Biocompatibility of The Photo-cross-linked Hyaluronate Gel (Gel-One®) for The Treatment of Knee Osteoarthritis

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Disclosures:

Introduction: Osteoarthritis (OA) of the knee is a common joint disorder in the aging population. Intra-articular hyaluronan (HA) products have been widely accepted as a viscosupplementation for the treatment of knee OA pain. HA products are divided into 2 major types, native HA products and cross-linked HA products. Native HA products are injected 3 to 5 times per one treatment course and its safety has been established based on long time clinical experiences1. In the cross-linked HA category of the US market, there are three products, Gel-One®, Synvisc® and Synvisc-One®. Gel-One® is the most recently approved single injection HA product and it is composed of Gel-200 which is a novel cross-linked hyaluronate hydrogel manufactured by Photo-Gelation technology. Synvisc® and Synvisc-One® are composed of hylan G-F 20 which is a mixture of two cross-linked HA derivatives. Synvisc-One® is the first approved single injection product in USA and it contains triplicate hylan G-20 of Synvisc® requiring three injections. There is growing clinical evidence to suggest that hylan G-F 20 may be associated with an increased incidence of pseudosepsis or granulomatous synovitis2,3,4,5,6,7. The purpose of this study is to compare differences in biocompatibility and immunogenicity between Gel-200 from Gel-One® and hylan G-F 20 from Synvisc® series.

Methods:
Biocompatibility was investigated using a rat subcutaneous air pouch model and the knee joint of normal rabbit.
1. The rat subcutaneous air pouch model has been accepted as a biocompatibility model for evaluating hyaluronan products, because the pouch membrane structure closely resembles synovium8. The sterile air was injected subcutaneously into the intrascapular area of the rat (Male, SD rats, 8 weeks of age, n=6) back. Six days after injection, 2 mL of test material (Gel-200, hylan G-F 20, 1% carrageenan or saline) was injected into the pouch. Animals were sacrificed on days 14, 28 and 56 after the injection of test materials. Histological examination and cell count in pouch fluid were performed.
2. For the evaluation in the knee joint, 0.25 mL of test material (Gel-200, hylan G-F 20 or saline) was injected singly or 3 times on a weekly basis into both knee joints of normal rabbit (Male, NZW rabbits, 11 weeks of age, n=6). Animals were sacrificed on day 15 after the initial injection. Histological examination and cell count in synovial fluid were performed.
3. In the investigation of immunogenicity, guinea pigs (Male, Hartley, 6 weeks of age, n=6 or 4) were immunized by 3 weekly subcutaneous injections of test materials (Gel-200, Gel-200 with equal volume of Freund’s complete (or incomplete) adjuvant (+FCA), hylan G-F 20, hylan G-F 20+FCA, non-immunized, and ovalbumin (OVA) +FCA ). Immunized animal sera were analyzed by homologous passive cutaneous anaphylaxis (PCA) assay and by ELISA for anti-Gel-200 or anti-hylan G-F 20 IgG.

Results: 1. In the rat subcutaneous air pouch model, from 14 days after administration, in addition to inflammatory changes, formation of the fibrous belt was characteristic in all groups. In the hylan G-F 20 group, many granulomatous nodules primarily composed of macrophages, multinucleated giant cells, and eosinophils accompanied with the test article in the center of the nodules were seen in the fibrous belts. On the other hand, in the Gel-200 group, no such granulomatous inflammation was seen, and many foamy macrophages containing the test article were observed.
2. In the knee joints of normal rabbits, the entire surfaces of the synovium were slightly rough, and slight proliferation of the synovial cells was seen in some parts in both Gel-200 and hylan G-F 20 groups. In the hylan G-F 20 group, slight to moderate infiltration of inflammatory cells (lymphocytes, macrophages, and eosinophils) and fragments of the test material were seen in the adipose tissue. In addition, obvious granulomatous inflammation was seen in one animal. The total cells, monocytes and heterophiles of synovial fluid increased in hylan G-F 20 group. These findings observed in hylan G-F 20 group were more severe in 3 times doses than in single dose. However, in the Gel-200 group, only a little infiltration of macrophages was seen in the adipose tissue in which some fragments of test material remained. The number of each cell of synovial fluid was not different from control in the Gel-200 group.
3. For the immunogenicity, the sera of hylan G-F 20-immunized animals showed positive reaction in the homologous PCA assay and high amount of anti-hylan G-F 20 IgG was detected in these sera. On the other hand, the sera of Gel-200-immunized animals showed neither the reaction in the homologous PCA assay nor production of anti-Gel-200 IgG.

Discussion: The results demonstrated the differences between Gel-200 and hylan G-F 20 in biocompatibility and immunogenicity. In the rat air pouch model, tissue reaction in the fibrous belt induced by hylan G-F 20 was obviously more severe than that by Gel-200. Hylan G-F 20 induced, possibly as an allergic reaction, granulomatous inflammation accompanied with prominent eosinophil infiltration and multinucleated giant cells. Gel-200 did not induce such a granulomatous reaction and only phagocytosis of Gel-200 by foamy macrophages was observed. In the knee joint of normal rabbit, heterophil/ eosinophil
infiltration was observed in only hylan G-F 20 group. Further, hylan G-F 20 exhibited immunogenicity (positive PCA reaction and production of antibody) in guinea pigs. These findings suggest that the adverse events by hylan G-F 20 may be caused by allergic reactions. On the other hand, Gel-200 did not cause such an allergic reaction and exhibited no immunogenicity.

**Significance:** These results demonstrated differences in biocompatibility and immunogenicity between Gel-200 and hylan G-F 20. Gel-200 showed favorable biocompatibility and no immunogenicity compared to hylan G-F 20. Gel-One™ is expected as a patient-friendly single injection HA product for the treatment of knee OA pain.

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**References:**

![Histological appearances of the rat air pouches injected with the four different study substances](image-url)