INTRODUCTION: Lumbar disc degeneration is a primary cause of chronic low back pain and radiolucre. Furthermore, surgical procedures, such as microdiscectomy and laser or other decompressive procedures, induce intervertebral disc (IVD) degeneration after surgical treatment. Treatments that strategize to regenerate or decelerate disc degeneration hold great promise.

In the connective tissue field, esculetin (6,7-dihydroxycoumarin) has been reported to decrease proteoglycan release induced by interleukin-1 in monolayer cultures of rabbit, bovine nasal chondrocyte and human chondrocyte cell lines [1, 2]. However, when esculetin, which is derived from the plants, Artemisia scoparia and Fraxinus japonica Blume, is orally administered to animals, the blood concentration soon reaches a maximum level and then rapidly disappears. CPA-926 (6-[2-(acetyl-amino)-2-deoxy-B-D-glucopyranosyl]oxy]-7-hydroxy-coumarin), a prodrug of esculetin, shows a long-lasting pharmacokinetic profile in the blood circulation. Several interesting biological and biochemical activities, such as anti-inflammatory [3, 4] and antitumorigenesis effects [5], have been reported for CPA-926. A recent in vivo finding showed that the prophylactic oral administration of CPA-926 appears to provide some protection against cartilage destruction in a short-term rabbit experimental osteoarthritis model [6]. However, there has been no in vivo study to test the hypothesis that the oral administration of CPA-926 changes the course of degenerative disc diseases.

The purpose of this study was to monitor, radiographically and histologically, the effects of the oral administration of CPA-926 on IVD degeneration in the rabbit annular needle puncture model.

MATERIALS AND METHODS: Protocol: Sixteen New Zealand White rabbits (3 kg) were used with institutional animal care committee approval. An annular puncture model was established using defined needle gauges and depths [7]. Under general anesthesia, puncture of the IVD (5 mm depth) was accomplished using an 18 gauge needle, that had a stopper attached to control the depth, on lumbar IVDs (L2/3 and L4/5) using the left posterolateral retroperitoneal approach. These rabbits were divided equally into two groups; one group received the oral administration of the vehicle solution, 0.5% methylcellulose and 0.05% polysorbate [Control group]; the other group received the oral administration of 400 mg/kg of CPA-926 formulated in the vehicle [Kureha Chemical Industry, Co. Ltd, Tokyo Japan, (CPA-926 group)]. The drug or vehicle was orally administered to the rabbits once daily beginning on the day of surgery (before surgery) and for 56 days post-surgery. Lateral X-rays of the lumbar spine were taken before and at 2-, 4-, 6- and 8-weeks after surgery to measure IVD height. At 8-weeks after the annular puncture, rabbits were euthanatized and the IVDs were assessed histologically. As an internal control, the non-punctured discs (L3/4) were also assessed histologically.

Radiographic Analysis: Vertebral body height and disc height were measured using Scion Imaging Software (Frederick, M.A.). Intervertebral disc height was expressed as the disc height index (%DHI) [7]. Percent DHI (% DHI = (postoperative DHI / preoperative DHI) x 100) was also calculated.

Histological Assessment: Discs were harvested and sagittal sections of IVDs were stained with Hematoxylin and Eosin, and Safranin-O. The histological data were analyzed by the Mann-Whitney test

RESULTS: Change in body weight: The change in body weight of rabbits that received CPA-926 was 11.4% (3.14 ± 0.02 kg, on day 0; 3.50 ± 0.06 kg on day 56) while that of Control rabbits was 15.3% (3.06 ± 0.03 kg, on day 0; 3.54 ± 0.07 kg, on day 56, not significant). There were no significant differences in body weight within each group at each time point.

DHI: Control Group: Rabbits in the Control group showed a significant decrease in the disc height of punctured discs during the experimental period (%DHI: 2W, 76.8 ± 3.7; 8W, 70.7 ± 2.3, p<0.001, vs. the non-punctured control discs). The disc height of non-punctured discs of the Control group (L3/4) showed a slight, but significant decrease (%DHI: 2W, 98.2 ± 1.3; 4W, 93.6 ± 2.0; 6W, 92.2 ± 1.6; 8W, 90.5 ± 2.3, p<0.01).

CPA-926 Group: On the other hand, rabbits in the CPA-926 group did not show a statistically significant decrease in the disc height of punctured discs during the first four weeks. At later time points, those discs showed a slow, but progressive disc narrowing (%DHI: 6W, 85.5 ± 2.0; 8W, 82.2 ± 1.8, p<0.05, vs. non-punctured control discs). Importantly, at each time point, a significant difference in the disc height between the punctured discs in the Control group and those in the CPA-926 group (p<0.001) was observed (Figure). Interestingly, the DHI of non-punctured discs in the CPA-926 group decreased significantly (8W, 95.1 ± 2.3, p<0.05) but this change was significantly less than that of the non-punctured discs of the Control group (8W, 86.5 ± 3.7, p<0.05).

Radiographic Score: At the 8-week time point, the histological scores of punctured degenerative discs were significantly higher in the Control group (9.4 ± 0.7) than in the CPA-926 group (6.4 ± 0.6) (p<0.01).

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