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Liposome accumulation in irradiated tumors display important tumor and dose dependent differences

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Abstract

Radiation therapy may affect several important parameters in the tumor microenvironment and thereby influence the accumulation of liposomes by the enhanced permeability and retention (EPR)-effect. Here we investigate the effect of single dose radiation therapy on liposome tumor accumulation by PET/CT imaging using radiolabeled liposomes. Head and neck cancer xenografts (FaDu) and syngenic colorectal (CT26) cancer models were investigated. Radiotherapy displayed opposite effects in the two models. FaDu tumors displayed increased mean accumulation of liposomes for radiation doses up to 10 Gy, whereas CT26 tumors displayed a tendency for decreased accumulation. Tumor hypoxia was found negatively correlated to microregional distribution of liposomes. However, liposome distribution in relation to hypoxia was improved at lower radiation doses. The study reveals that the heterogeneity in liposome tumor accumulation between tumors and different radiation protocols are important factors that need to be taken into consideration to achieve optimal effect of liposome based radio-sensitizer therapy.

Key words: Liposome; PET; Radiotherapy; Radio-sensitizer; Hypoxia

External beam radiation therapy (RT) is a central part of the treatment regimen for more than half of all cancer patients. Liposomal drug delivery systems that carry radio-senzitizers to tumors can potentially improve therapeutic efficacy of RT without increasing loco-regional side effects in the irradiated region.[1,2] Combining targeted RT and targeted drug delivery can therefore increase regional tumor control.[3] Moreover, liposomes are flexible in regards to the selection of drugs that can be encapsulated, transported and released within tumors. Liposomes can therefore serve as optimal delivery systems for targeting radiosensitizers to malignant tissue.[1,2] However, liposome accumulation in solid tumors has been demonstrated to depend on multiple factors, including interstitial pressure, tumor vasculature and perfusion.[4–6] Liposome extravasation by the enhanced permeability and retention (EPR) effect is primarily driven by transvascular convection and their accumulation is

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inversely correlated to interstitial fluid pressure (IFP) and directly correlated to regional blood perfusion and leakiness.[4,6–8] RT influences these parameters; however, results on the effect on tumor accumulation levels of nano-sized particles are not clear.[6]

Molecular oxygen is the most important radio-sensitizer and hypoxic tumor cells are highly radio-resistant and display increased malignancy. Tumor hypoxia is generally divided in acute perfusion limited, chronic diffusion limited and anemic hypoxic.[9] The nature of tumor hypoxia is closely related to vascular parameters and liposomes may therefore distribute poorly to hypoxic regions. In both experimental and clinical tumors the IFP is increased and associated with an increased malignant phenotype.[10,11] RT has been associated with increased vascular leakiness, and high total radiation doses can potentially increase the extravasation of macromolecules.[6] Pretreating tumors with cytotoxic agents has been identified to increase tumor blood flow and decrease IFP, potentially being the results of a reduction in tumor cell density to alleviate tumor blood vessels compressions and increase the vascular surface area which subsequently increases liposome accumulation.[12,13] Following these observations the effects of RT could also mediate a beneficial effect for macromolecular extravasation by reducing cell density.[11,14] Importantly, single radiation doses >10 Gy, are known to cause significant damage to neoangiogenic tumor vasculature and increase hypoxia and mediate significant secondary cancer cell death following vascular damage.[15] On the contrary, single doses <10 Gy cause mild vascular damage and may potentially increase vascular perfusion and thereby decrease hypoxia after irradiation.[15–17] Few studies of the effect of RT on liposome uptake have been conducted. Single-fraction irradiation had no effect on liposome uptake in human KB cancer xenografts when evaluated by gamma counting radiolabeled liposomes.[18] Considering this and that important tumor dependent differences and responses may exist, we investigated the effect of single fraction radiation therapy on liposome accumulation. This was evaluated by non-invasive PET imaging in regard to i) the potential for improving liposomal drug delivery by RT 24 h prior to liposome administration, ii) the influence of RT on vascular tumor parameters, cellular density and necrosis and iii) locoregional liposome accumulation in hypoxic tumor regions, in a human head and neck cancer xenograft model and in a syngenic murine colon cancer model.

Methods

Tumor model

FaDu (human head and neck cancer) xenografts were established by subcutaneous injection of ~5×10^6 cells suspended in 100 μl of culture medium and Matrigel over the thigh/flank of 7 weeks old female NMRI nude mice. Tumors were allowed to grow for 12-14 days. CT26 (murine colon cancer) syngenic tumors were established by subcutaneous injection of ~3×10^5 cells suspended in 100 μl of culture medium over the thigh/flank of 6 weeks old female Balb/c mice. Tumors were allowed to grow for 18 days. The National Animal Experiments Inspectorate approved all study procedures.

Radiolabeled liposomes

Pegylated liposomes consisting of HSPC:CHOL:DSPE-PEG2k (56.5:38.2:5.3) were remote loaded with the PET isotope $^{64}Cu^{2+}$. Briefly, 100 nm 50 mM pegylated liposomes entrapping 10 mM DOTA were prepared as previously described.[19] Radiolabelling was achieved by adding a volume of liposomes to dried $^{64}CuCl_2$ followed by incubation at 55 °C for 75 min. The loading efficiency was afterward evaluated by Thin Layer Chromatography (Radio-TLC) and Size Exclusion Chromatography (Radio-SEC),[19] which showed a loading efficiency of >98% for both techniques. The liposomes were prepared at either 3.3 mM or 6.6 mM lipid concentration and an activity concentration of 62.5 MBq/ml or 125 MBq/ml (activity at the time of injection) for the FaDu and CT26 tumors respectively. Each animal was dosed with a volume corresponding to 22 μmol/kg and an activity of ~12.5 MBq/animal.

Radiation therapy

Mice carrying FaDu xenografts were randomized into four treatment groups; non-irradiated controls (n = 11), 5 Gy (n = 11), 10 Gy (n = 10) and 20 Gy (n = 11). Mice carrying CT26 tumors were randomized into four treatment groups; non-irradiated controls (n = 8), 2 Gy (n = 8), 5 Gy (n = 8) and 10 Gy (n = 8). Radiation therapy was delivered as a single fraction at a dose-rate of 1 Gy/min (320 kV, 12.5 mA) using a small animal irradiator (X-rad320, pXi, CT, USA). Mice were irradiated in a dedicated fixation device securing that only the tumor bearing leg was exposed to irradiation and the remaining body shielded.

MicroPET/CT imaging

PET/CT imaging was performed on an Inveon® small animal PET/CT system (Siemens Medical Systems, PA, USA) approximately 24 h after completion of RT. Mice were anesthetized by inhalation anesthesia (~3% sevoflurane) and ^{64}Cu-liposomes injected into a tail vein. ^{64}Cu-liposomes were allowed to distribute for 1 h before commencing a 5-min PET scan (1-h scan) followed by a corresponding CT scan. A similar PET/CT scan (15 min acquisition) was performed after a distribution period of 24 h (24-h scan). Emission data were corrected for dead time and decay and attenuation correction was performed based on the corresponding CT scan. PET scans were reconstructed using a maximum a posteriori (MAP) reconstruction algorithm (0.815 × 0.815 × 0.796 mm). Image analysis was performed using Inveon® software (Siemens Medical Systems, PA, USA). 3D regions of interest (ROIs) were manually constructed and decay corrected data (%injected dose per gram tissue (%ID/g)) reported.

Immunohistochemistry CD31, cell density and necrosis

Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded 4 μm tumor sections that were stained with H&E for histological evaluation and with CD31 antibodies for tumor blood vessels. CD31 staining was performed by