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Rosiglitazone-loaded nanospheres for modulating macrophage-specific inflammation in obesity

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ABSTRACT

PPARγ nuclear receptor agonists have been shown to attenuate macrophage inflammatory responses implicated in the metabolic complications of obesity and in atherosclerosis. However, PPARγ agonists currently in clinical use, including rosiglitazone (RSG), are often associated with severe side effects that limit their therapeutic use. Here, 200 nm PLGA/PVA nanospheres were formulated for the systemic delivery of RSG specifically to macrophages. RSG was encapsulated with over 50% efficiency in the hydrophobic PLGA core and released specifically within the acidifying macrophage phagosomes. In bone marrow derived macrophages, RSG-loaded nanoparticles (RSG-NPs) induce a dose dependent upregulation (1.5 to 2.5-fold) of known PPARγ target genes, with maximal induction at 5 μM; and downregulate the expression of genes related to the inflammatory process, with a maximum effect at 10 μM. In $Ldlr^{-/-}$ mice fed high fat diet, treatment with RSG-NPs alleviated inflammation in white adipose tissue and liver but, unlike treatment with free RSG, did not alter genes associated with lipid metabolism or cardiac function, indicating a reduction in the RSG side effect profile. These biocompatible, biodegradable RSG-NPs represent a preliminary step towards the specific delivery of nuclear receptor agonists for the treatment of macrophage-mediated inflammatory conditions associated with obesity, atherosclerosis and other chronic disease states.

1. Introduction

Macrophages are critical determinants of metabolic disorders, including diabetes and obesity, and in atherosclerosis [1–3]. Obesity-induced macrophage inflammatory responses are associated with altered adipocyte function, insulin resistance and increased atherosclerosis. Therefore, controlling macrophage inflammation activities can prevent, attenuate, and possibly reverse such disorders (Fig. 1). In this respect, the activation of nuclear receptors, such as the peroxisome proliferator-activated receptor γ (PPARγ), has been proved beneficial, in large part, by inhibiting macrophage inflammation [4,5]. Molecular compounds have been designed to target PPARγ, such as the clinically available rosiglitazone (RSG) and pioglitazone [6] for the oral treatment of type 2 Diabetes. However, in addition to attenuating inflammation and promoting insulin sensitivity, these compounds are often associated with severe side effects including weight gain, edema, bone fracture, and congestive heart failure, which have severely limited their clinical application [7,8].

Over the last two decades nanoparticles (NPs) have been demonstrated to be useful for systemic delivery and controlled release of therapeutic agents in a number of biomedical applications [9–11]. The NPs geometrical and physico-chemical properties can be finely tuned during their synthesis process to optimize drug loading and enhance specificity in reaching the biological target [12–14], thus increasing the therapeutic efficacy and limiting side effects. The nuclear receptor PPARγ is expressed in most cells and tissues, however, this ubiquitous distribution is problematic because of the unwanted side effects. Thus, specific activation of macrophage PPARγ to induce a systemic anti-inflammatory response by down-regulating the secretion of cytokines would be highly desirable. Based on this rationale, the development of NPs for the preferential delivery of PPARγ agonists, such as RSG, to macrophages represents a promising and novel therapeutic strategy for controlling the development and progression of many metabolic disorders and also in atherosclerosis.

Spherical NPs with a diameter of a few hundreds of nanometers are easily and rapidly engulfed by professional phagocytic cells, such as circulating monocytes and macrophages [15–22]. As such, a systemically

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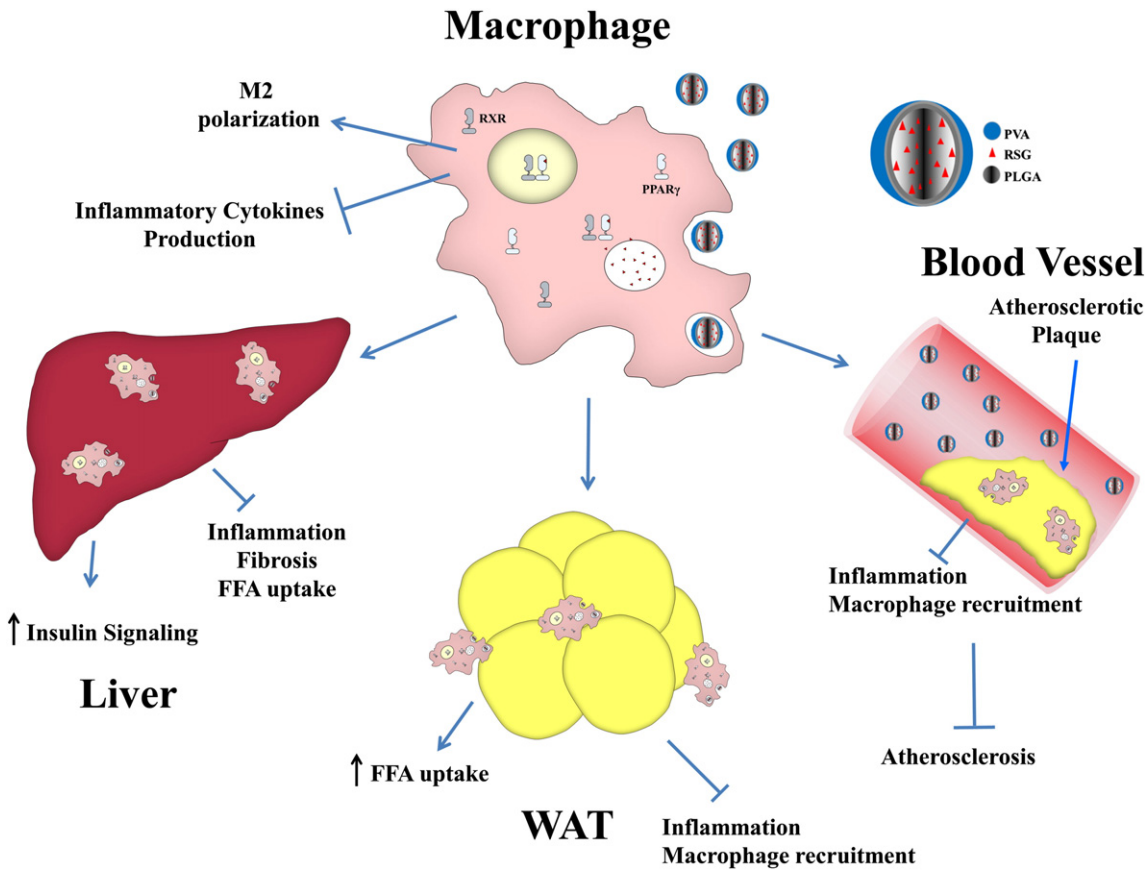

Fig. 1. Expected effects of macrophage-targeted RSG delivery in inflammatory processes in different tissues. After adhering to macrophage cell membrane, NPs are entrapped in endocytic vesicles and progress through endosome maturation until endosome acidification causes rapid degradation of the NPs, resulting in RSG release from the NPs polymeric matrix and its diffusion to the cytosol and nucleus where it can bind PPARγ to regulate gene expression. The higher magnification shows a schematic of a RSG-NPs consisting of an internal hydrophobic core, which entraps the RSG cargo, covered by a hydrophilic PVA layer. In WAT, RSG-NPs would normalize adipose function by decreasing macrophage-mediated inflammation and cytokine secretion, thereby decreasing macrophage recruitment and excess lipolysis to improve normal lipid storage. Improved WAT lipid storage would reduce pro-inflammatory lipid accumulation in the liver and vascular macrophages, reducing liver inflammation and atherosclerosis development. Monocyte/macrophage phagocytosis of RSG-NPs would also attenuate cytokine secretion by vascular macrophages to inhibit further recruitment of macrophages to existing atherosclerotic plaques and thus attenuate atherosclerosis progression.

injected NPs loaded with RSG would be preferentially taken up by circulating monocytes, that eventually infiltrate developing vascular plaques and by macrophages, residing in the liver (Kupffer cells) and in the white adipose tissue (WAT). This targeted approach to specifically release RSG into monocytes and macrophages would be useful to control diverse inflammatory processes orchestrated by these cells, yet mitigate RSG accumulation in hepatocytes, cardiomyocytes, renal tubular and other cells causing the severe undesired effects [23]. Note that this approach is different, and possibly more general, than developing nanoparticles for the specific treatment of atherosclerotic plaques [24,25], and it could be used for the effective treatment of any macrophage-mediated inflammatory disease [26].

In the present investigation, spherical polymeric NPs with a diameter of about 200 nm were synthesized and characterized for the systemic, macrophage-selective delivery of RSG. These NPs consisted of an inner hydrophobic poly(lactic-*co*-glycolic acid) (PLGA) matrix, where the poorly water soluble RSG molecules [27,28] are encapsulated, surrounded by an outer layer of polyvinyl alcohol (PVA), creating a hydration layer to reduce NPs aggregation by steric repulsion and attenuate protein modification and opsonization in the blood [29,30]. The RSG-loaded NPs (RSG-NPs) were characterized for their geometrical and physico-chemical properties; loading and release performance; and macrophage internalization capabilities. Then, gene induction was studied in primary phagocytic cells, mouse bone marrow derived macrophages (BMDMs), and in the WAT, liver and heart of $LDLR^{-/-}$ mice injected bi-weekly with RSG-NPs. As a control, a separate group of mice received dietary RSG daily for the duration of the study.

2. Experimental section

2.1. Materials

PLGA (50:50, Carboxy Terminated, MW ~60 kDa) and PVA (MW ~50 kDa) were purchased from Sigma Aldrich (St. Louis, MO). Rosiglitazone was purchased from SST Corp. (Clifton, NJ) and used without further purification. Analytical grade dimethyl sulfoxide (DMSO), acetonitrile (ACN), chloroform and other solvents were obtained from Fisher Scientific. D-275 (3,3′-dioctadecyloxacarbocyanine perchlorate, DiOC18(3)) was purchased by Invitrogen. J-774 and RAW 264.7 cells were purchased from American Type Culture Collection (ATCC, Bethesda, MD) and cultured according to the manufacturer's protocol. Phosphate buffered saline (PBS) and cell culture media were purchased from Sigma Aldrich.

2.2. Synthesis of RSG-NPs

30 mg of PLGA (50:50) Carboxy terminated was dissolved in chloroform and mixed with 6 mg of RSG dissolved in 600 μl of ACN to obtain a homogeneous solution. The single emulsion nanoparticle (NPs) fabrication technique was adapted to synthesize RSG loaded