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Low-Dose Vascular Photodynamic Therapy Decreases Tumor Interstitial Fluid Pressure, which Promotes Liposomal Doxorubicin Distribution in a Murine Sarcoma Metastasis Model[1]

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Abstract

INTRODUCTION: Solid tumors are known to have an abnormal vasculature that limits the distribution of chemotherapy. We have recently shown that tumor vessel modulation by low-dose photodynamic therapy (L-PDT) could improve the uptake of macromolecular chemotherapeutic agents such as liposomal doxorubicin (Liporubicin) administered subsequently. However, how this occurs is unknown. Convection, the main mechanism for drug transport between the intravascular and extravascular spaces, is mostly related to interstitial fluid pressure (IFP) and tumor blood flow (TBF). Here, we determined the changes of tumor and surrounding lung IFP and TBF before, during, and after vascular L-PDT. We also evaluated the effect of these changes on the distribution of Liporubicin administered intravenously (IV) in a lung sarcoma metastasis model. *MATERIALS AND METHODS:* A syngeneic methylcholanthrene-induced sarcoma cell line was implanted subpleurally in the lung of Fischer rats. Tumor/surrounding lung IFP and TBF changes induced by L-PDT were determined using the wick-in-needle technique and laser Doppler flowmetry, respectively. The spatial distribution of Liporubicin in tumor and lung tissues following IV drug administration was then assessed in L-PDT–pretreated animals and controls (no L-PDT) by epifluorescence microscopy. *RESULTS:* L-PDT significantly decreased tumor but not lung IFP compared to controls (no L-PDT) without affecting TBF. These conditions were associated with a significant improvement in Liporubicin distribution in tumor tissues compared to controls ($P < .05$). *DISCUSSION:* L-PDT specifically enhanced convection in blood vessels of tumor but not of normal lung tissue, which was associated with a significant improvement of Liporubicin distribution in tumors compared to controls.

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Introduction

The efficacy of drug therapy is partly related to the ability of the therapeutic agent to reach its target. The delivery of chemotherapeutics to tumors was shown to be influenced by the tumor blood supply, the drug transport through the vascular wall, and the drug diffusion/convection through the interstitial space [1,2]. Various methods have been tested to improve drug distribution, including isolated organ

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perfusion, drug physiochemical property changes, and tumor vessel modulation [3–5].

Photodynamic therapy was initially designed to destroy tumor cells and the tumor vasculature. It consists of the administration of a photosensitizer that, after activation by nonthermal light, produces a variety of changes at the cellular level in the treated area [6]. Recently, low-dose photodynamic therapy (L-PDT) was shown to enhance the extravasation of macromolecular compounds into tumors [7,8]. For example, vascular L-PDT of sarcoma metastasis in a murine model resulted in a significant and selective enhancement of liposomal doxorubicin (Liporubicin; Regulon Inc, Athens, Greece) in tumors. The enhanced drug penetration in tumors was observed with different modes of Liporubicin administration (IV and isolated lung perfusion). Similar results were found in a different murine model of colon cancer, lung adenocarcinoma, and mesothelioma [7–10]. However, the precise mechanism by which L-PDT improves drug transport through the tumor vasculature remains unknown.

For macromolecular drugs (<100 nm in diameter), it was recently demonstrated that convection is the main promoter of drug extravasation between the intravascular and extravascular spaces [11]. The latter is dependent on the Starling equation that includes two main parameters, namely, tumor hydrostatic and oncotic pressures. A hallmark of malignant cancer is the angiogenic switch that primarily occurs through vascular endothelial growth factor. High levels of vascular endothelial growth factor were shown to alter the tumor vascular organization, to increase vascular permeability and the interstitial fluid pressure (IFP) thus hindering convection and drug delivery [1,2,4]. Many methods have been suggested to improve drug uptake and selectivity in tumors among which is vasculature "normalization." The latter was shown to occur with low doses of antiangiogenic therapy given at appropriate intervals, which caused a transient decrease in tumor vascular permeability and IFP. This made the vessels function in a more "normal" way and improved convection and concomitant drug delivery to tumors [2,4].

In the present study, we hypothesized that L-PDT caused a transient improvement in the function of tumor vasculature in a somewhat similar way to "vascular normalization." In a rodent model of sarcoma metastasis, we studied the changes in tumor and lung tissue (IFP) as well as TBF before, during, and up to 1 hour after low-dose Visudyne (Novartis, Hettlingen, Switzerland)–mediated L-PDT. In parallel, the uptake of Liporubicin administered IV was determined by epifluorescent microscopy in tumor and lung tissues.

Material and Methods

Study Design

Thirty-eight Fischer rats (Charles River Laboratories, France) underwent subpleural sarcoma implantation in their left lower lobe. This was followed 10 days later by a re-thoracotomy. Tumor L-PDT was performed using Visudyne and laser light. This was directly followed by the administration of Liporubicin, which was allowed to circulate for 1 hour. IFP was measured in tumor and normal lung in 10 and 8 animals, respectively, before and during 1 hour following L-PDT. In a separate set of five animals, TBF was measured in tumors before and during 1 hour following L-PDT. Liporubicin concentration and distribution in tumors and surrounding lung were assessed by epifluorescence microscopy performed on samples embedded in a cryogenic gel (OCT; Electron Microscopy Sciences, Hatfield, PA, USA) in the different treatment groups (n = 5 per group, total = 10). Finally, five animals were used as controls with no L-PDT. In these, all procedures including Visudyne and Liporubicin were injected, but no light was delivered.

Animals and Housing

Male Fischer rats weighing 250 to 300 g were treated in accordance with the Animal Welfare Act and the National Institutes of Health "Guidelines for the Care and Use of Laboratory Animals" and according to the Local Ethical Committee of the University of Lausanne.

Tumor Cell Line

A syngeneic methylcholanthrene-induced sarcoma (MCA) cell line was used as previously described [3]. It was cultivated at 37°C with 5% CO_2 in 20 ml of Roswell Park Memorial Institute (RPMI) medium 1640 medium containing glutaril, 10% FBS, and 1% penicillin/streptomycin (Invitrogen Corporation/Gibco/Life Technologies Ltd, Paisley, United Kingdom).

Subpleural Tumor Generation in the Left Lower Lung Lobe

This procedure was performed as described previously [3]. Briefly, animals were anesthetized by pentobarbital sodium (50 mg/kg), and oro-tracheal intubation was performed using a 16-gauge polyethylene Angiocath (Becton Dickinson, Sandy, UT). Animals were ventilated with a mixture of oxygen and isofluran (0.5%-2%, Forene; Abbott, Zug, Switzerland) using a tidal volume of 10 ml/kg and a respiratory rate of 75 to 90/min. A left-sided minithoracotomy was performed through the seventh intercostal space, and 0.1 ml of MCA cell solution containing 5×10^7 viable tumor cells was injected subpleurally into the left lower lobe using a 27-gauge needle [12]. The thoracotomy was closed layer by layer, and the endotracheal tube was removed.

L-PDT of Lungs Bearing Sarcoma Metastasis

Treatment was initiated when the tumors had reached a size of approximately 4 to 6 mm in diameter (approximately 7 days) as previously described [13]. The animals were anesthetized, and a left-sided thoracotomy was performed through the fourth intercostal space. The left lung was freed from its adhesions. A left cervical incision was performed to cannulate the external jugular vein. Visudyne was dissolved in NaCl (0.9%) and glucose (5%) and injected at a dose of 0.0625 mg/kg. After 15 minutes, laser light was applied to the exposed lower lung at a wavelength of 689 nm by an optical fiber–based frontal light distributor (Medlight, Ecublens, Switzerland) coupled to a diode laser (4-W laser diode; Biolitec, Germany). Noncontact, nonthermal surface irradiation was performed to the tumor and the surrounding normal lung tissue with the incident laser beam directed perpendicular to the lung surface and centered on the tumor. The treatment spot had a diameter of 30 mm, and the treated area was exposed to an irradiance of 35 mW/cm^2 and a light dose of 10 J/cm^2 corresponding to a treatment time of approximately 5 minutes. The irradiances and the light doses were measured in real-time as previously described [7,12].

IV Administration of Liporubicin

Immediately after laser light delivery, 400 μg of Liporubicin dissolved in 0.5 ml of 6% Hydroxyethyl Starch (HAES) was injected through the external jugular vein catheter. The time interval between Liporubicin administration and harvesting of the left lung (Liporubicin circulation time) was 60 minutes. Control animals underwent exactly the same operative procedure (including Visudyne injection) but had no light therapy (no laser and kept in the dark) before Liporubicin administration.