Introduction: Recently we described a slowly progressive intervertebral disc degeneration in the goat in which degeneration was induced by means of injecting 0.25 U/ml Chondroitinase ABC (CABC), a proteoglycan side chain degrading enzyme in the nucleus pulposus (NP). The chemically induced loss of proteoglycans might mimic the process of human disc degeneration. There have been reports in literature on spontaneous regeneration of degenerated discs after chemical induction of degeneration. This is relevant for the practicability of the goat degeneration model. The evaluation of treatment modalities, e.g. stem cell therapy, might be compromised by these spontaneous regenerative effects. Similarly, the development of the degenerative signs in the goat model over a longer period of time are of interest. Finally, the reproducibility of the chemically induced degeneration is important. To study the reproducibility, spontaneous recovery and the development of the degenerative signs over time, degeneration was induced in twelve goats exactly similar as described before and these goats were followed for 18 weeks (n=6) and 26 weeks (n=6). Degeneration was analyzed by means of X-ray, MRI, macroscopic analysis and histology.

Materials and Methods: Twelve goats were used in this study. All research protocols have been approved by the Animal Ethics Committee. 0.25 U/ml (Sigma Aldrich, Saint Louis, USA) of CABC was injected directly in to the NP in a retroperitoneal surgical procedure described in detail previously.1 The development of degeneration was studied after 18 and 26 weeks. The induced degeneration was compared to results from a previous study, in which degeneration was induced similarly and analysis was performed after 12 weeks.1 X-ray analysis was performed by measuring the Disc Height Index (DHI). The percentage of the original height at the time-points was calculated. MRI analysis used a measure called MRI index, which measures the surface and signal intensity of the NP. These values were expressed as relative to the PBS injected disc to correct for inter-MRI and animal differences. The macroscopic analysis was performed using a macroscopic grading score. Finally, histologic scoring was performed using a grading scale developed by our group previously.

Results: The severity of the degenerative signs was mild and was consequent- ly present in all parameters analyzed. After twelve weeks the DHI had decreased insignificantly to 88% of the original height (100%), see fig. 1A. After 18 weeks the DHI decreased to 84% of its original height (p < 0.0001) and decreased further to 80% after 26 weeks (p < 0.0001). After 26 weeks osteophyte formation was observed in 4/6 goats.

The CABC injected discs all had a lower MRI Index compared to 1.0, the “normal” value of the PBS levels by definition (see Fig. 1B). It was previously shown that this was significant after twelve weeks (p = 0.04). In the present study this was also significant after 18 weeks (p = 0.0015) and 26 weeks (p = 0.0162).

The macroscopic analysis also revealed that some of the discs in the 26 weeks follow-up groups demonstrated more severe signs of degeneration like osteophyte formation and ruptures of the AF. After twelve weeks, the macroscopic score was increased to 2.6 (p = 0.03), see fig. 1C. The macroscopic score of these CABC injected discs was 2.4 after 18 weeks (p = 0.002) and 2.2 after 26 weeks (p = 0.0005). No differences were detected between the different time-points (p > 0.71).

The histologic score of the PBS injected discs was 0.8 after 12 weeks and 1.3 after both 18 and 26 weeks (see Fig. 1D). The CABC injected discs however were scored significantly higher: after 18 weeks the score was 3.8 (p = 0.0005) and after 26 weeks it was 3.5 (p = 0.0005). The discs that were followed for twelve weeks had a histological score of 4.2 (p = 0.03). There were no statistically significant differences between the different follow-up time points (p = 0.6).

Discussion: Discussion: The induced degeneration was compared to results from a previous study, in which degeneration was induced similarly and analysis was performed after 12 weeks. No significant differences were found in any of the parameters between the different time-points, although the severity seemed to decrease slightly. The induced degeneration apparently levels of after 12 weeks. However, osteophyte formation was only observed in the 26 weeks follow-up group, indicating that the severity of the degeneration does increase. Spontaneous recovery was not observed up to 6 months.

The severity of the degenerative signs was mild and was consequently present in all parameters analyzed. When compared to the results after twelve weeks, the degeneration was similar in the present study. From these observations we conclude that the reproducibility of the disc degeneration model is good. Based on the present study and previous results, the goat CABC model is established as a suitable large animal model to evaluate mild disc degeneration and potential new (cellular) therapies.


Acknowledgements: This study was financially supported by Cytori Therapeutics Inc, San Diego, California, USA.

Prof. PJM Wuisman has passed away 25 July 2007.