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RAPID COMMUNICATIONS

RES blockade: A strategy for boosting efficiency of nanoparticle drug

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Summary Nanoparticles have been intensely pursued for drug delivery for three decades, but their delivery efficiency remains low despite numerous innovations in nanoparticle manufacturing including materials, particle size, surface physicochemistry and targeting moieties. A major reason for this shortfall is their unintended uptake by reticuloendothelial system (RES) in the liver, spleen, lung, lymph nodes, *etc.*, which competes with the intended targets. Here we demonstrate a temporary blockade of RES by commercial liposome to substantially delay blood clearance and increase tumor accumulation of small sized, PEGylated nanoparticles. RES-blockade dramatically enhanced therapeutic efficacy of paclitaxel-containing nanoparticles against human prostate cancer: substantial tumor cell kill was detected within 48 h of administration by diffusion MRI and a remarkably longer tumor growth delay was achieved in comparison with nanoparticle treatment alone. Importantly, RES-blockade did not result in any weight loss or impairment of liver function, neither did it jeopardize RES-mediated host defense. Cellular and molecular studies have revealed blockade mechanisms, suggesting this safe and effective blockade strategy has broad utility.

Introduction

Drug-loaded nanoparticles (NP) have shown promise for improving clinical management of prostate cancer as they can facilitate treatment of primary and metastatic diseases by radio-, thermal- and chemotherapies [1—6]. However, rapid clearance of circulating NP by reticuloendothelial system (RES), also referred to as mononuclear phagocyte system, continues to pose a huge challenge for realizing their promise as a more effect treatment for all cancers [7,8]. Although the drug payload per NP may be high, the tissue accumulation of the drug can actually be lower than achieved by the free drug carried by a buffer solution because the vast majority of the intravenously injected NP are absorbed by RES-rich organs. Consequently, the

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therapeutic efficiency of NP is severely reduced at target cells unless they are macrophages; meanwhile, there is a risk of collateral drug damage to RES-rich organs, which are part of body's immune defense system.

The problem is difficult to solve because, following injection, surface of NP is rapidly covered by serum proteins (*i.e.*, opsonins), which are recognized and internalized by macrophages through a variety of receptors [9]. Strategies to delay/minimize opsonization by modifying physicochemical properties of NP, including size, charge and surface grafting hydrophilic polymeric molecules [7,10]—especially polyethyleneglycol (PEG) [11], have succeeded in prolonging the circulating time of liposome and other NP [12]. Indeed, PEGylated liposome are referred to as ''stealth liposome'' as they demonstrate a dose-independent pharmacokinetics (see review [13]). But even when size and PEGylation are optimized, the majority of injected NP are still lost to RES *en-route* leading to only a very low percentage (~5%) retained in the tumor [14]. This is not unexpected: extensive PEGylation makes the NP ''slippery'' thus slows down their binding to serum opsonins while reducing their binding/uptake by tumor cells as well. Therefore prolonged circulation merely delays the eventual capture of NP by RES but does not enhance their accumulation in the target tissue [8,12].

Alternatively, directly blocking or depleting RES macrophages with an array of inorganic and organic materials including silica, carbon, latex beads, methyl paltitate, gadolinium chloride, dextran sulfate, glycine and clodronate-containing liposome has shown effectiveness to various degrees [15—23], but the systematic toxicity brought by these agents excludes their regular usage in the therapeutic regimen [15]. A safer agent is phospholipids (PL): studies have shown that a large dose (10—2000 mg PL/kg body weight) can reversibly block the RES phagocytosis and increase the circulation half-life of subsequently injected liposome [20,24—28]. However, since PEGylation and small particle size were considered more effective in prolonging the circulation of NP than RES-blockade mediated by pretreatment of *empty* liposome, this approach has received little attention in the past 30 years.

Given the realization that small-sized, PEGylated, long-circulating NP (20—60 nm) do not accumulate in the target (*e.g.*, tumor) much higher than liposome (>100 nm), and the knowledge that NP design has already optimized PEGylation and other physicochemical features, direct blockade of RES may now offer a possible break from the stand-off for delivery of such NP. This approach has not been explored for these NP in the past, because its effectiveness is questionable in view of the NP's long circulation half-life, well beyond the time for any prudent temporary RES blockade. In this work, we demonstrated for the first time a partial and temporary RES blockade to be highly effective for delaying the blood clearance and increasing tumor accumulation of small, long circulating NP. The blockade became effective within 90 min and its therapeutic benefit evident within 48 h, as determined by *in vivo* magnetic resonance imaging (MRI); over a longer time period it obtained a remarkable *additional* 40 days of tumor growth delay. Importantly, our data show that liposome-mediated RES-blockade is safe without a negative impact on liver function or host defense capacity against foreign bacteria.

Results

Liposome-mediated RES-blockade: decreased liver uptake and blood clearance of test NP

To assess how pre-dosing with phosphatidylcholine:cholesterol (PC:Chol) liposome (a simple, commercial formulation with a size distribution of 0.3—3 μm) would affect subsequent RES uptake of 25 nm 3F-NP, a PEGylated nanoparticle (see Supplementary data [40—47]), we first injected liposome (376 mg PL/kg) intravenously (i.v.) *via* tail vein; 1.5 h later, we injected 3F-SPIO (10 mg Fe/kg, i.v.), a test NP encapsulating super paramagnetic iron oxide (SPIO), which reduces transverse relaxation time (T2) of the water. Using rapid MRI, we followed the immediate time course of 3F-SPIO's liver uptake and its clearance from blood in vena cava (inset of Fig. 1B). Compared the saline- *vs.* liposome-treated group, the initial liver uptake ($t \in [0,200]$ s) is almost completely blocked if mice were pre-treated with liposome (inset of Fig. 1A). Saturation of liver RES uptake is evidenced by the apparent plateau (red curve) reached after 20 min in the liposome-treated mice; in contrast, the MR signal continues to rise in saline controls (blue curve). Consequently, the AUC (area under the curve, for 3075 s or ~50 min) in livers of mice pretreated with liposome is less than half of that with saline ($P = 0.02$). The corresponding blood clearance of 3F-SPIO monitored by MRI signal (Fig. 1B) exhibits a 4-fold increase in AUC with liposome pretreatment (red) *versus* saline (blue, $P = 0.02$), suggesting an increased blood concentration and delayed clearance of the NP. At 24 h, measurement of liver T2, whose reduction is a robust indicator of SPIO accumulation, showed a significant reduction *only* with saline-pretreatment (Fig. 1C and Table 1). Prussian blue staining 24 h post-3F-SPIO injection confirmed much fewer blue spots (indicating SPIO) in liver and spleen in mice pretreated with the liposome (Fig. 1D). Kinetic MRI also suggests that RES-blockade takes effect at 1 h post-bolus injection of the liposome and lasted for ~2.5 h (Fig. S4).

To assess how the liposome would affect blood clearance of NP over a 24 h period, we injected 3F-DiD, a test NP encapsulating a fluorophore (DiD), 1.5 h after liposome injection. Compared to the saline control (blue trace in Fig. 2A), liposome pre-dosing resulted in minimal clearance of 3F-DiD in the 1st hour, extending the half-life of 3F-DiD to over 24 h (red trace in Fig. 2A). Taken together, these data indicate that PC:Chol liposome significantly reduced liver accumulation of small, PEGylated NP, leading to remarkably increased circulation half-life of such NP. From this point on, we will refer to this phenomenon as *RES-blockade*.

RES-blockade increases nanoparticle accumulation in tumor

A direct benefit of liposome-mediated RES blockade is a remarkable increase of NP accumulation in the tumor. In