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Inhalation delivery of Telmisartan enhances intratumoral distribution of nanoparticles in lung cancer models

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ABSTRACT

The purpose of the present study was to evaluate the effect of Telmisartan (Tel) and Losartan (Los) on nanoparticle intratumoral distribution and anticancer effects in lung cancer. A549 lung tumor cells were orthotopically and metastatically administered to Nu/nu mice. Fluorescent polystyrene nanoparticles (FPNPs, size ~200 nm) beads were used to study their intratumoral distribution after Tel and Los treatments. Animals were administered with FPNPs and after 2 h, FPNPs intratumoral distribution was studied by fluorescent microscopy. Tel (~1.12 mg/kg) and Los (~4.5 mg/kg) were administered by inhalation delivery at alternative days for 4 weeks to tumor bearing animals. Collagen-1, transforming growth factor beta 1 (TGF-β1), cleaved caspase-3, Vimentin and E-Cadherin expressions were studied by western blotting. To correlate the AT_1 receptor blockage to anticancer effects, VEGF levels and microvessel densities (MVD) were quantified. Los and Tel treated group resulted in the 5.33 and 14.33 fold increase respectively in the FPNPs intratumoral distribution as compared to the controls. Tel treatment attenuated 2.23 and 1.70 fold Collagen 1 expression compared to untreated control and Los groups, respectively. Further, in Tel and Los treated groups, the TGF-β1 active levels were significantly ($p < 0.05$) decreased. Tel (at four times less dose) was 1.89 and 1.92 fold superior in anticancer activity to Los respectively in A549 orthotopic and metastatic tumor models ($p < 0.05$) when given by inhalation route. Tel, by virtue of its dual pharmacophoric nature could be an ideal candidate for combination therapy to improve the nanoparticle intratumoral distribution and anticancer effects.

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1. Introduction

Non-small cell lung carcinoma (NSCLC) is a highly heterogeneous disease and the most common cause of cancer mortality worldwide with a 5-year survival rate of only 15%. Despite recent advances in cancer treatments, the clinical outcome among NSCLC patients is not impressive [1,2]. Chemotherapeutic drugs are rarely successful due to limited amount of the drug reaching the lung tumor cells, resistance development and the associated adverse side-effects [3]. Some of the biopharmaceutical and formulation hurdles in the anticancer drug delivery have been successfully overcome by novel drug delivery systems including nanoparticle approach [4,5]. Cancer chemotherapy that uses nanocarriers has been developed to improve the clinical treatment of solid tumors by obtaining selective high accumulation of drugs in tumor tissues with limited accumulation in normal tissues [6,7]. Cell penetrating peptides, monoclonal antibodies and tumor cell specific nanoparticles can induce targeted delivery of chemotherapeutic agents to tumor cells [4,8]. However, irrespective of the targeting nature of the nanoparticles, their intratumoral distribution is hindered by dense collagen network and highly fibrous interstitium observed in the solid tumors [9–11]. Solid tumors are characterized by pathologic desmoplasia resulting in increased extracellular matrix (ECM) deposition and tumor fibrosis [11,12]. The ECM is mainly composed of collagen networks and is responsible for the compartmentalization of tumors [12]. Increased ECM remodeling and stiffening by collagen and fibronectin enhances tumor cell survival and proliferation [13,14]. Tumor fibrosis correlates with the progression and invasion of various cancer types [14]. The increased ECM deposition directly contributes to tumor growth by stimulating tumor cell proliferation, increasing angiogenesis, and promoting invasion [15]. In addition to tumor progression, tumor fibrosis also plays crucial role in the intratumoral delivery and distribution of macromolecules. High interstitial fluid pressure, found in various types of tumors is associated with vessel leakiness, lymph vessel abnormalities, and perivascular fibrosis, leading to matrix rigidity and fibroblast contractility and resulting in increased fiber tension. This leads to

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decreased transcapillary transport and limiting the chemotherapeutic drug delivery to tumors [15].

Use of antifibrotic agents has been reported to decrease tumor interstitial fibrosis and promote nanoparticle intratumoral distribution [6,10]. The role of transforming growth factor beta (TGF-β1), a multifunctional cytokine in tumor fibrosis has been well documented and plays a pivotal role in regulation of progression of cancer through effects on tumor microenvironment as well as on cancer cells [16,17]. TGF-β system acts as a tumor suppressor in early stages and as a tumor promoter in late stages of tumor progression. Most lung cancers have intact TGF-β signaling but develop resistant mechanisms against TGF-β mediated growth inhibition, suggesting the tumor-promoting role of TGF-β. Expression of TGF-β is frequently upregulated in NSCLC and many other cancers and is correlated with enhanced invasion and metastasis [18]. TGF-β inhibitors have recently been shown to prevent the growth and metastasis of certain cancers [19,20]. Improvement of nanocarriers cancer-targeting therapy by inhibition of TGF-β signaling was also reported in intractable solid tumors [21]. Peroxisome Proliferator-Activated Receptor-γ (PPAR γ) activation also inhibited the tumor metastasis by antagonizing TGF-β and Smad3 [18,22]. A recent study demonstrated that AT_1 receptor blocker Los through TGF-β inhibition improved the penetration and therapeutic efficacy of drug loaded nanoparticles [10]. Tel, another AT_1 blocker also demonstrated PPAR γ activity and several studies demonstrated the beneficial effects of Tel over other AT_1 blockers [23–25]. Previous studies have also suggested the role of Tel in controlling diabetes associated cardiovascular complications by virtue of its additional PPAR γ agonistic activity [26]. Further, Tel has also been reported to reduce TGF-β induced ECM production in various disease conditions [23,27]. The combined antifibrotic effects of PPAR γ activators and AT_1 receptor blockers (ARB) may be more beneficial in ameliorating tumor fibrosis which could be successfully used with Tel [28–30].

Angiotensin II (Ang-II) is the biologically active peptide of the renin-angiotensin system (RAS) involved in blood pressure control, tissue remodeling and angiogenesis as well as in vascular and inflammatory pathologies. However, compelling evidence indicates that the Ang-II peptides also play a role in cell proliferation and metastasis [31]. Ang-II is a vasoconstrictor, a mitogen, and an angiogenic factor, while angiotensin (1–7) has vasodilator, anti-proliferative, and anti-angiogenic properties [32–35]. Major functions attributed to Ang-II (inflammation, angiogenesis and migration) are also related to cancer progression [31]. Most components of the RAS including angiotensinogen, angiotensin converting enzyme (ACE) and angiotensin receptors are expressed locally in a wide variety of tumors [31,36]. Angiogenesis is essential for tumor growth and metastasis and vascular endothelial growth factor (VEGF) is the most potent angiogenic factor identified to date and is produced by RAS through AT_1 receptor activation [37]. TGF-β1 acts as an indirect angiogenic agent by regulating the VEGF production [38]. Recently various reports have demonstrated the anticancer effects of angiotensin receptor (AT_1) blockers (ARBs) and AT_2 receptor activators and Ang 1–7 peptides [13,34,39–41]. Considering the important role of AT_1 receptors in tumor development and metastasis, and signal cross talk between TGF-β1, AT_1 receptors in tumor fibrosis [42], the current study has been designed to study the antifibrotic effects of Los and Tel in lung cancer models [42]. Tumor fibrosis nature was characterized by estimating collagen levels and masson trichrome staining. Role of TGF-β1 in collagen induced tumor fibrosis was studied by quantifying the TGF-β1 levels. The mechanism of anticancer effects induced by Los and Tel was characterized by analyzing the apoptosis (cleaved caspase 3) and MMP-9 expressions. The role of ARBs on tumor angiogenesis was studied by VEGF and microvessel density (MVD) levels. Further, the antimetastatic effects of Los and Tel were studied by Vimentin, E-Cadherin and MMP-9 expressions.

In this study, we have proposed to treat the lung tumors with Los and Tel by inhalation route prior to administering the nanoparticles to solid lung tumors. We hypothesize that prior treatment with Tel will make poorly penetrable fibrous tumors into easily nanoparticle penetrable loose interstitial networks allowing for better intratumoral distribution of the nanotherapeutics leading to their superior anticancer effects.

2. Materials and methods

2.1. Materials

Tel, and Los were purchased from Alpha Aesar (MA 01835), and Fluorescent polystyrene sulfate modified Latex beads (L9902-1 ML) were procured from Sigma Aldrich (St. Louis, MO 63178). Human non-small cell lung cancer cells (A549 and H1650) and normal lung fibroblasts WI-38 were procured from ATCC. The antibodies (TGF-β1 was from R&D systems MN 55413), Collagen-1, Cleaved caspase-3, CD31, E-cadherin, AT_1 receptor (F-3) Vimentin and MMP-9 were procured from Santa Cruz Biotechnology Inc (CA 95060). VEGF kit was purchased from Thermo Fisher scientific Inc (IL 61101). TGF-β1 ELISA kit was purchased from R&D systems (MN 55413). Masson trichrome staining kit was purchased from Polysciences Inc (PA 18976).

2.2. In vitro anticancer effects of Los and Tel

Non-small cell lung cancer cell lines A549 and H1650 were plated in 96 well plates at 10,000 cells/well density. After 24 h, cells were treated with various concentrations of Tel and Los, 0.1% DMSO treated cells were considered as control cells. After 72 h of treatment, cells were fixed in 0.25% glutaraldehyde and stained with 0.05% crystal violet staining. Percent of cell viability or cell kill was calculated by considering DMSO treated control cells as 100% viable (zero% cell kill).

2.3. Effect of Los and Tel on normal lung fibroblasts

To study the effect of Tel on normal lung fibroblast cells (WI-38), dose dependent cytotoxic effects were studied. 10,000 cells/well were plated in 96 well plates and allowed to attach and grow for 24 h, then cells were treated with different doses of Los and Tel (in DMSO). After 72 h of treatment, cytotoxicity was studied by crystal violet staining method. Percent of cell viability or cell kill was calculated by considering DMSO treated control cells as 100% viable (zero% cell kill).

2.4. Aerodynamic size distribution analysis

Particle size distribution was measured using an 8-stage Anderson cascade impactor, Mark II connected to the PARI LC STAR jet nebulizer mouth piece. The impactor plates were coated with 10% Pluronic L10 in ethanol solution to prevent particle bounce. The aqueous formulation or solution was nebulized using PARI LC STAR jet nebulizer using dry compressed air for 5 min into the cascade impactor which was operated at a flow rate of 28.3 L/min according to USP guidance [43]. To determine the aerodynamic properties of Los and Tel, the inhaled aerosol on the nebulizer, throat, jet stage, plates on impactor stages 0–7, and filter was collected by washing with 5 ml of HPLC grade water. The analysis of Los and Tel was performed on a Waters HPLC system using a Symmetry C18 column (5 μm, 4.6 × 250 mm) with a Nova-Pack C8 guard column at corresponding wavelengths and flow rate of 1 ml/min. The HPLC system consisted of a Waters autosampler (model 717 plus), Waters binary pump (model 1525), and Waters UV photodiode array detector (model 996). All samples were analyzed in triplicate. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were obtained from impactor data using established software. Impactor experiments were repeated at least two times. Data was expressed as the percentage of the total drug deposited on all stages