Characterization of alendronic- and undecylenic acid coated magnetic nanoparticles for the targeted delivery of rosiglitazone to subcutaneous adipose tissue

Katayoun Saatchi, PhD\textsuperscript{a}, Sarah E. Tod\textsuperscript{b}, Donna Leung\textsuperscript{a}, Kenton E. Nicholson\textsuperscript{b}, Irene Andreu\textsuperscript{c}, Christian Buchwalder\textsuperscript{a}, Veronika Schmitt\textsuperscript{a}, Urs O. Häfeli, PhD\textsuperscript{a,}*, Sarah L. Gray, PhD\textsuperscript{b,}*

\textsuperscript{a}Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada
\textsuperscript{b}Northern Medical Program, University of Northern British Columbia, Prince George, BC, Canada
\textsuperscript{c}Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain

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Abstract

Obesity is a state of positive energy balance where excess white adipose tissue accumulates to the detriment of metabolic health. Improving adipocyte function with systemic administration of thiazolidinediones (TZDs) improves metabolic outcomes in obesity, however TZD use is limited clinically due to undesirable side effects. Here we evaluate magnetic nanoparticles (MNPs) as a tool to target rosiglitazone (Rosi) specifically to adipose tissue. Results show Rosi can be adsorbed to MNPs (Rosi-MNPs) with hydrophobic coatings for which we present binding and release kinetics. Rosi adsorbed to MNPs retained the ability to induce PPAR\textsubscript{γ} target gene expression in cells. Biodistribution analysis of radiolabeled Rosi-MNPs revealed a fat-implanted magnet significantly enhanced localization of Rosi to the targeted adipose tissue when administered by subcutaneous injection to obese mice. We propose MNPs for targeted delivery of anti-diabetic agents to superficially located subcutaneous adipose tissue.

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Key words: Obesity; Type 2 diabetes; Adipose tissue; Magnetic nanoparticles; Drug targeting; PPAR\textsubscript{γ}

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*Corresponding authors.

E-mail addresses: urs.hafeli@ubc.ca (U.O. Häfeli), sarah.gray@unbc.ca (S.L. Gray).

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Thiazolidinediones (TZDs) are a class of anti-diabetic drugs that have been shown to potently enhance insulin sensitivity by inducing positive effects on adipose tissue function. The molecular target for TZDs is peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear transcription factor that regulates transcription of genes involved in lipid metabolism and adipocyte differentiation. The TZDs specifically activate PPARγ, initiating gene expression that enables previously saturated adipose tissue to take up more lipids, reducing lipotoxicity and inducing an insulin sensitizing adipokine profile.

Despite these highly desirable anti-diabetic outcomes, the use of TZDs in clinical medicine is limited because each causes adverse side effects when administered systemically. Pioglitazone increases risk of bladder cancer in T2D patients, while Rosiglitazone (Rosi) induces peripheral edema increasing the risk of congestive heart failure. Rosi also increases bone loss and subsequent bone fracture, especially among women, likely because PPARγ promotes bone remodeling at the level of cell differentiation.

Given the clinical risks associated with systemic TZDs and knowledge that many of the anti-diabetic effects of TZDs are mediated through adipose tissue, we propose targeted delivery of Rosi specifically to adipose tissue as a desirable strategy for the treatment of T2D. Targeted drug delivery is expected to reduce or eliminate adverse side effects while maintaining effective treatment of T2D. The expansive body of literature associated with the effects of TZDs on metabolic health provides a prototype for adipose tissue-targeted drug delivery that could be applied to a myriad of other molecular targets. To target drug delivery to adipose tissue we propose superparamagnetic iron oxide nanoparticles, which can be directed to specific sites using magnetic fields. The magnetic nanoparticles (MNPs) most commonly used consist of a magnetite (Fe₃O₄) core, which is biocompatible, non-toxic, non-immunogenic, able to bind various drugs and has been shown to be effectively retained in the target tissue through interactions with the cell membrane and phagocytic uptake by cells.

Despite the rising popularity of MNPs for use in medicine, there is a lack of knowledge with respect to their impact on health. Here we assessed MNP retention within targeted adipose tissue, evaluated cell viability when exposed to MNPs, and tested the functional activation of gene expression in live cells to characterize drug activity when adsorbed to MNPs. This work demonstrates a critical step in assessing the potential use of MNPs for targeted delivery of anti-diabetic drugs to adipose tissue before evaluating physiological responses of the drug formulation in in vivo animal models of obesity and T2D.

Methods

Synthesis of magnetic nanoparticles (MNPs)

MNPs were made by a slow nitrate intermediate oxidation process to make large single domain particles. Briefly, NaNO₃ (225 mg) was added to NaOH (25 mM, 475 mL). Upon dissolution of the nitrate salt, FeCl₃•4H₂O (0.1 M, 25 mL) prepared in deionized and deoxygenated H₂O was added. Reactions, performed under inert atmosphere, were stirred at 60 rpm for 22–24 h at room temperature (RT). Magnetic separation was performed for 2 min, the supernatant removed, and sample washed with 10 mL of 0.1 M HCl for 30 secs. Coating was performed by adding 10 mM alendronic acid (Al) (TCI, Portland, Oregon, USA) or ethanol-dissolved undecylenic acid (Un) (Sigma-Aldrich, Oakville, Ontario, Canada) to the particle suspension with stirring at 400 rpm for 2 h. After coating, all samples were washed (10 mL dH₂O or ethanol ×3) to remove excess coating material. Particles were redispersed in 1 mL H₂O or ethanol accordingly. An ultra-sensitive scale was used to accurately determine the concentration (mass/vol) of each batch of coated MNPs.

Characterization of MNPs

Transmission Electron Microscopy (TEM) images were taken to characterize the size and morphology of the coated MNPs. Drops of dilute MNP suspensions were evaporated on top of carbon-coated Cu grids. Observations were made on a Joel 2100 TEM working at an acceleration voltage of 120 kV. The size of the MNPs was determined by measuring the diameter of 300 particles from different images. The hydrodynamic size and zeta potential of the coated MNPs was characterized in dilute aqueous suspensions by Dynamic Light Scattering using a Malvern Zetasizer (Westborough, MA, USA). First magnetization curves of the coated MNPs at 300 K and between 0 and 5 T were acquired using a SQUID magnetometer MPMS-XL (Quantum Design, CA, USA). The solvent of the coated MNP suspensions was evaporated on a polycarbonate special-purpose capsule. The saturation magnetization (Mₛ) taken as the magnetization value at 5 T, and the initial susceptibility (χ) were calculated after removing the diamagnetic contribution of the capsule, considering the mass of the coated MNP.

Ligand modification and ⁶⁷Ga-Radiolabeling of MNPs

The Al coated particles were modified with the chelator p-SCN-bz-NOTA (Macrocyclics, Dallas, Texas, USA) in a single step taking advantage of the free amine group on Al and then radiolabeled with Gallium-67 (⁶⁷Ga, T½ = 78.3 h) by incubation. 100 μL of the Al coated particles were washed magnetically with NaHCO₃ (1 mL, 0.1 N ×2) and incubated with 1 mg/mL chelator p-SCN-bz-NOTA solution at 21 °C overnight and magnetically washed and re-suspended in NH₄OAc (0.1 N, 600 μL). NOTA, a neutral molecule of about 630 Da is a very well investigated ligand complex is thus expected over time. Particles were radiolabeled by adding ⁶⁷GaCl₃ (233.5 MBq) to the suspension and incubating at RT for 20 min with mixing, followed by magnetic filtration and washing twice with buffer (96.03% labeling efficiency). The radiolabeled particles were dispersed in PBS for further use.

Adsorption of rosiglitazone to magnetic nanoparticles (Rosi-MNPs)

A 350 μg/mL solution of Rosiglitazone-HCl (Rosi) (Ontario Chemicals Inc., Guelph, Ontario, Canada) was prepared in RT dH₂O and then filtered (0.22 μm syringe filter). Al-MNPs (200 μg), or
Un-MNPs (200 μg) were washed with RT dH2O in a 2 mL glass vial. Particles were resuspended in 1 mL Rosi (350 μg/mL) and binding was allowed to proceed for 23 h at RT on a microplate shaker (750 rpm) to discourage particle aggregation. Following binding, particles were filtered magnetically and resuspended in 1 mL PBS at 37 °C. The suspension was vortexed at 37 °C for 7 h to wash away loosely associated Rosi. PBS was removed as above and particles were resuspended in aqueous medium (H2O or PBS) at the desired concentration for subsequent experiments.

To generate adsorption curves, adsorption was carried out as above, and 2 μL of solution were collected periodically after attracting the particles to a strong magnet to prevent the MNPs from interfering with the measurement. The concentration of Rosi in the samples was measured at an absorbance of 318 nm (Nanodrop ND-1000, Thermo Scientific, Rockford, IL, USA) and calculated from the equation of a standard curve constructed from a serial dilution of Rosi dissolved in 100% ethanol. Percentage of Rosi adsorbed was then plotted against time. To generate release curves after 23 h of adsorption, MNP were attracted to the bottom of the vial with a strong magnet and the solution carefully decanted. The adsorbed was then plotted against time. To generate release curves from the equation of a standard curve constructed from a serial dilution of Rosi dissolved in 100% ethanol. Percentage of Rosi adsorbed was then plotted against time. To generate release curves after 23 h of adsorption, MNP were attracted to the bottom of the vial with a strong magnet and the solution carefully decanted. The adsorbed was then plotted against time.

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or 500 μg/mL MNP, in a final volume of 100 μL and incubated for 48 h at 37 °C, after which the MTT assay was performed (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide) (EMD Millipore).

**Luciferase assay (PPRE-Luc Construct)**

To evaluate the biological activity of Rosi when adsorbed to Un-MNPs, a gene expression system expressing firefly luciferase under control of a PPAR response element (PPRE) was developed in HEPA1–6 cells. Since endogenous PPARγ levels are low in HEPA1–6 cells, PPARγ and the PPRE- inducible luciferase reporter construct were expressed. Specifically, HEPA1–6 cells were cultured in 96 well plates at 37 °C and 5% CO2 in media containing DMEM supplemented with 10% (v/v) Bovine Calf Serum (AXK49947, Thermo Scientific, Logan UT, USA), 200 mM L-glutamine (Sigma), 100 U/mL Penicillin–Streptomycin (Sigma), and 10 mM HEPES (G7513, Sigma). At 50% confluence HEPA1–6 cells were cultured in antibiotic-free media and transfected with 200 ng pcDNA-flag-PPARγ (7042 bp, Plasmid8895, Addgene) and 100 ng PPAR-responsive luciferase construct and a constitutively expressing Renilla element (40:1)/well (Qiagen, Valencia CA, USA) using Lipofectamine 2000 (Invitrogen, Burlington ON, Canada). Control cells receiving a mixture (40:1:1) of constructs expressing GFP, constitutively expressing firefly luciferase, and constitutively expressing Renilla luciferase allowed measurement of transfection efficiency (70% or greater) and served as a positive control for the luciferase assay (Cignal positive control (luc), Qiagen, Valencia, CA, USA). Cells were then incubated for 16 h with media containing no treatment, 1 μM Rosi, 400 ng MNPs or 1 μM Rosi-Un-MNP (400 ng) (stably adsorbed Rosi-Un-MNPs, preincubated in PBS for 24 h) and harvested for the Dual-Glo® Luciferase Assay (Promega, Madison WI, USA) and luminescence measured (Biotek’s Synergy 2 Multi-mode Microplate reader, BioTek, Winooski VT, USA).

**PPARγ target gene expression**

To directly assess induction of PPARγ target gene expression by Rosi-Un-MNPs, HEPA1–6 cells were cultured, transfected with pcDNA-flag-PPARγ and treated as above. RNA was extracted (RNeasy kit, Qiagen, CA, USA), concentration and purity assessed (Nanodrop ND-1000) and RNA integrity assessed by visualizing intact 18S and 28S rRNA bands on a native 1.5% agarose gel. RNA (500 ng) was reverse transcribed (Superscript III; Invitrogen) and quantitative real-time PCR performed in accordance with the MIQE guidelines31 using SYBR Green chemistry. Primers (IDT, Coralville, IA, USA) were designed (Beacon Designer software, Premier Biosoft, CA, USA), or taken from the literature (sequences available upon request). 25 μL reactions contained forward and reverse primers (300 nm; Sigma), nuclease-free H2O, and 1/10 cDNA (3 μL) in iQ SYBR Green Supermix (1X)iQ5 thermocycler, BioRad Laboratories, Hercules, CA, USA).

Expression levels of PPARγ target genes (fatty acid translocase (CD36), stearoyl-CoA desaturase (SCD1), uncoupling protein 2 (UCP2), pyruvate dehydrogenase kinase isozyme 4 (PDK4), and acyl-CoA oxidase (AOX)) were normalized to three stably expressed reference genes (β-actin, TATAA-box binding protein (TBP) and ribosomal protein L19 (Rpl19), assessed by geNorm (QBase + software, Biogazelle, Gent Belgium)) and expressed as fold change above the no treatment group.

**Analysis of data**

Plots and statistical analyses were performed using GraphPad Prism version 6.0b (La Jolla California USA, www.graphpad.com). In all cases, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. Statistical differences in biodistribution of 67Ga and 3H were determined with a Two-way ANOVA followed by Bonferroni’s multiple comparisons test. Statistical differences between groups in cell viability assays were identified with a One-way ANOVA followed by Dunnett’s multiple comparisons test. Differences in luciferase reporter gene expression between groups were identified with a One-way ANOVA followed by uncorrected Fisher’s Least Significant Difference. Differences in gene expression data between groups were identified using Student’s t-test.

**Results**

**MNPs coated in different hydrophobic substances adsorb similar quantities of Rosi, but produce different adsorption and release curves**

Both types of MNP consisted of superparamagnetic magnetite/maghemite cores sized 21 ± 4 nm, as determined by TEM (Figure 1, A), with an ~2 nm shell of coating. Each magnetic core was nicely coated with Al or Un, but seemed to loosely aggregate in a bimodal fashion. A first hydrodynamic peak for both particles was around 50 nm, while a second peak was around 150 nm. The Un-MNPs showed a broader size distribution in this second peak than the Al-MNPs. The zeta potential of the Al-MNPs was −42 mV, and similar for the Un-MNPs at −46 mV. The MNPs’s saturation magnetization Ms was 24.6 and 27.0 emu/g for Al-MNP and Un-MNP, respectively, and the M at 1.5 T (magnetic field generated at the surface of the implanted magnets) was 18.2 and 21.1 emu/g, respectively.

As a first step toward determining whether these coated MNPs would serve as a viable strategy for targeted drug delivery to adipose tissue, we assessed the ability of each type of particle to efficiently adsorb Rosi in water at room temperature (Figure 1, C and D). Al-MNPs and Un-MNPs bound 45.1% and 46.5% of the starting amount of Rosi that was in solution (350 μg), respectively, yielding 0.8 μg of Rosi adsorbed per 1.0 μg of MNPs. Despite the similar Rosi adsorption capacity and adsorption kinetics, the different MNP coatings resulted in characteristically different release curves (Figure 1). When maximally adsorbed MNPs were suspended in PBS at 37 °C, Al-MNPs slowly and steadily released Rosi, losing 44.7% of the adsorbed Rosi by 31 h (Figure 1, D). Over the same period of time, the Un-MNPs only released 9.3% of the adsorbed drug (Figure 1, D).

**An internally implanted magnet assists retention of Rosi-MNPs in the target adipose tissue**

Since Al-MNPs are more conducive to labeling with 67Ga compared to Un-MNPs, they were radiolabeled with 67Ga to characterize the biodistribution of the particles via SPECT/CT imaging and 67Ga counting (Figures 2 and 3). Interestingly,
imaging data and $^{67}$Ga counts revealed that the MNPs remained at the site of injection, over the right inguinal adipose tissue pad, for up to 24 h in both Magnet and Sham mice (Figure 2), although the $^{67}$Ga counts indicated that the presence of a magnet assisted in tighter localization. Importantly, the left inguinal adipose tissue pad, which served as an internal control, showed only background levels of $^{67}$Ga counts, similar to all other tissues collected and counted (Figure 3).

In order to track the biodistribution of Rosi, $^3$H-Rosi was adsorbed to the MNPs and counted in each of the collected tissues. Rosi accumulated in tissues associated with the digestive system (stomach and small intestine) to similar levels at 2 and 24 h post-injection, and in the liver, the main organ involved in Rosi clearance, at 2 h, but not 24 h post-injection, in Magnet and Sham groups (Figure 3). Notably, very little Rosi was found in the kidneys or bone, tissues where systemically administered Rosi is known to act to induce fluid retention and bone loss, respectively. The presence of an implanted magnet improved Rosi retention in the targeted, right subcutaneous adipose tissue pad compared to the untargeted, left subcutaneous adipose tissue pad at 2 h post-injection, however, at 24 h post-injection no substantial difference in $^3$H counts were observed in the right and left subcutaneous adipose tissue pads in Magnet and Sham mice.

**Toxicity of Al- and Un-MNPs in a Pre-Adipocyte cell line**

Since the biodistribution studies described above showed MNPs remained at the site of injection for 24 h, it was important to assess their effect on adipocyte viability. While Un-MNPs did not significantly reduce cell viability at any concentration tested (0.5, 5, 50, and 500 μg/mL) and AI-MNPs only caused a significant reduction in cell viability at 500 μg/mL, there is a trend that indicates Un-MNPs impact cell viability as concentration increases and AI-MNPs impair cell viability at all concentrations tested. Cell viability was 62.4% and 65.8% for AI- and Un-MNPs, respectively, at the highest concentration of MNP tested (500 μg/mL), and was 66.3% and 100% at the lowest concentration tested (0.5 μg/mL), respectively (Figure 4). Further functional experiments utilized the less toxic Un-MNPs at a concentration of 4 μg/mL.

**Biological activity of Rosi as not impaired when adsorbed to Un-MNPs**

Using a PPRE-luciferase reporter construct in HEPA1–6 cells overexpressing PPARγ (HEPA1–6-PPARγ) we assessed the biological activity of Rosi adsorbed to Un-MNPs compared to free Rosi. Importantly, treatment of HEPA1–6-PPARγ cells with Un-MNPs alone did not induce PPARγ activity compared to cells receiving no treatment (Figure 5, A). As expected, free Rosi (1 μM) significantly induced PPARγ activity compared to no treatment. Rosi-Un-MNPs induced PPARγ activity to the same extent as free Rosi with luciferase activity significantly increased compared to untreated and Un-MNP treated cells. These results demonstrated that the Rosi-Un-MNPs are as effective as free Rosi in activating PPARγ in a cell system (Figure 5, A).

To provide further validation that biological activity of Rosi was retained when adsorbed to Un-MNPs, we assessed the expression of five PPARγ target genes (CD36, SCD1, UCP2, PDK4, and AOX) in HEPA1–6 cells overexpressing PPARγ and treated with free Rosi (1 μM), Un-MNP alone (400 ng), or Rosi-Un-MNP (1 μM Rosi/400 ng Un-MNP) (Figure 5, B). Three (CD36, AOX and PDK4) of five target genes were significantly elevated when treated with free Rosi and Rosi-Un-MNPs, but not in cells treated with Un-MNPs alone. These results strongly validate that Rosi adsorbed to Un-MNPs retains its biological activity and provides support that Rosi-Un-MNPs can be used to activate PPARγ in adipose tissue.
Discussion

The mechanism of action, stability and clearance, and undesirable side effects of Rosi have been characterized extensively over the last two decades. Consequently, Rosi serves as a valuable tool to evaluate the efficacy of new approaches to tissue-targeted delivery of anti-diabetic and anti-obesity therapies and was used here as a proof-of-principle to demonstrate a tool for adipose tissue targeting. In order to target Rosi to subcutaneous adipose tissue, we developed a protocol to adsorb Rosi to lipid-coated MNPs and characterized the adsorption and release profiles of Rosi to and from the nanoparticles. We then demonstrated that a subcutaneous injection of Rosi-MNPs into obese mice with a magnet implanted in the target adipose tissue depot was successful at localizing Rosi to the target adipose tissue. Importantly, Rosi bound to the MNPs retained the same level of biological activity as free Rosi, as measured by upregulation of PPAR\(\gamma\) target gene expression in a cell system.

While this is the first attempt to target anti-diabetic agents to adipose tissue using MNPs, MNPs are currently used in medicine as contrast in Magnetic Resonance Imaging, and they are being developed as tools for other medical applications including delivery of chemotherapeutic agents to various tumors. More recently, MNPs have been incorporated into larger magnetic microspheres with the goal of magnetically directed intrapulmonary inhalation drug therapy.

Along with the shape and size of an MNP, the chemical properties of the MNP coating plays a critical role in determining the suitability of a particular MNP for a given application. In our study we selected two hydrophobic coatings to encourage adsorption of lipophilic Rosi. While the two MNP coatings resulted in similar loading kinetics, their release profiles illustrated the importance of coating selection, with Un-MNPs providing reduced drug release in an aqueous environment compared to Al-MNPs (Figure 1). This was expected, given the greater hydrophobicity of Un compared to Al.
In vivo imaging studies revealed MNPs were retained close to the injection site with or without the presence of an implanted magnet, although direct measurement (counts) of the 67Ga-labeled particles in tissue samples suggested subcutaneous injection of Rosi-Al-MNPs in combination with an implanted magnet allowed tighter localization of the particles to the targeted adipose tissue depot (Figure 2). As predicted by the known binding affinity and clearance of free 67Ga and 67Ga-NOTA, respectively, at 24 h post-injection we detected small amounts of 67Ga in other tissues such as kidney and bone (Figure 3, A). While 67Ga-NOTA-MNPs are cleared via the liver or spleen, free 67Ga will accumulate in bone, and 67Ga-NOTA will be cleared via the kidneys. Some of these counts thus likely represent degradation products of 67Ga-NOTA-MNPs.

The chemical and physical properties of the coated MNPs and Rosi used in this study resulted in the generation of a drug formulation with strong lipophilic features and significant mass. As such this localization may have been due to hydrophobic attraction of the particles to adipose tissue, settling at the site of injection due to the mass of the particles, or a combination of both.

Levels of 3H-Rosi in the right subcutaneous adipose tissue depot of mice with an implanted magnet were significantly higher than in the same tissue of mice without a magnet at 2 h post-injection. The absence of 3H-Rosi in the right subcutaneous adipose tissue at 24 h in both the sham and magnet groups indicates uptake and clearance of rosiglitazone by the cells according to the known biological half-life of 3–4 h.39 Notably, the internally implanted magnet also resulted in greater 3H counts in the right subcutaneous adipose tissue depot compared to the left subcutaneous adipose tissue depot, a result not observed in animals without an implanted magnet. Based on this observation we speculate that when a magnet was present, the attraction of Rosi-Al-MNPs to adipose tissue after injection was enhanced, allowing greater uptake of Rosi directly into adipocytes.

To evaluate the extent at which Rosi dispersed to other peripheral tissues within the animal, we measured 3H counts in...
16 peripheral tissues and blood. Analysis of \(^3\)H counts revealed elevated, but not statistically significant levels of Rosi in the stomach and small intestine after 2 and 24 h, likely representing radiolabelled breakdown products in urine and feces and may be amplified in the intestine due to the presence of lipid rich, high fat diet in the lumen of these tissues. Rosi also accumulated in the liver in both the Sham and Magnet groups at 2 h. This was expected since Rosi is metabolized primarily by the liver.\(^{32,33}\)

Taken together, the results from the biodistribution studies demonstrate subcutaneous injection of Rosi-MNPs in combination with an implanted magnet can be used to successfully enhance localization of Rosi to adipose tissue (Figure 3).

It is well known that systemically administered Rosi induces adverse effects on fluid balance and bone metabolism.\(^{15,17}\) Specifically, Rosi has been shown to increase the risk of congestive heart failure due to Rosi-induced edema, which results from increased vascular permeability, vasodilation, and fluid retention in the kidneys.\(^{19}\) Rosi also increases bone loss and thus fracture risk. Our goal is to determine if adipose tissue specific activation of PPAR\(\gamma\) can replicate the anti-diabetic effects of Rosi without inducing the undesirable side effects encountered with systemic administration. Results of the biodistribution studies showed Rosi did not accumulate in the kidney. Rosi also did not accumulate in bone, suggesting that targeted delivery of Rosi to adipose tissue could also reduce adverse effects such as edema and bone loss experienced with systemic Rosi administration.

PPAR\(\gamma\) interacts with a diverse set of ligands ranging from natural free lipids to pharmacological agents such as TZDs and novel small molecule agonists.\(^{40-44}\) The ability to bind such diverse compounds has been attributed to PPAR\(\gamma\)’s relatively large binding pocket of approximately 1300 Å\(^3\), with Rosi occupying approximately 40% of this space.\(^{45}\) The TZD makes several specific interactions with helices 3, 4, 10, and the AF-2 helix by adopting a U-shaped conformation.\(^{41}\) In order to confirm MNPs do not sterically interfere with Rosi binding at the PPAR\(\gamma\) ligand binding site, reducing Rosi activity when adsorbed to MNPs, we assessed PPAR\(\gamma\)-mediated transcriptional activation in response to Rosi-MNPs. Results from the PPRE-luciferase reporter assay conclusively showed similar activity of Rosi adsorbed to Un-MNPs compared to free Rosi.

The induction of 3 of 5 endogenous PPAR\(\gamma\) target genes in cells treated with free Rosi or Rosi-Un-MNP further validated the ability of the Rosi-Un-MNPs to induce PPAR\(\gamma\) \textit{in vivo} (Figure 5). The
fact that Rosi-adsorbed to Un-MNPs can induce gene expression, supports the use of a strong hydrophobic surface that retains Rosi bound to the MNP, minimizing drug leakage into systemic circulation. We thus favor Un-MNPs for future in vivo studies.

In summary, we have developed hydrophobic-coated nanoparticles (Un-MNPs) that adsorb Rosi, and subsequently release only MNPs in a cell system. Future implanted magnet, and reporter and gene expression assays confirmed Rosi retained full biological activity when adsorbed to MNPs in a cell system. Future in vivo studies are required to confirm if biologically active Rosi-MNPs can induce PPARγ activation in adipose tissue in vivo, and if activation of PPARγ at the adipose tissue alone is enough to induce the potent anti-diabetic effects of systemically administered TZDs in part or in full.

References


