked to fibrotic diseases. Enhanced TGF-β1 expression and an associated increase in the production of collagen I, III, and VI have been documented in tissues of patients with systemic sclerosis, postburn hypertrophic scar tissue, and keloids.11,12 Conversely, inhibition of TGF-β decreased collagen deposition and scarring. The application of neutralizing antibodies to TGF-β in rat incisional wounds successfully reduced cutaneous scarring.13 Mannose-6-phosphate is a natural inhibitor of TGF-β, with a similar structure to that of β-glycan. Mannose-6-phosphate can competitively bind TGF-β1 and reduce TGF-β activity and scar formation.14

The purpose of this study was to investigate the preventive effect of mannose-6-phosphate on flexor tendon adhesion formation.

Materials and Methods

All rabbit experiments were performed according to protocols conducted in strict accordance with the guidelines for caring for laboratory animals formulated by the Ministry of Science and Technology of the People’s Republic of China.

Rabbit Model of Zone II Flexor Tendon Repair

Eighty-four adult New Zealand White rabbits (weight range, 4.0-4.5 kg) were anesthetized with an intramuscular injection of acepromazine (0.01 mg/kg), xylazine (5 mg/kg), and ketamine (50 mg/kg). A longitudinal incision was made on the volar surface between the metacarpo- and proximal interphalangeal joints of the middle digit. Tissues were carefully dissected under loupe magnification until the flexor sheath was identified. The sheath was sharply opened in the midline. The flexor digitorum profundus tendon was isolated between the A2 and A4 pulleys and sharply transected. An immediate tendon repair was performed with 5-0 Prolene suture (Ethicon, Inc, Somerville, New Jersey) in the modified Kessler fashion with 2 strands crossing the repair site by using a published protocol with minor modifications.15

Thirty-six of the rabbits were randomly divided into 2 groups (n=18). With a tuberculin syringe, 100 µL of the control substance (phosphate-buffered saline solution) or test substance (mannose-6-phosphate) was applied to the tendon repair site and the surrounding tissue and allowed to infiltrate for 1 minute. The skin was then reaproximated with a running 4-0 Prolene suture. Tendons were harvested at 4 and 8 weeks to conduct biomechanical testing, histological evaluation, and scanning electron microscopy observation.

The remaining 48 rabbits were randomly divided into 2 groups (n=24), and 100 µL of the control substance or test substance was applied to the tendon repair site. Tendons were harvested at 1, 2, 4, and 8 weeks and analyzed by in situ hybridization to determine the mRNA expression of TGF-β1 and collagen I using an image analysis technique. The number of positive cells in the unit area was also calculated, and the average value of 10 positions in each specimen was obtained.

Statistical Analysis

Statistical analysis was performed with SPSS version 10.0 software (SPSS, Inc, Chicago, Illinois), and data were expressed as mean±SD. Intergroup comparison was conducted using the t test, and a level of P<.05 was considered statistically significant.

Results

Biochemical Detection

The gliding excursion ratio of the tendon was shortened and the simulated active flexion ratio was less in the saline group than in the mannose-6-phosphate group at 4 and 8 weeks postoperatively (P<.05).

Tendon anastomosis breaking strength showed no significant differences between the 2 groups (P<.05) (Table 1).

Histological Observation

Hematoxylin-eosin staining revealed that collagen fibers increased and disorganized in the saline group but arranged regularly in the mannose-6-phosphate group parallel to the tendon longitudinal axis at 4 weeks postoperatively. The fibroblasts...
reduced and blood capillaries proliferated. At 8 weeks postoperatively, the collagen fibers arranged irregularly at the anastomosis site but regularly in the mannose-6-phosphate group (Figures 1, 2).

**TGF-β1 and Collagen I mRNA Expression**

The in situ hybridization examination revealed that TGF-β1 and collagen I mRNA expression in the mannose-6-phosphate group was lower than that in saline group at each time point \((P<.05)\) (Table 2). Transforming growth factor-β1 and collagen I mRNA expression reached the peak at 2 to 4 weeks, respectively, and the addition of mannose-6-phosphate significantly reduced mRNA expression.

**DISCUSSION**

Flexor tendon repair in the hand can be complicated by pathologic scar formation. Adhesions form between the tendon and sheath, impairing the gliding mechanism necessary for tendon function. A debate exists among researchers over in vivo mechanisms of flexor tendon wound healing. Extrinsic and intrinsic mechanisms of repair may occur. Proponents of an extrinsic mechanism, whereby tendon sheath fibroblasts and inflammatory cells migrate inward by the vincula system to promote repair, include Peacock \(^1^8\) and Potenza \(^1^9\). In the mid-1970s, Lundborg and Rank \(^2^0\) observed the healing of isolated flexor tendons within the knee joint synovium, thus suggesting that healing intrinsic to the tendon itself was possible. A recent study demonstrated differential collagen expression by cells in 3 distinct areas: the tendon sheath, the epitenon, and the endotenon. \(^1^9\) The traditional tendon-healing paradigm involving extrinsic and intrinsic mechanisms can thus be expanded to include all 3 sites. \(^1^9\) However, it remains unclear whether a single mechanism occurs in vivo or if multiple, possibly redundant pathways exist. Further investigation is required to define the molecular events that occur after tendon injury and the relative contributions to adhesion formation in each location.

In the past decade, multiple peptide growth factors and their roles in tissue repair have been characterized. Previous studies on flexor tendon healing have implicated basic fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor in the tendon-healing process. \(^2^0\) Reports have recently shown a similarly important role for TGF-β1 in tendon healing. \(^2^1^-^2^3\) The upregulation of

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**Table 1**

<table>
<thead>
<tr>
<th>Gliding Excursion Ratio, cm</th>
<th>Simulated Active Flexion Ratio, deg</th>
<th>Tendon Anastomosis Breaking Strength, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>4 wk</td>
<td>8 wk</td>
</tr>
<tr>
<td>Saline</td>
<td>0.43±0.08</td>
<td>0.74±0.06</td>
</tr>
<tr>
<td>M6P</td>
<td>0.95±0.12(^b)</td>
<td>1.25±0.14(^b)</td>
</tr>
</tbody>
</table>

Abbreviations: deg, degrees; M6P, mannose-6-phosphate.

\(^a\)\(x\)±s; \(n=9\).

\(^b\)Compared with the saline group \((P<.05)\).

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**Figure 1:** Histological examination of the tendon 4 weeks postoperatively revealing fibroblasts and regular collagen fibrils (arrow) in the mannose-6-phosphate digit (A) and fibroblasts and irregular collagen fibrils (arrow) in the saline digit (B) (hematoxylin-eosin stain \(×40\)).

**Figure 2:** Histological examination of the tendon 8 weeks postoperatively revealing fewer fibroblasts and regular collagen fibrils (arrow) in the mannose-6-phosphate digit (A) and some fibroblasts, irregular collagen fibrils (arrow), and abnormal collagen diameter in the saline digit (B) (hematoxylin-eosin stain \(×40\)).
TGF-β1 mRNA in repair specimen began immediately on the first postoperative day and continued to the last time points examined (56 days). The results illustrated the multiple roles that TGF-β1 played throughout wound healing, the initial inflammatory response, angiogenesis, collagen deposition, and eventual tissue remodeling. Therefore, early modulation of TGF-β1 may be a useful strategy to control unnecessary peritendinous adhesion formation.

Transforming growth factor-β antagonists are likely candidates to reduce scarring and improve clinical outcome. One antagonist is TGF-β–neutralizing antibody, which consistently and significantly reduced TGF-β–induced collagen I production in all 3 components of the cultured flexor tendon, namely the sheath, the epitenon, and the endotenon cells. The application of TGF-β1–neutralizing antibody improved postsurgical ROM at the time of flexor tendon surgery in a rabbit model. However, TGF-β antibodies have a short biologic half-life, and continuous supplementation of exogenous TGF-β antibodies is not practical. The current study investigated the preventive effect of mannose-6-phosphate on flexor tendon adhesion formation.

Studies have shown that TGF-β1 expression can be observed in noninjured tendon tissues, but at a low level. Following tendon injury, the expression increased. Early modulation of TGF-β1 has not been used clinically, and the side effects are unclear.

**REFERENCES**


