Prevention of Synovial Membrane Inflammation & Cartilage Degradation by a Novel Chitosan Hydrogel in a Rabbit Model of Osteoarthritis

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OBJECTIVES
Current treatments of osteoarthritides (OA) are mainly based on the alleviation of painful symptoms but they are unable to prevent or to reverse cartilage degradation. The development of new hydrogels for intra-articular viscosupplementation is a promising approach. Herein, we reported the effects of a novel chitosan hydrogel in OA induced surgically in rabbit.

MATERIALS & METHODS
A biodegradable thermosensitive hydrogel was prepared with ultrapure and animal-free chitosan according to a proprietary formulation and process (VI002, Vegetech inside, Synolyne Pharma, Herstal, Belgium). OA was surgically induced by the transection of the anterior cruciate ligament (ACLT) of the right knee of female Hyla albino rabbits aged 20 weeks. One week after surgery, animals were randomly divided into 2 groups and injected intra-articularly (right knee) with 600 microliters saline solution (control, n = 10) or with 600 microliters VI002 hydrogel (n = 10) (Figure 1, Table 1). Standard radiographs were acquired in extension and scored with the Kellgren and Lawrence (K&L) scale 9 weeks after surgery. Animals were euthanized at that time and the right knee joint was dissected. A macroscopic evaluation of cartilage was done. The size and severity of the lesions were recorded according to the OARSI recommended scores (1). Synovial membrane and cartilage (femoral condyles) were processed for histologic evaluation. Synovial membrane sections were stained with hematoxylin/eosin and cartilage with Safranine-O/fast green. Histologic evaluation was performed according to the OARSI scores (1). Data were analyzed using Mann-Whitney U test. They are presented as box and whisker plots. Results are given either as global (entire joint) or for specified compartment or parameter. P value below 0.05 was considered significant.

RESULTS
Tolerance and safety
Except one rabbit lost during narcose, no loss was recorded throughout the whole duration of the study. The I.A. treatment was well tolerated. No adverse reaction was reported.

Imaging
Standard X-rays showed characteristic OA manifestations (Figure 2). The analysis showed a significant decrease (p=0.0079) of the K&L score in rabbits injected with VI002 hydrogel compared with controls (Figure 3).

Macroscopic analysis
All animals developed OA lesions. The observation of the joint structures revealed lesions with different grade and size on the different compartments (Figure 4). The size and the severity of the macroscopic OA cartilage lesions significantly decreased in the lateral compartment in animals treated with VI002 compared to controls (p=0.0041) (Figure 5). The same tendency was observed with the global score. This result is in favor of a protective effect of VI002 against the development of OA lesions.

Histology of the synovial membrane
The synovial membrane is the site an inflammatory reaction characterized by synovial hypertrophy, villous hyperplasia and inflammatory infiltrate (Figure 6). VI002 hydrogel significantly improved the global score of synovial membrane (p=0.0040) (Figure 7). This effect was mostly due to the significant reduction of synovocytes hyperplasia and inflammatory cells infiltrate criteria. VI002 was indeed able to reduce the synovial inflammation.

Histology of cartilage
Cartilage lesions were of mild to moderate intensity (Figure 8). VI002 treatment induced a slight but not significant improvement in the global score of cartilage. However a significant effect was shown on the cartilage structure score with VI002 treatment (p = 0.0017) (Figure 9).

CONCLUSIONS
VI002 hydrogel administered intra-articularly in rabbit after ACLT surgery was well tolerated. Altogether these results are in favor of a beneficial effect of the treatment on the development of OA. VI002 was indeed able to prevent cartilage degradation and to decrease synovitis. The results confirm the high potential of a chitosan-based hydrogel in preventing OA.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>VI002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Adult female Hyla albino rabbit</td>
<td></td>
</tr>
<tr>
<td>Age on delivery</td>
<td>20 weeks</td>
<td></td>
</tr>
<tr>
<td>Weight at surgery</td>
<td>3.3 ± 0.1 kg</td>
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</tr>
<tr>
<td>Number of rabbits</td>
<td>9</td>
<td>10</td>
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<tr>
<td>Treatment</td>
<td>Single I.A. 600 µl</td>
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<tr>
<td>follow-up period (days)</td>
<td>61-68</td>
<td>61-68</td>
</tr>
</tbody>
</table>

Notes: *One animal lost in narcose.