Inhibition of osteolysis and increase of bone formation after local administration of siRNA-targeting RANK in a polyethylene particle-induced osteolysis model

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1. Introduction

During the periprosthetic osteolysis process, wear particles released from bearing surfaces result in a local inflammatory response challenged by proinflammatory macrophages (M1) that produce interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) [1]. The largest particles (>10 μm) coated with proteins may act as damage-associated molecular pattern molecules (DAMPs) and are sensed by macrophagic toll-like-receptors (TLRs) [2–4], leading to the activation of an innate inflammatory immune response such as a “foreign body reaction” [5,6]. Otherwise, the smallest particles (<10 μm) and ions are up taken, activating the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome, leading to an adaptive immune response [3,5–7]. Both immune pathways activate the transcription factors NF-κB and NF-κB, increasing synthesis of the receptor activator of nuclear factor kappa-B ligand (RANKL) and pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) [8,9]. These cytokines were recognized as the main molecules responsible for maintaining the periprosthetic inflammatory environment and for increased osteoclastogenesis [8].

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Inhibition of the RANKL–RANK axis is an efficient therapeutic approach for decreasing osteoclast differentiation/activation. Antiresorptive drug-based therapies, such as bisphosphonates and the anti-RANK monoclonal antibody (Denosumab™), have evolved from experimental models of osteolytic bone diseases [10–13] to clinical uses [14–16]. In addition, new inhibitors, such as small peptides designed to target a specific region of the RANK membrane, are currently in preclinical development [17]. The prevention of bone loss by blocking the RANK–RANKL axis was reported in an experimental model of particle-induced osteolysis using a recombinant protein of RANK (RANK-Fc) [18] and by inducing the osteoclast apoptosis using zoledronic acid (ZOL) [19]. Despite these encouraging preclinical results, they have still not been transferred to clinical use [20].

Small interference ribonucleic acid (siRNA) regulates the synthesis of proteins by means of a specific gene silencing mechanism [21–23]. The use of siRNA-based therapy is a specific and biocompatible approach that has led to significant advances in cancer, age-related macular degeneration and viral diseases [24]. Two key aspects need to be considered in this strategy: the identification of clinically relevant targets and the use of efficient delivery vectors. Targeting of the key RANK–RANKL–axis was first reported in vitro in murine cells by Wang et al. [25] and Ma et al. [26] with effective inhibition of RANK expression, osteoclast differentiation and osteolysis using Rank-siRNAs and Rank-shRNAs, respectively, which target the mouse Rank transcript [25–27]. The systemic delivery of therapeutic siRNAs using biological and synthetic vectors was reported in bone disease experimental models, including bone-metastatic cancer (targeting Luciferase (LucF) and delivered by a recombinant adenoviral vector was reported [31]. Similarly, a siRNA targeting of the type I bone morphogenetic protein receptor transcript (BMPR-IB) systematically delivered by a recombinant adeno viral vector was reported [31]. However, the nature of aseptic loosening by wear debris seems to be a confined condition that requires a local intervention. The local delivery of siRNAs is then a logical strategy for bypassing the anatomical barriers and optimizing its biotransformation during its transport. In this context, the local delivery of Rankl-siRNA by the cationic liposome DMA-PAP/DOPE in a murine model of osteosarcoma [32] and a local lentiviral delivery of p1100-siRNA, targeting a subunit of the PI3K/AKT pathway in a particle-induced osteolysis model, were reported [33]. However, there are no scientific reports using siRNA-based technology targeting the key RANKL–RANK axis by local delivery using a synthetic vector in an in vivo model. We hypothesized that siRNA targeting RANK, locally delivered in situ by a cationic liposome might be an effective approach for inhibiting osteoclastogenesis in vivo. The aim of our study was to unveil the therapeutic effect of three doses of siRNA targeting both human RANK and mouse Rank transcripts (RANK-811 siRNA) in a mouse model of polyethylene (PE) particle-induced osteolysis.

2. Material and methods

2.1. SiRNAs

All siRNAs were ordered from Eurogentec (Angers, France) with 3’ overhanging dTdT and with annealed sense and reverse strands. The primer sequence sense strand 5’-GUGCGAAAUAGCAGUGCUCC-3’ was designed to target Homo sapiens Rank mRNA (NM_003839; Tumour Necrosis Factor Receptor Superfamily 11A, TNFRSF11A) at start positions 811 and was named RANK-811. The antisense strand of RANK-811 siRNAs presents perfect complementarity with Mus musculus Rank mRNA (NM_009399.2) at start positions 804. RANK-811 siRNA was selected based on its efficacy for decreasing RANK expression in RANK-overexpressing human embryonic kidney 293 (HEK 293) cells [17] and Rank in murine RAW 264.7 monocyte cells (American Type Culture Collection, Promochem, Molsheim, France) (Supplementary Information Fig. S1).

The siRNA duplex (sense strand 5’–UUUCUGCAACGGUGUACC-3’) which did not show significant homology with any mouse mRNA sequence according to BLAST database searches, was used as a negative control and designated Ct-siRNA. A previously validated siRNA (LucF-siRNA sense strand 5’–CUACCCGUAGACCU-3’) was used in vivo as an innocuous siRNA [32].

2.2. Particles of polyethylene

Pure PE particles (Ceridust 3610™) were purchased from Clarient (Gersthofen, Germany). The morphology of particles was assessed by scanning electron microscopy (JEOL, model 6400F). The particle size and distribution were determined by five consecutive measurements obtained with a Coulter Counter™ (Beckman Coulter Inc., USA). The mean particle size was 7.23 μm and with a distribution of d10 = 1.15, d25 = 3.94, d50 = 7.14, d75 = 10.28 and d90 = 13.05. To eliminate endotoxins, the particles were washed in ethanol, dried and then aliquoted until use [34]. Endotoxin levels were measured using a quantitative Limulus Amebocyte Lysate (LAL) assay (Lonza, Belgium). The threshold of positivity was 0.25 EU ml−1.

2.3. Implantation of polyethylene particles in mouse calvaria

The mice (Elevages Janvier, Le Genest Saint Isle, France) were housed in pathogen-free conditions at the Experimental Therapy Unit (Faculty of Medicine, Nantes) in accordance with the institutional guidelines of the French Ethical Committee (CEEA Pdl 06 ethical committee, authorization no. 1280.01) and under the supervision of the authorized investigators. All surgical procedures were also performed according to international ethical guidelines for animal care (authorization no. 2012-198). Twenty-one C57BL/6 male mice (Janvier, Le Genest-Saint Isle, France) aged 10 weeks were randomly divided in two groups (Table 1). Eighteen of the mice were surgically implanted using the adapted mouse calvaria model [35] with 20 mg of dried PE particles (PE-implanted group). Briefly, under general anaesthesia (2–3% isofluorane in 100% oxygen), the calvaria was exposed by a midsagittal incision in previously shaved and anesthetized mice (Betadine, France). The dried PE powder was uniformly spread over the periosteum with a sterile surgical spoon. The surgical approach was carefully closed with 5–0 non-absorbable sutures. A subcutaneous injection of buprenorphine (Buprecar 0.1 mg kg−1) was performed after the surgical procedure (Palier 1 protocol). One group of three mice underwent the same surgical procedure but without the PE particle implantation (Sham group).

2.4. Local injections of formulated siRNAs

For in vivo injections, siRNAs were premixed with an equal quantity of a deoxyribonucleic acid (DNA) adjuvant in 150 mM sodium chloride as described in Ref. [36] and mixed with an equivalent volume of cationic liposome DMA-PAP/DOPE at a ratio of 6 nmol of cationic lipid per microgram of nucleic acid as previously described [37]. The lipopolysomes were formed at room temperature for at least 30 min. Injections of 50 μl of lipopolysomes containing 2.5, 5 or 10 μg of siRNAs were used in vivo.

The PE-implanted mice were randomly divided in four groups which received siRNA injections, while the Sham group (non-implanted; n = 3) received 50 μl of saline solution (NaCl 0.9%) (Table 1). Three groups of PE-implanted mice received a total of
three injections (at days 1, 4 and 7 after surgery) in the calvaria site, of either 2.5 or 5 or 10 μg of \textit{RANK}-811 siRNA and respectively named PE-2.5 μg \textit{RANK}-811 siRNA (n = 5), PE-5 μg \textit{RANK}-811 siRNA (n = 5) and PE-10 μg \textit{RANK}-811 siRNA group (n = 5). One PE-implanted group received three injections of 2.5 μg \textit{LucF}-siRNA and was named the PE-2.5 μg \textit{LucF}-siRNA group (n = 3). The injection site was determined intermediate to the full cephalo-caudal length of the sutured surgical approach and 2 mm laterally to the right of it. The inclination of the 26-gauge needle was oblique, pointing medially until a soft contact with the bone surface was made. A continuous and slow pressure injection, to avoid the reflow of injected solution, was performed. All animals were killed by cervical dislocation following isofluorana anaesthesia at day 9 after the surgical procedure.

### 2.5. Micro-computed tomography assessment

The analysis of bone microarchitecture was performed in fixed calvariae at the time corresponding to the necropsy (day 9) using a high-resolution X-ray micro-computed tomography (micro-CT) system for small-animal imaging (SkyScan-1076, SkyScan, Kontich, Belgium) (Table 1). All calvariae were scanned using the same parameters (pixel size 9 μm, 50 kV, 0.5 mm Al filter and 0.8° of rotation step). Three-dimensional reconstruction and quantification of bone parameters were performed in a cylindrical volume of interest (VOI) (ratio 0.5 mm, height 1.143 mm, VOI = 0.89 mm³; Fig. 2A) using ANI and CTvol software (Skyscan). The assessment of the bone volume density was measured by the fraction of the VOI (i.e. the total volume, TV) that is occupied by mineralized bone (bone volume, BV) (BV/TV) expressed as a percentage (%).

### 2.6. Histological analysis

Harvested calvariae were fixed in 4% buffered formaldehyde for 24 h and decalcified with 4.13% ethylenediaminetetraacetic acid (EDTA) and 0.2% paraformaldehyde in phosphate-buffered saline (PBS) for 96 h using a KOS microwave histostation (Milestone, Kontich, Belgium) (Table 1). All calvariae were scanned using the same parameters (pixel size 9 μm, 50 kV, 0.5 mm Al filter and 0.8° of rotation step). Three-dimensional reconstruction and quantification of bone parameters were performed in a cylindrical volume of interest (VOI) (ratio 0.5 mm, height 1.143 mm, VOI = 0.89 mm³; Fig. 2A) using ANI and CTvol software (Skyscan). The assessment of the bone volume density was measured by the fraction of the VOI (i.e. the total volume, TV) that is occupied by mineralized bone (bone volume, BV) (BV/TV) expressed as a percentage (%).

### 2.7. RNA extraction from calvaria tissue and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from three implanted calvaria samples of each group using homogenizer Ika Ultra-Turrax T 25 (Janke and Kunkel) and TRIzol reagent (Invitrogen) (Table 1). Synthesis of the first strand of complementary DNA and the polymerase chain reaction were carried out in triplicate using the same protocol as described above. The listed oligonucleotides were used to amplify \textit{Mus musculus} receptor activator of nuclear factor kappa-B (\textit{RANK}, 5'-TGACGCTCTCTGATCGACTG-3' and 5'-CAGGTTACTATACGACAGATTG-3'), Cathepsin K (\textit{CatK}, 5'-GAGGCCGCTATATAGCACTGAAAAGAAG-3' and 5'-GCTGAGGATTCTGACCAGAAAG-3'), tumour necrosis factor alpha (\textit{TNF-α}, 5'-GGGTTATCGCTGCTTCCAAAAGGGAA-3' and 5'-TGGTTTGCTCACGACTGTTGACAC-3'), interleukin-6 (\textit{IL-6}, 5'-TCTGCTCTCTTCTACCCCAATTTCC-3' and 5'-TTGGTCTCTTTACGCTC-3'), interleukin-1β (\textit{IL-1β}, 5'-TTGACCGACCCAGAAGAAG-3' and 5'-GATGCTCTGCTGCGAGATT-3'), interleukin-1 (\textit{IL-1}, 5'-TTGACCGACCCAGAAGAAG-3' and 5'-GATGCTCTGCTGCGAGATT-3'), cytochrome c-1 (\textit{Cy C 1}, 5'-TGTGCTACA CGAGAAGAAA-3' and 5'-CACATCATTAGGGCCATCC-3') and 60S ribosomal protein L19 (\textit{Rpl19}, 5'-TGGTTGCAGAAAACAC-3' and 5'-AGGTACCTCTTCTACGAC-3'). Cyc and \textit{Rpl19} were used as housekeeping genes. Target gene expression was normalized to a housekeeping gene in respective samples as an internal standard, and the comparative cycle threshold method was used to calculated relative quantification of target messenger RNAs. Analyses were then performed using the Vandesompele method [41].

### 2.8. Statistical analysis

All analyses were performed using GraphPad InStat version 3.02 software (GraphPad Software, La Jolla, CA, USA). In vitro experimentation and micro-CT results were analyzed with the unpaired non-parametric Mann–Whitney U-test using two-tailed P values. Each \textit{RANK} siRNA group was compared independently with the \textit{LucF} siRNA group. The histological results were analyzed with
ANOVA and an unpaired parametric two-tailed $t$-test. Results are given considering a $P$ value <0.05 as representing significance.

3. Results

3.1. RANK-811 siRNA prevents the bone loss induced by implanted PE particles by inhibiting osteoclastogenesis and osteoclast activation

A first assessment of the anti-resorptive effect of RANK-811 siRNA in vivo at day 9 after implantation of PE particles using micro-CT showed that the inner cortical of the calvaria was protected from osteolysis in the groups treated with 5 or 10 μg of RANK-811 siRNAs. However, an increase in the bone volume was only observed at a dose of 10 μg of RANK-811 siRNA (BV/TV = 19.11% ± 2.3) compared to the control siRNA (BV/TV = 10.57% ± 0.6; $P = 0.006$). However, severe bone loss of the inner cortical area was observed for the group treated with 2.5 μg of RANK-811 siRNAs (Fig. 1A, B).

TRAP staining revealed that osteoclasts detected in the groups injected with 5 or 10 μg of RANK-811 siRNAs were smaller than those in the groups injected with 2.5 μg LucF- or RANK-811 siRNAs (Fig. 2A). In addition, RANK-811 siRNAs inhibited osteoclast differentiation in a dose-dependent manner. Indeed, the percentage of TRAP-positive cells was reduced by 70% with 2.5 μg RANK-811 siRNA ($P = 0.003$) and by 90% with 5 ($P = 0.0009$) and 10 μg ($P = 0.0007$) RANK-811 siRNA administration (Fig. 2B).

The inhibitory effect of RANK-811 siRNAs on osteoclast activity was also confirmed by the downregulation of Rank expression after 5 ($P = 0.0001$) and 10 μg ($P = 0.006$) RANK-811 siRNA administration (Fig. 2B). In addition to the inhibition of osteoclastogenesis, RANK-811 siRNAs was also decreased Cathepsin K expression (Fig. 2B), demonstrating the inhibition of the osteoclast activity with 5 ($P = 0.001$) and 10 μg ($P = 0.001$) RANK-811 siRNA. In contrast, 2.5 μg RANK-811 siRNA did not modify Rank expression (not significant (NS)) and induced an increase of Cathepsin K expression ($P = 0.01$) (Fig. 2B).

![Fig. 1. RANK-811 siRNA exhibits a significant protective effect against PE particle-induced osteolysis in vivo. PE particles were implanted or not (Sham-NaCl) over the periosteum of calvaria for 9 days. Increasing doses (2.5, 5 and 10 μg) of RANK-811 siRNA were injected locally and bone quantity was followed, compared to the control LucF siRNA. PE particle-induced osteolysis and the efficacy of RANK-811 siRNA was followed qualitatively by micro-CT scan (A) and quantitatively determined by the measure of the BV/TV (B). Bone preserved areas were recognized in spongy bone as well as in external and inner corticals (black arrows). **$P<$ 0.01.](image-url)
3.2. Local treatment with the formulated siRNA induces an anabolic bone response characterized by the total renewal of osteoblastic cell lineage

PE-implanted mice exhibit osteonecrotic and osteolytic lesions. Thus, we confirmed the presence of empty lacunae, without osteocytes, in the external cortex of the calvaria of PE-implanted mice compared with non-implanted mice (Fig. 3A). However, characteristic osteocytes located in their cavities were observed in the inner cortex of the calvariae in all groups assessed (Fig. 3A). Interestingly, new bone formation was observed in the PE-implanted and siRNA-treated mice compared to the Sham group, as demonstrated by ALP (Fig. 3A) and osterix (Fig. 3B) positive immunostaining in the area of interest.

3.3. RANK-811. siRNA reduced the inflammatory environment without depletion of macrophages

Because it is known that wear debris induces an inflammatory reaction [7,42], we assessed the consequence of a RANK blockade on the inflammatory process. Histological analysis showed that the implantation of PE particles resulted in a reaction characterized by the thickening of an inflammatory membrane more infiltrated by cells compared with the non-implanted group (Fig. 4A). F4/80+ macrophages were detected either in the Sham group (loosely distributed in the subcutaneous connective tissue) as well as in the implanted groups (highly localized within the inflammatory membrane and surrounding the implanted particles) (Fig. 4A). Similarly, we observed the proinflammatory effect of PE particles by the increased expression of proinflammatory cytokines genes (Tnf-α, Il-6 and Il-1β) from the PE-LucF-siRNA group compared with the Sham–NaCl group (Fig. 4B). We observed that the injections of 2.5 μg RANK-811 siRNAs decreased Tnf-α expression (P = 0.007) and slightly increased the expression of Il-1β (P = 0.04) compared to the LucF siRNA injections. Il-6 expression was not modulated using the same dose of siRNA (NS). In contrast, the injections of 5 and 10 μg of RANK-811 siRNAs decreased Tnf-α expression (P = 0.001) and the expression of Il-1β (P = 0.04) compared to the LucF siRNA injections. Il-6 expression was not modulated using the same dose of siRNA (NS). In contrast, the injections of 5 and 10 μg of RANK-811 siRNAs decreased the expression of Tnf-α (P = 0.0001 and P = 0.0002, respectively), Il-6 (P = 0.0001 and P = 0.0009, respectively) and Il-1β (P = 0.0001 and P = 0.008, respectively) (Fig. 4B). Interestingly and despite the down-regulation of proinflammatory genes associated with the injection of 5 and 10 μg of RANK-811 siRNA and the variable response of the same parameters for the same genes using 2.5 μg RANK-811 siRNA, the macrophage infiltration was not repressed (Fig. 4A).

4. Discussion

Targeting the components of the triad OPG/RANKL/RANK by interference of RNA seems to be a promising approach. Experimental studies blocking RANK–RANK signalling by siRNAs targeting
M. musculus Rank have previously been reported in in vitro systems with an effective inhibition of Rank expression, osteoclast differentiation and osteolysis [25–27]. Furthermore, the efficacy of siRNAs targeting Rankl was reported in in vitro culture of RANKL-expressing HEK 293 cells and in a murine model of osteosarcoma [32,37]. Our study provides strong evidence of the efficacy of three formulated cross-species sequences of RANK siRNAs in both human RANK-expressing HEK 293 cells and in murine RAW 264.7 cells in vitro (Fig. S1). Then, once the down-regulation of RANK and Rank was confirmed, we selected the most efficient sequence among the three evaluated (RANK-811 siRNA with 48.7% of inhibition) to assess its effect on osteolysis in vivo (Fig. S1).

Our study shows for the first time an effective inhibition of particle-induced osteolysis in an in vivo model using a RANK-directed siRNA sequence. We observed a large reduction in the number of TRAP+ activated cells in the parasagittal area on implanted calvariae using three different doses of RANK-811 siRNAs. This observation revealed a high ability of the osteoclast precursors to uptake the injected lipoplex (RANK-811 siRNA with synthetic vector, cationic liposome DMAPAP/DOPE and plasmid). This synthetic vector has also shown efficacy in the local delivery of formulated Rankl-directed siRNA by intra-tumour injection in previous reports [32,37]. Moreover, Khoury et al. [29,30], reported a successful systemic delivery of siRNAs targeting proinflammatory cytokines (Tnfα, Il-1β, Il-6 and Il-18) in a mouse model of rheumatoid arthritis using the same siRNA formulation [29,30].

While the doses of 5 and 10 μg of RANK-811 siRNAs decreased the number of TRAP+ cells and the expression of osteolytic genes (Rank and Cathepsin K), confirming the inhibition of osteoclastogenesis and osteolysis, we observed a contradictory response with the dose of 2.5 μg. These findings may be explained by an imbalance between the amount of Rank transcript and the weak dose of siRNA (2.5 μg). Supporting this observation, the volumetric assessment by micro-CT showed that only the maximal dose assessed, 10 μg of RANK-811 siRNA, prevented bone loss compared to the control group (PE-LucF-siRNA). Furthermore, a major effectiveness of lipoplex in the external cortical area rather than in the inner cortical area of the calvaria was also observed. These differences may be explained by a reduced diffusion of the injected volume to the inner cortical area due to the modified local anatomy (eroded surface of bone and trabecular spaces in the diploe), by the local inflammatory reaction and/or the viscosity of the...
lipoplex. The present results were consistent with a direct antiresorptive effect of RANK-811 siRNA, presumably on RANK+ pre-osteoclasts and/or mature osteoclasts, demonstrated by the inhibition of osteoclastogenesis and the decrease of osteoclast activity (Figs. 1 and 2) similarly to the zoledronic acid treatment (Fig. S2). Interestingly, the unexpected bone formation observed in siRNA-treated mice suggests that the injection of this lipoplex might create a favourable local anabolic microenvironment. Thus, a complete renewal of osteoblastic lineage characterized by a sequence of localized osteolysis/osteonecrosis induced by PE particles, followed by a pulse of bone formation induced by the RANK-811 siRNA may be proposed. The osteogenic signs observed in the central area of calvariae may be secondary to the inflammatory microenvironment generated by particle implantation and/or possibly, the immunogenic effect of lipoplex. The link between inflammation and osteogenesis was recently proposed and might be regulated by oncostatin M signalling produced by monocytes/macrophages [43]. In addition, a STAT3 activation in mesenchymal stem cells was reported [44]. Further studies need to be performed to elucidate the osteogenic effect showed in our report.

On the other hand, the dramatic inhibition of the expression of proinflammatory cytokines in harvested tissues of RANK-811 siRNA-treated mice (including RANK+ pre-osteoclasts and osteoclasts) could also actively participate in the modulation of the inflammatory response. We also observed the persistence of macrophages both in non-implanted (Sham) and in RANK-811 siRNA-treated groups. While those observed in the Sham group exhibited a heterogeneous distribution in connective tissue and could be associated with the inflammatory process after a surgical approach, those observed in the RANK-811 siRNA-treated groups were always closely related to the PE particles (presumably phagocytosed by them). This fact could be related with the paradoxical rise of the cytokine expression observed with the highest dose (10 µg) of siRNA. A potential immunoactivation role of the lipoplex [45,46] (or some of its components, i.e. cationic liposome or nucleic acids) could explain these findings. However, in a previous study, we showed that the same construct used in an intra-osseous site did not activate the systemic immune response [32]. These contradictory data reinforce our hypothesis for the existence of non-identified cellular sources for pro-inflammatory mediators (i.e. osteoclast precursors, dendritic cells, lymphoid cells and/or stromal cells).

The difficulty in determining the cell(s) type(s) that effectively internalize(s) the RANK-811 siRNA administered is the first limitation of the present study. The use of carboxyfluorescein-labelled-siRNAs (FAM-siRNAs) would allow the identification of the cells incorporating this siRNA. The interest of FAM-siRNAs delivered by cationic liposomes to detect its trafficking in bone tissue was previously shown by Zhang et al. [47]. In addition, the cationic

![Figure 4](image-url)

**Fig. 4.** RANK-811 siRNA does not modulate the invading macrophages. PE particles were implanted or not (Sham-NaCl) over the periosteum of calvaria for 9 days. Increasing doses (2.5, 5 and 10 µg) of RANK siRNA-811 were injected locally and inflammatory status was followed compared to the control LucF siRNA. Macrophages were identified by anti-F4-80 antibody in all groups (implanted and sham) mice (A). The relative expression of Tnf-α, Il-6 and Il-1β was determined in explanted tissues by qRT-qPCR. While 5 and 10 µg RANK-811 siRNA decreases the expression of pro-inflammatory Tnf-α, Il-6 and Il-1β cytokines, the dose of 2.5 µg induces a variable response decreasing Tnf-α, maintaining Il-6 and increasing Il-1β expression. (B). *P < 0.05; **P < 0.01; ***P < 0.001.
liposome used in Zhang et al.’s study has been combined with a peptide for delivering siRNAs specifically to bone-forming surfaces. In our study, the liposome DMAPAP/DOPE was not functionalized for such bone targeting. However, we observed a delivery of RANK-siRNAs by the cationic liposome in osteoclasts of implanted calvaria as demonstrated by the marked decrease in the TRAP cell number. Moreover, the internalization of siRNAs using the cationic liposome DMAPAP/DOPE was confirmed in monocyte/macrophages in a murine model of rheumatoid arthritis [30]. Further studies will be necessary to reveal the specific cell(s) that uptake RANK-811 siRNA and to determine the intracellular sites of interaction. Finally, we will present consistent evidence about the anti-resorptive effects of siRNA in experimental conditions, the promise of using anti-inflammatory and bone anabolic effects needs to be confirmed in a large number of samples.

5. Conclusions

Following local treatment with three doses of RANK-811 siRNA delivered by the cationic lipid DMAPAP/DOPE in a PE particle-induced osteolytic mouse model, the bone volume was preserved delivered by the cationic lipid DMAPAP/DOPE in a PE particle-

References

Acknowledgements

Financial support for this research was provided by the Agence Nationale de la Recherche, Grant 2007 Pathophysiology of Human Diseases Project No. RO 7196N, INSERM, University of Nantes, France and by CONICYT—Becas Chile, Chile.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2014.10.042.

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