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Collagenase-1 injection improved tumor distribution and gene expression of cationic lipoplex

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ABSTRACT

Elevated interstitial fluid pressure (IFP) in a tumor is a barrier to tumor accumulation of systemic delivery of nanocarriers. In this study, we investigated whether intravenous injection of type I collagenase (collagenase-1) reduced IFP in tumors and increased the accumulation and gene expression of cationic liposome/plasmid DNA complex (lipoplex) in tumors after intravenous injection into mice bearing mouse lung carcinoma LLC tumors. Collagenase-1 reduced the amount of type I collagen in the tumor, and significantly decreased IFP by 65% at 1 h after injection. Therefore, collagenase-1 induced 1.5-fold higher accumulation and 2-fold higher gene expression of lipoplex in tumors after intravenous injection. These findings indicated that intravenous injection of collagenase-1 improved the accumulation of lipoplex by decreasing IFP in tumors. These results support the potential use of collagen digestion as a strategy to improve systemic gene delivery into tumors.

1. Introduction

Systemic delivery of anticancer drugs or therapeutic genes to tumors requires transport through the extracellular space in tumors, also called the tumor interstitium. Compounds with low molecular weight are mainly transported by diffusion, which is dependent on concentration differences. Meanwhile, macromolecules such as proteins and nanocarriers are mainly transported by convection flow, which is driven by differences between hydrostatic pressure in the vessel (microvascular pressure, MVP) and interstitial fluid pressure (IFP) in tumors (Bouzin and Feron, 2007; Rippe and Haraldsson, 1994). Therefore, transport of macromolecules and nanocarriers into tumors is impeded by imbalances between MVP and IFP. Generally, normal pressure from the capillaries into surrounding tissues is about 1–3 mm Hg; however, IFP in tumors is often increased (Young et al., 1950), and high IFP in tumors is an obstacle to the delivery of nanocarriers such as liposomes (Jain, 1987a,b). Many studies of human and rodent tumors have observed a relationship between decreased drug uptake and elevated IFP in tumors (Curti et al., 1993).

The mechanism for increased IFP in tumors involves the unique microenvironment of solid tumors. Tumor tissues contain leaky vessels which have deficient coverage by pericytes (Dvorak et al., 1995), and the leakiness of tumor vessels increases the outflow of osmotic proteins from the vessels. Furthermore, the lack of a lymphatic system (Alitalo and Carmeliet, 2002; DiResta et al., 2000; Leu et al., 2000; Padera et al., 2002; Ribatti et al., 2007) reduces the mechanism to drain excess proteins from the tumor interstitium, and these accumulated proteins elevate IFP in tumors. If osmotic proteins were accumulated in tissues, IFP in normal tissues are maintained by changes in the volume of the tissues such as edema in patients with inflammation or burn injuries; however, IFP in solid tumor could not be maintained, because the tumor interstitium has a denser network of connective-tissue molecules such as collagen, hyaluronan and proteoglycans. Consequently, increased IFP in tumors is persistent (Heldin et al., 2004). The tumor interstitium is enriched with tumor-associated fibroblasts (TAF), endothelial cells, pericytes, infiltrating inflammatory cells and extracellular matrix (ECM) (Fleming et al., 2010; Park, 2010). Platelet-derived growth factor (PDGF), which is released from tumor cells, stimulates the activity of TAF, which produces ECM (Desmouliere et al., 2004). Interstitial hydraulic conductivity has been found to correlate inversely with collagen and glycosaminoglycan content (Swabb et al., 1974; Weinberg et al., 1997), as well as with IFP (Jain and Baxter, 1988). The interstitial diffusion coefficient also correlated inversely with collagen (Netti et al., 2000).

Recently, the use of collagenase or hyaluronidase for modulation of ECM in tumors has proven useful in reducing IFP in animal models (Eikenes et al., 2004, 2005) and has succeeded in improving the accumulation of antibody (Eikenes et al., 2004) and liposomal doxorubicin in tumors (Eikenes et al., 2005; Zheng et al., 2011). In cancer gene therapy, cationic liposomes were used as a carrier for the delivery of therapeutic genes such as plasmid DNA (pDNA) into tumors; however, the application of these enzymes for gene

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delivery by cationic liposome has not been reported. Type I collagen is a major constituent of ECM in tumors and plays a central role in impeding solute transport within the tumor interstitium (Magzoub et al., 2008). The interstitial collagen network is heterogeneous, with a substantial amount of collagen surrounding the microvessels. Type I collagenase (collagenase-1), known as matrix metalloproteinase-1 (MMP-1), is a protease with substrate specificity for mainly type I and III collagens. In this study, we focused on collagenase-1 for the reduction of IFP in tumors, and investigated whether collagenase-1 treatment could improve accumulation and gene expression in tumors after intravenous injection of cationic liposome/pDNA complex (lipoplex).

2. Materials and methods

2.1. Materials

N-[1-(2,3-dioleoyloxy) propyl]-*N*,*N*,*N*-trimethylammonium methyl-sulfate (DOTAP) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Cholesterol (Chol) and type I collagenase from *Clostridium histolyticum* (230 U/mg, EC 3.4.24.3) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Methoxy-poly (ethyleneglycol)–distearylphosphatidylethanolamine (PEG_{2000}–DSPE, PEG mean molecular weight, 2000) was purchased from NOF Inc. (Tokyo, Japan). Lissamine™ rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (rhodamine-DHPE) was purchased from Invitrogen (Carlsbad, CA, USA). All other chemicals were of the finest grade available.

2.2. Plasmid DNA

pCpG free-Luc encoding the *firefly* luciferase gene under the control of human elongation factor 1 alpha promoter and mouse cytomegalovirus enhancer was constructed by inserting the *firefly* luciferase cDNA fragment from pMOD-LucSh (Invivogen, San Diego, CA, USA) into the *Bgl*II/*Nhe*I site of pCpG-mcs (Invivogen), and was then amplified in *Escherichia coli* GT115. A protein-free preparation of the plasmid DNA (pDNA) was purified following alkaline lysis using the EndoFree Plasmid Max Kit (Qiagen, Hilden, Germany).

2.3. Cell culture

Mouse Lewis lung carcinoma (LLC), mouse Colon 26, mouse sarcoma 180 (S180), mouse neuroblastoma Neuro2a and mouse melanoma B16 cells were supplied by the Cell Resource Center for Biomedical Research, Tohoku University (Miyagi, Japan). The cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and kanamycin (100 μg/mL) at 37 °C in a 5% CO_2 humidified atmosphere.

2.4. Tumor model

All animal experiments were performed with approval from the Institutional Animal Care and Use Committee of Hoshi University. To generate tumors, 1×10^6 cells suspended in 50 μL RPMI-1640 medium were inoculated subcutaneously into the flank of mice (7 weeks of age). LLC and B16 cells were implanted into female C57BL/6NCrSlc mice (Sankyo Lab. Service Corp., Tokyo, Japan), Neuro2a into female A/J Jms Slc mice (Sankyo Lab. Service Corp.), Colon 26 into female CDF1 mice (Sankyo Lab. Service Corp.) and S180 into female ddY mice (Sankyo Lab. Service Corp.).

2.5. Toxicity by injection of collagenase

C57BL/6NCrSlc mice were intravenously injected with 100 μL of 0.3% collagenase-1 (0.3 mg) or 0.5% collagenase-1 (0.5 mg) in PBS. Twenty-four hours after injection, the lung was were immediately frozen, sectioned 16-μm thick and mounted. The sections were stained with hematoxylin and pure eosin (H&E staining) (Muto Pure Chemicals Co. Ltd., Tokyo, Japan).

2.6. Immunohistochemistry

When the average volume of LLC, B16, S180, Neuro2a and Colon 26 tumors reached 100–200 mm^3, the mice were sacrificed and the tumors were frozen on dry ice. The frozen tumors were embedded in O.C.T. compound and processed by frozen sectioning at 16 μm. Each frozen section was mounted on silane-coated slides. After protein blocking for 1 h at room temperature with PBS containing 5% rabbit serum (Sigma–Aldrich, St. Louis, MO, USA), the sections were incubated with goat polyclonal antibody against type I collagen (COL1A1 (D-13); Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), followed by incubation with Alexa 555-labeled rabbit anti-goat IgG (Invitrogen) as the secondary antibody. Fluorescence was examined microscopically using an ECLIPSE TS100 microscope (Nikon, Tokyo, Japan).

2.7. Western blotting

When the tumor volume reached approximately 100–200 mm^3, LLC tumor-bearing mice were intravenously injected with 100 μL of 0.3% collagenase-1 (0.3 mg) in PBS or intratumoraly injected with 50 μL of 0.6% collagenase-1 (0.3 mg) in PBS. One hour after injection, the tumors were excised and then homogenized in 10 mM Tris buffer (pH 8.0) containing 250 mM sucrose and phenylmethanesulfonylfluoride (PMSF). As a control, untreated LLC tumors were homogenized and then incubated with 0.3 mg collagenase-1 at 37 °C for 1 h. For the detection of type I collagen and β-actin proteins, 30 μg proteins were separated by 7.5 and 12.5% SDS-polyacrylamide gel, respectively, and then transferred to a polyvinylidene difluoride membrane (iBlot™ Gel Transfer Stacks PVDF, Regular®; Invitrogen) by a device (iBlot™ Gel Transfer Device®; Invitrogen). Type I collagen protein was identified using antibody against type I collagen (COL1A1 (D-13)) and rabbit anti-goat IgG horseradish peroxidase conjugate (Santa Cruz Biotechnology Inc.) as the secondary antibody. Expression of β-actin protein was identified using mouse anti-β-actin monoclonal antibody peroxidase conjugate (Santa Cruz Biotechnology). Type I collagen and β-actin proteins were detected with peroxidase-induced chemiluminescence (Super Signal West Pico Chemiluminescent Substrate; Pierce, Rockford, IL, USA).

2.8. IFP measurement in tumors

When the tumor volume reached approximately 200 mm^3, LLC, Colon 26 and Neuro2a tumor-bearing mice were intravenously injected with 100 μL of 0.3% collagenase-1 in PBS. One and twenty-four hours after injection, mice were anesthetized with isoflurane, and then interstitial fluid pressure (IFP) of tumors was measured with a needle probe pressure monitor, fitted with an 18-gauge side-ported needle (Intra-Compartmental Pressure Monitor System; Stryker, Kalamazoo, MI, USA) connected to a syringe filled with 0.9% saline, as previously reported (Tailor et al., 2010). The needle probe was inserted into the center of the tumor or normal muscle, and IFP was recorded. The IFP in tumors was normalized to that in muscle (normalized IFP = IFP (mm Hg) of tumor/IFP (mm Hg) of muscle).