Pharmacokinetic Analysis of Oligodeoxynucleotide after an Injection into Intervertebral Discs of Normal Rabbits

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INTRODUCTION: Degenerative disc disease is a common clinical problem. To treat degenerative disc diseases or to prevent further degeneration, direct injection into the intervertebral disc (IVD) is one of various methods used to deliver drugs. The intradiscal injection of growth factors, including osteogenic protein-1 (OP-1 or bone morphogenetic protein-7) and growth and differentiation factor-5 (GDF-5), has shown positive effects on degenerated IVDs [1, 2] in preclinical studies. To develop therapeutic drugs and frequency with which the drug is distributed during the first 28 days after injection [3]. The direct injection of oligodeoxynucleotide (ODN) containing the NFκB cis element into degenerated IVDs partially restored disc height and improved histology and MRI appearance [2]. Because ODN is a nucleotide, its molecular and binding properties may differ from proteins, such as OP-1. To date, there are no reports describing how injected ODN distributes in the IVD and how long it remains in the tissue. The purpose of this study was to perform a pharmacokinetic analysis of intradiscally-injected NFκB-ODN, specifically, to determine, using multilmod quantification techniques, its distribution, elimination, and disposition (Fig. 1). Intradiscal delivery of ODN can transport the treatment to target cells at day 16. (Fig. 2).

METHODS: Animals and Experimental Groups: NZW rabbits (n=6) were used with IACUC approval. Under general anesthesia, rabbits received intradiscal injections of various ODN solutions using an Ino microsyringe (MS*GEN25 w/XX*MS16, OD=52/0.43 mm, tapered, 27 gauge equivalent).

Pharmacokinetics Study: Thirty female rabbits received a single 10 µL injection of ODN solution (30 µg/disc) into the nucleus pulposus (NP) of the L3/L4 disc. Animals were euthanized at 8 and 24 hours, and 7, 14 and 28 days after injection, n=6 per time point. Immediately upon euthanasia, the entire IVD was dissected and frozen at -80°C. The quantity of ODN in each entire IVD was performed by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Plasma was also collected at 0.5, 1, 8 hours and 7, 14 and 28 days after injection.

Whole Body Autoradiography: Five male animals received a single 10 µL injection of 1H-ODN solution (54 µCi/animal, 100 µg/disc) into the NP of the L3/L4 disc. One animal at each time point (6, 24 hours, 14, 7 and 28 days after injection) was euthanized. After euthanasia, whole bodies of animals were prepared for cryo-sectioning. Sections were exposed to imaging plates (Fuji), the plates were scanned, and radioactivity was quantified.

Cellular Level Distribution Analysis: Female animals received a single 10 µL injection of fluorescein amide (FAM)-labeled ODN (FAM-ODN) solution (100 µg/disc) into the NP of the L2/3 and L3/4 discs. Three animals were euthanized 24 hours and 7 days after injection and two animals were euthanized 16 days after injection. Immediately upon euthanasia of animals, entire IVDs were dissected and analyzed by confocal microscopy.

RESULTS: Pharmacokinetic Study: LC/MS/MS quantification of non-labeled ODN showed that the amount of ODN remaining in the IVD after injection was fit well by a double exponential decay equation, e = C1e^(-t/τ1) + C2e^(-t/τ2) (Fig. 1 blue line). The corresponding distribution half-lives were 11.9 hours and 618 hours. At the 30 minute time point after injection, only one plasma sample out of 6 had a detectable level of ODN (5.81 ng/ml). All other plasma samples were below the detectable limit (<4.00 ng/ml) of ODN.

Whole Body Autoradiography: The radioactivity of 1H-ODN in the IVD after injection showed a double exponential decay similar to that obtained by LC/MS/MS measurements (Fig. 1 red line). At 24 hours after injection, 1H-ODN is clearly seen at the injection site as well as in the bladder (Fig. 2); this indicates that IVD-injected ODN circulates to, and is eliminated by, the kidney. Seven days after injection, 1H-ODN was detectable only in the injected IVD and not elsewhere. 1H-ODN was detected in the IVD at least up to 28 days after injection (Fig. 2, black boxed-area).

Cellular Level Distribution Analysis: FAM-ODN was seen in cells from the right frontal part to the left posterior part of IVDs at 24 hours (Fig. 3A and B). The injection was performed from the right side of the rabbits; the distribution of FAM-ODN conforms to the direction of injection (Fig. 3A). At 7 and 16 days after injection, FAM-ODN was detected in similar locations; however, the same gain condition for fluorescence used at and after 24 hours after injection was not sufficient to visualize FAM-ODN, suggesting that the amount of FAM-ODN had decreased. Fig. 3B presents a detailed distribution of FAM-ODN at day 16 after injection. FAM-ODN distributed in entire cells and strongly in nucleus.

DISCUSSION: Although the intradiscal distribution of OP-1 has been reported, the pharmacokinetics of compounds with different molecular properties and binding capacities may vary greatly. We report, for the first time, the distribution, elimination, and disposition kinetics of ODN injected into a healthy IVD. The ODN decayed with a typical two phase elimination/distribution pattern well fitted to a double exponential decay equation. The results indicate that in the first distribution phase, ODN spreads in the IVD and some could either be rapidly distributed to the circulation through the end plate, while some may leak out through the injection hole, as seen in Fig. 2. In the second elimination phase, ODN was gradually eliminated. Interestingly, the injected ODN (15%) was still detectable in the IVD 28 days after injection. These findings provide a foundation for planning the dosage and formulation of ODN. In a degenerated IVD, the vascular condition and cellular matrix differ from that of the normal rabbit IVD. To apply ODN clinically, the investigation of the pharmacokinetics of ODN in degenerated rabbit IVDs may shed light on the actual pharmacokinetics in the human IVD. The biological effects of ODN that persist long term in the IVD (especially inside cells) should be elucidated.

SIGNIFICANCE: Using radioisotope-labeled ODN, fluorescence-labeled ODN and LC/MS/MS measurements, a significant amount of ODN injected into normal rabbit IVD remains up to 28 days. The amount of ODN remaining in the IVD after injection fits well by a double exponential decay equation.


Fig. 1: The amount of ODN remaining in the IVD post-injection obtained by LC/MS/MS measurements.

Fig. 2: Whole Body Autoradiography at 24 hours after injection. 1H-ODN is clearly seen at the injection site and in the bladder. Injection sites at days 7 and 28 are superimposed on the 24 hour time point picture in highlighted black areas.

Fig. 3: Representative confocal microscopy images. A: Entire IVD at 24 hours. (x10, tile-merged). Yellow dots line presents outline of IVD. Allow indicates needle direction. B: Nucleus pulposus cells at day 16. (x63). Green signal is FAM-ODN.