In Vivo Efficacy of Rosiglitazone-Loaded Hybrid Nanoparticles for the Treatment of Cardiovascular Diseases

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ABSTRACT SUMMARY
We report the synthesis and characterization of an insulin sensitizer – Rosiglitazone (RSG) – loaded lipid-polymer hybrid, biomimetic nanoparticle (NP) by single step nanoprecipitation. These NPs were systemically administered for 1 month, twice a week, to LDLR−/− mice on high-fat diet. The expression of genes regulating lipid metabolism and inflammation was analyzed in different organs, including the white adipose tissue (WAT), liver and heart. As compare to the freely administered RSG (oral RSG), the hybrid nanoparticle formulation resulted in reduced inflammation and less pronounced side effects.

INTRODUCTION
Drug delivery systems (DDS), including polymeric nanoparticles (NPs), metal/ceramic NPs, liposomes, and dendrimers have been exploited as carriers for drugs and other bioactive substances in recent decades. In this context, biodegradable and biocompatible polymer and lipid combination, the lipid-polymer hybrid NPs, offer many advantages over the other more traditional carriers. This includes the versatility in surface modification with specific targeting ligands or homing devices such as IgG or IgM. Also, the components used are non-immunogenic, biodegradable, and biocompatible; they freely circulate throughout the body and offer ease of preparation. These NPs have the capacity to carry large amounts of drugs, and can behave as a slow-release, long-acting system [1].

Given the clinical success of polymeric and liposomal NPs, we aim to use lipid and polymer hybrid NPs to deliver rosiglitazone (RSG), which activates PPARγ target genes. RSG is a member of the thiazolidinedione class of drugs, which reduces glucose, fatty acid, and insulin blood concentrations. Unfortunately, RSG has been also found to increases fatalities from heart dysfunction and eventually failure. It has been reported that the risk of heart attack increased as much as 43% [2]. Due to the extreme cytotoxic effect of RSG, the US Food and Drug Administration (FDA) issued an alert of having higher risk of death from cardiovascular diseases and revised its prescribing information and medication guides for all RSG containing medicines. Considering the fact that the RSG is one of the important classes of drug that targets peroxisome proliferator-activated receptors (PPARγ), we proposed to reformulate the drug in a nanoparticle envelope in order to minimize its toxic effect. We hypothesize that encapsulating RSG into the nanoenvelope may reduce the side effects and enhance the therapeutic efficacy of RSG by conferring cell selective drug delivery, particularly to macrophages residing within the WAT and atherosclerotic plaques.

EXPERIMENTAL METHODS
L-α-phosphatidylcholine hydrogenated (EGG chicken, Avanti polar Lipid), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy (polyethylene glycol) -2000] (ammonium salt, DSPE-PEG-COOH), and carboxy terminated poly(DL-lactide-co-glycolide) (PLGA-COOH) was used to prepare lipid-polymer hybrid NPs by nanoprecipitation technique [1]. Male LDLR−/− mice were purchased from The Jackson Laboratory and maintained as reported earlier [3]. Mice were injected RSG-hybrid (10 ug of RSG twice a week) and bare (control, same concentration of polymer as in drug treated mice) lipid-polymer hybrid NPs systemically for a period of one months. After one month period, mice were euthanized by isoflurane overdose and the organs were collected for the gene expression studies.

RESULTS AND DISCUSSION
In this study, the NP consists of inner hydrophobic PLGA polymeric core surrounded by a lipid monolayer in which the small hydrophobic molecules of RSG were embedded. Further the NPs were PEGylated in order to prevent from aggregation in physiological environment by creating steric repulsion that evade protein opsonization in vivo leading to higher plasma residence time. NPs were characterized for their
hydrodynamic diameter, surface property and drug release behavior. The dynamic light scattering (DLS) measurement confirmed the formation of 100±6 nm size NPs with very narrow PDI of 0.18±0.02 (Figure 1A). The NP spherical morphology was also confirmed by scanning electron microscopy (SEM), returning a uniform size of 70±3 nm (Figure 1B). SEM measured size is slightly smaller than DLS due to the different states of the measurements. The long term stability of RSG-loaded NPs was estimated over the time period of 11 days. As shown in Figure 1A, these NPs are highly stable in PBS at pH 7.4.

Next we focused our attention on in vitro drug release kinetics of RSG-hybrid NPs (loading efficiency of 3.0 wt%, Figure 1B-inset) at pH 7.4 and 5.0. These two different ionic strengths were selected to mimic physiological and acidic endosomal environment, respectively. Only 20% of drug was released in first 5h at pH 7.4, whereas at pH 5.0, 50% of the drug was released within the first 5h as shown in Figure 1B. Drug release at pH 5.0 was relatively fast, which can be attributed to the erosion of outer stabilizing lipid layer and the degradation of PLGA in acidic medium. These results could highlight that the pharmokinetic profile in acidic pH is dramatically increased, so that this NP, after internalization via endocytotic pathway, can immediately release RSG thereby increasing the local drug concentration. As observed in in vitro gene expression studies with bone marrow derived monocytes (BMDM), low dose (10 µM) of RSG-hybrid NPs up regulates the CD36, FABP4, ABCG1 and PPARγ to the same extent to that of Free RSG (Figure 2). Considering the slow releasing profile of the NP system, the treatment with NP would further facilitate in vivo transcriptome profile. As expected, we observed reduced expression of inflammatory genes in liver, WAT, kidney and heart as compared to oral RSG, and no alteration in the expression of lipid metabolism genes.

CONCLUSION
In conclusion, the lipid-polymer hybrid NPs mediated the sustained delivery of RSG and modulated the overall inflammatory response. We expect this approach to have potential in the treatment of cardiovascular diseases by enhancing the therapeutic efficacy of thiazolidinedione-based drugs.

REFERENCES