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Highly site-selective transvascular drug delivery by the use of nanosecond pulsed laser-induced photomechanical waves

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ABSTRACT

Photomechanical waves (PMWs), which were generated by irradiation of a light-absorbing material (laser target) with nanosecond laser pulses, were used for targeted transvascular drug delivery in rats. An Evans blue (EB) solution was injected into the tail vein, and laser targets were placed on the skin, muscle and brain. Each laser target was irradiated with a laser pulse(s) and 4 h later, the rat was perfused and the distribution of EB fluorescence in the targeted tissues was examined. We observed laser fluence-dependent and hence PMW pressure-dependent extravasation of EB selectively in the tissues that had been exposed to a PMW(s). Uptake of leaked EB into cells in extravascular space was also observed in the targeted tissues. Tissue damage or hemorrhage was not apparent except in the brain exposed to the highest laser fluence used. The results for the brain indicated opening of the blood–brain barrier (BBB). Reverse-order (PMW application before EB injection) experiments showed that the BBB was closed in the duration from 8 h to 12 h after PMW application at a laser fluence of 0.5 J/cm^2. Since EB molecules are strongly bound with serum albumin in blood, the results indicate that the present method can be applied not only to small molecules but also to macromolecules.

1. Introduction

Among various drug delivery systems, transvascular delivery is a golden standard for many diseases. However, its efficiency and outcome are not always satisfactory, since transportation of the drug molecules from blood vessels to the lesion is often limited. For solid tumors, delivery of macromolecular anticancer drugs usually relies on an enhanced permeability and retention (EPR) effect [1,2]; various intelligent nanocarriers, such as those based on liposomes and micelles, have been developed to maximize the effect [2–5]. However, further improvement in delivery efficiency is needed. Moreover, an efficient EPR effect is not expected in many other diseases.

For brain diseases, transvascular drug delivery is often difficult due to the blood–brain barrier (BBB). Thus, the development of a method to safely control the permeability of blood vessels in targeted tissue is one of the most important challenges for the next-generation drug-based medicine. For this purpose, the use of an external physical energy, such as ultrasound [6–13], electric field [14,15], light [16,17] or radiation [18,19], has been receiving much attention in the past few decades. Especially, there have been many reports on the use of focused ultrasound (FUS) [6,7,10,11,13]. Since ultrasound-related technology is well-established and widely used for medical imaging, its adaptability to theranostic application would be highly attractive. However, there remain problems associated with the use of a contrast agent; the region for delivery depends on the distribution of a contrast agent, and safety of the use of a contrast agent has not been established for therapeutic application. Thermal side effects are often observed in ultrasound-based drug delivery. In *in vivo* use of an electric field (electroporation), electrodes must be placed near the target tissue; their insertion into the tissue is often needed. Even doing so, the region of discharge is affected by the distribution of electric impedance in the tissue, causing limitation in site selectivity of delivery. For BBB opening, moreover, electroporation has been used in the irreversible mode, which is accompanied by cell death [14,15].

We have been investigating the use of nanosecond pulsed laser-induced photomechanical waves (PMWs) for gene delivery *in vivo* [20–25], and we found that PMWs can increase the permeability of blood vessels [26]. In this study, we investigated characteristics of PMW-based targeted transvascular drug delivery. In this method,

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neither a contrast agent nor electrodes are needed, and the thermal effect is negligible due to extremely short interaction time. Thus, a process with low invasiveness is possible. In addition, delivery is not affected by uncontrollable factors, such as the distributions of a contrast agent and electrical impedance in the tissue, enabling highly site-selective delivery.

In the present experiments, Evans blue (EB) as a test drug was injected into the rat tail vein, and then PMWs were applied to the skin, muscle and brain. Observation of the distribution of EB-originating fluorescence in targeted tissues showed laser fluence-dependent and hence PMW pressure-dependent leakage of EB molecules from the vessels and incorporation of EB molecules by cells in extravascular spaces. For the brain, duration of leakage was evaluated by reverse-order (PMW application before EB injection) experiments. In this paper, we describe the results of these experiments and discuss the mechanism and characteristics of the method as well as potential medical applications.

2. Materials and methods

The Ethics Committee of Animal Care and Experimentation, National Defense Medical College, Japan approved all requests for animals and the intended procedures of the present study (Permission number: 09074, 10042). All animal experiments were performed under anesthesia, and all efforts were made to minimize suffering.

2.1. Generation and characteristics of PMW

The experimental setup used in this study was the same as that used for gene delivery studies [20–25]. Briefly, a laser target, which was a light-absorbing material (0.5-mm-thick natural black rubber disk) to which an optically transparent material (1.0-mm-thick transparent polyethyleneterephthalate sheet) was attached, was placed on the target tissue (Fig. 1a). For acoustic impedance matching, ultrasound gel (Echo Jelly, Aloka, Tokyo, Japan) was used between the target bottom surface and the tissue. The laser target was irradiated with a nanosecond laser pulse to induce plasma, and its expansion was accompanied by a strong pressure wave called a photomechanical wave (PMW). As a laser source, nanosecond pulsed laser is suitable, since its laser pulse temporally overlaps well with its own inducing plasma. In this study, the second harmonics of a Q-switched Nd:YAG laser (Brilliant b, Quantel, Les Ulis Cedex, France; wavelength, 532 nm; pulse width, 6 ns FWHM) was used.

Fig. 1b shows typical temporal pressure profiles of PMWs generated at different laser fluences on the laser target (spot diameter, 3 mm). The waveforms are characterized by a fast rise time and high peak pressure. However, durations of PMWs are as short as a few hundreds of nanoseconds (FWHM) and their impulses are therefore relatively small. In addition, pressures of PMWs are dominated by a positive (compressive) component. Due to these properties, the invasiveness of PMWs is low. Both the peak pressure and impulse increase almost linearly with increase in laser fluence. The diameter of a PMW can be easily changed by changing the defocused distance of the laser beam. Thus, pressure

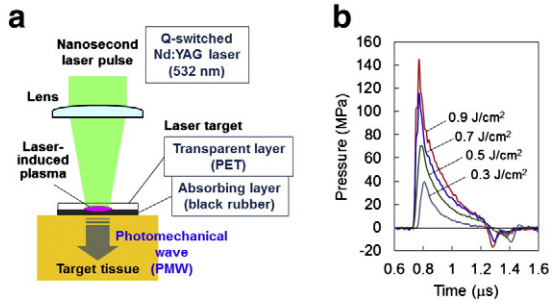

Fig. 1. Generation and characteristics of a photomechanical wave (PMW). (a) Experimental arrangement. (b) Waveforms of PMWs at various laser fluences. PET is polyethyleneterephthalate.

characteristics of PMWs are highly controllable, both temporally and spatially.

2.2. Test drug

We used Evans blue (EB) (Wako Pure Chemical Industries, Tokyo, Japan) as a test drug. Evans blue is an azo dye with a molecular mass of ~961 Da. However, EB molecules are strongly bound with serum albumin (~66 kDa) in blood [27], and EB molecules in blood can therefore be regarded as macromolecules. Under normal conditions, vascular leakage of EB-bound albumin (EBA) is limited in tissue, especially in the brain due to the BBB, but EBA can leak out of vessels when the permeability of vessel walls increases. Thus, EB can be used as a nontoxic marker to indicate permeation of vessels and disruption of the BBB. Since EB efficiently emits red fluorescence peaked at ~680 nm, the distribution of EB in tissue can be evaluated by measuring or imaging fluorescence intensity.

2.3. Animal preparation, drug administration and application of PMWs

The dorsal skin, the tibialis anterior muscle and the brain were chosen as targeted tissues. Nine- to twelve-week-old Sprague–Dawley male rats (Japan SLC, Hamamatsu, Japan) were anesthetized with pentobarbital sodium (50 mg/kg animal weight i.p.), and the dorsal skin, leg skin and scalp were shaved. To apply PMWs to the tibialis anterior muscle, a laser target was placed on the skin over the muscle and PMWs were thus applied transcutaneously. A PMW can be applied to the brain through the scalp, transcranially or directly through a cranial window. In this experiment, we applied a PMW to the brain through a cranial window to examine the direct effect of PMW on the brain. We exposed the parietal bone and made a cranial window of ~5 mm in diameter in the right parietal bone; a laser target was put on the brain surface.

A 5% saline solution of EB was injected into the tail vein at a dose of 1 ml/kg animal weight. About 5 min after injection, the laser target on each targeted tissue was irradiated with a laser pulse (spot diameter, 3 mm). In each experiment, 3 laser pulses were applied for the skin and muscle, while a single laser pulse was applied for the brain. After all laser irradiations to the rat, laser targets were removed and the incised scalp was sutured, and then the rat was returned to its individual cage. The experiment was repeated at different laser fluences for each target tissue.

2.4. Evaluation of the distribution and quantity of EB in target tissues

About 4 h after PMW application, the rat was anesthetized again in the same manner as that described above and perfusion fixation was performed by intracardiac infusion of 10% formalin solution or 4% paraformaldehyde solution. Punch biopsy was performed for the dorsal skin and the tibialis anterior muscle, and the whole brain was extracted and a coronal section was made at the site of PMW application. For the brain, white light photographs were taken before and after sectioning to observe blue staining with EB on the brain surface and cross-section. All tissues were post-fixed in the same fixative for about 2 h and then the tissues were frozen in an OCT compound (Sakura Finetek USA Inc., Torrance, CA) and sectioned into 10-μm-thick slices using a cryostat microtome. The sections were observed by a fluorescence microscope (Axiovert 200, Carl Zeiss, Göttingen, Germany; excitation wavelength, 534–558 nm or 539-585 nm; observation wavelength, >590 nm or 600-682 nm) to determine the distribution of EB in the tissues.

For semi-quantified evaluation of leaked EB, 3 vertical observation lines were randomly placed in the region of the tissue that had interacted with a PMW(s) on each fluorescence image. Intensity distribution was measured and integrated along each line by ImageJ software, and the three data sets were averaged; the values were further averaged in each condition (averaged integrated EB fluorescence intensity). Fluorescence was negligibly weak in all of the negative control