Development Of Aptamer-functionalized Osteoblast-targeting Lipid Nanoparticles Encapsulating Osteogenic Sirnas For Bone Anabolic Therapy: Investigation Of Tissue-selective Distribution, Dose-response Pattern And Persistence Of Gene Silencing

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Introduction: Our established osteogenic siRNAs delivery system approaching bone formation surface for osteogenic cells at tissue level, i.e. (Asp-Ser-Ser)6-liposome (Zhang G et al. 2012), still has concerns on efficacy and safety for RNA interference-based bone anabolic therapy due to lack of direct osteoblast-specific delivery. Recently, using cell-SELEX technology, we obtained an aptamers L6 which could target osteoblasts but not hepatocytes and PBCs in vitro (Liang C et al. 2012). CKIP-1 has been reported as a negative regulator of osteoblastic bone formation by our group (Lu K et al. 2008). So, we linked L6 to the surface of PEG-modified lipid nanoparticles (LNPs) encapsulating osteogenic CKIP-1 siRNA (Zhang G et al. 2012), i.e. L6-LNPs-siRNA, which showed satisfactory serum stability and no significant cytotoxicity in vitro. Furthermore, L6 facilitated the intracellular uptake of siRNA (encapsulated in LNPs) and subsequently gene knockdown in osteoblast other than in hepatocyte and PBCs in vitro (Wu H et al. 2012). However, there is still lack of in vivo evidence to advance the above in vitro findings. So, it is necessary to investigate L6-mediated change in tissue distribution of osteogenic siRNA, dose-response pattern and persistence of gene silencing in normal rats.
Methods: To investigate L6-mediated change in tissue distribution of osteogenic siRNA, seventy-two six-month-old healthy female Sprague-Dawley rats were divided into three groups and subjected to Cy3-labeled CKIP-1 siRNA with different formulations via tail vein injection: (1) LNPs-siRNA, (2) Rd-LNPs-siRNA and (3) L6-LNPs-siRNA (n=24 for each group), respectively. Six rats in each group at 2, 4, 6 and 12 hours were sacrificed and the major organs (heart, liver, spleen, lung, kidney and bilateral femur/tibia) were collected. The fluorescence signal of major organs from the rats at each time point was detected using Xenogen IVIS imaging system. Liver and bone of the rats at each time point were homogenized for determining fluorescence by microplate reader. To determine L6-mediated changes in dose-response pattern of gene silencing, nine six-month-old female Sprague-Dawley rats were sacrificed as baseline. Forty-five six-month-old female Sprague-Dawley rats were subjected to L6-LNPs-siRNA at the siRNA dosages of 0.5, 0.8, 1.0, 3.0 and 5.0 mg/kg (n=9 for each dosage) via tail vein injection. Two days after the administration, all the rats were sacrificed and distal femurs were collected for preparation of cryosections. The CKIP-1 mRNA expression of osteocalcin-positive staining osteoblasts was quantified by real-time PCR analysis in combination with laser capture microdissection (LCM) (Wang X, et al. 2013). To determine the L6-mediated changes in persistence of gene silencing, ninety-nine six-month-old female Sprague-Dawley rats were injected with L6-LNPs-siRNA at the optimal siRNA dosage determined by the above dose-response experiments. All the rats were sacrificed and the distal femurs were collected for preparation of cryosections at 0, 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 days (n=9 for each time point) after the injection. The CKIP-1 mRNA expression in osteocalcin-positive staining osteoblasts was quantified by real-time PCR analysis in combination with LCM.
Results: Biophotonic imaging data showed that, when compared with LNPs-siRNA or Rd-LNPs-siRNA group, the fluorescence signal intensity of bone in L6-LNPs-siRNA group was significantly higher across all the examined time points, whereas the fluorescence signal intensity in liver decreased in time-dependent manner and almost disappeared at 12 hours (Figure 1A). The quantification data from the fluorescence microplate readers was also consistent with the findings from the biophotonic imaging (Figure 1A, 1B). The fluorescence signal of siRNA was hardly detected in the hearts, spleens, lungs and kidney in rats with administration of all these three formulations across all the examined time points (Figure 1A). CKIP-1 mRNA level was efficiently decreased in a dose-dependent manner and the CKIP-1 gene silencing efficiency was almost achieved toward 80% after the administration with the L6-LNPs-siRNA at 1mg/kg of siRNA (Figure 2A). In addition, after the injection of the L6-LNPs-siRNA at 1mg/kg of siRNA, the highest CKIP-1 gene silencing was observed at 2 days and the effective duration for CKIP-1 at a level lower than 50% of the baseline lasted for 7 days (Figure 2B).
Discussion: L6 aptamer facilitated the distribution of the siRNAs in bone and reducing their accumulation in liver in normal rats. The effective duration for CKIP-1 at a level lower than 50% of the baseline lasted for 7 days after the injection of L6-LNPs-siRNA at 1mg/kg of siRNA.
Significance: Aptamer-functionalized osteoblast-targeting delivery system for osteogenic siRNAs will help to update the targeted

delivery from tissue level toward cellular level for accommodating RNAi-based clinical bone anabolic strategy in efficiency and safety to metabolic skeletal disorders associated with impaired bone formation.

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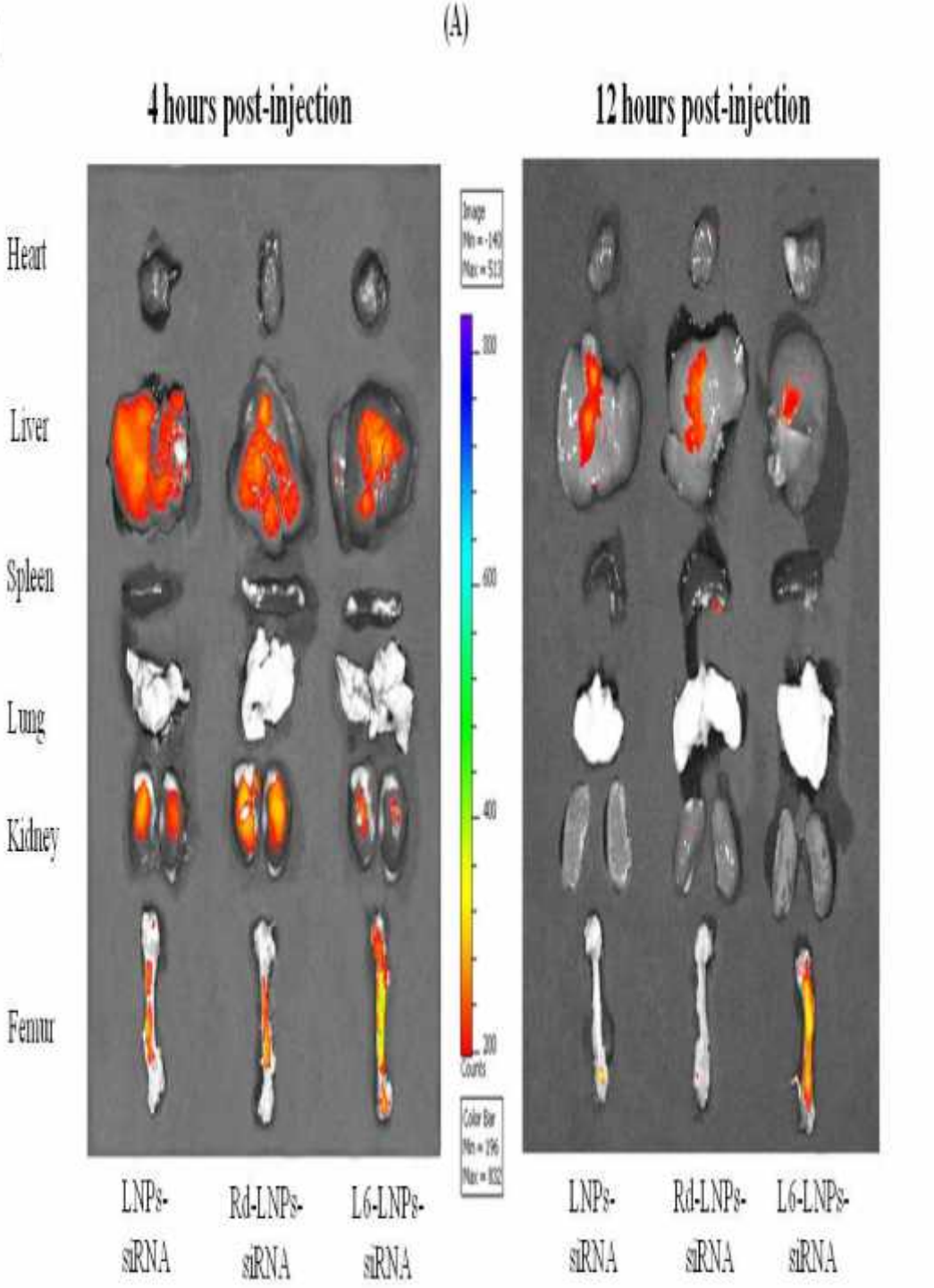
formation. Nat Med. 2013;19(1):93-

Figure 1


(A)
4 hours post-injection
12 hours post-injection
Heart
Liver
Spleen
Lung
Kidney
Femur
LNPs-siRNA
Rd-LNPs-siRNA
L6-LNPs-siRNA
LNPs-siRNA
Rd-LNPs-siRNA
L6-LNPs-siRNA
(B)
Percentage of dose in bone (%)
Percentage of dose in liver (%)

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