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Microvascular biodistribution of L19-SIP in angiogenesis targeting strategies

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ABSTRACT

Introduction: Various strategies using L19-mediated fibronectin targeting have become useful clinical tools in anti-tumour therapy and diagnostics. The aim of our study was to characterise the microvascular biodistribution and binding process during tumour angiogenesis and after anti-angiogenic therapy.

Materials and methods: SF126 glioma and F9 teratocarcinoma cells were implanted into dorsal skin fold chambers (SF126: $n = 4$; F9: $n = 6$). Using fluorescence and confocal intravital microscopy the biodistribution process was assessed at $t = 0$ h, $t = 4$ h and $t = 24$ h after intravenous application of Cy3-L19-SIP. Sunitinib treatment was applied for six days and microscopy was performed 2 and 6 days after treatment initiation. Analysed parameters included: vascular and interstitial binding, preferential binding sites of L19-SIP, microvascular blood flow rate, microvascular permeability. Histological analysis included CD31 and DAPI.

Results: L19-SIP showed a specific and time-dependent neovascular binding with a secondary extravasation process reaching optimal vascular/interstitial binding ratio 4 hours after iv administration (F9: L19-SIP: vascular binding: 74.6 ± 14.5; interstitial binding: 46.8 ± 12.1; control vascular: 22,2 ± 16.6). Angiogenic sprouts were preferred binding sites (F9: L19-SIP: 188 ± 15.5; RTV: 90.6 ± 13.5). Anti-angiogenic therapy increased microvascular hemodynamics (SF126: Su: 106.6 ± 13.3 μl/sec; Untreated: 19.7 ± 9.1 μl/sec) and induced increased L19-SIP accumulation (SF 126: t24; Su: 92.6 ± 2.7; Untreated: 71.9 ± 5.9) in therapy resistant tumour vessels.

Conclusion: L19-SIP shows a time and blood-flow dependent microvascular biodistribution process with angiogenic sprouts as preferential binding sites followed by secondary extravasation of the antibody. Microvascular biodistribution is enhanced in anti-angiogenic-therapy resistant tumour vessels.

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1. Introduction

Antibody-based targeted delivery of pharmaceuticals has become a promising approach for specifically targeting and supplying diagnostic and therapeutic molecules to solid tumours.[1] The Extradomain B (ED-B) of fibronectin represents one of the most promising neovascular markers. It is typically absent in human plasma and normal adult tissues, but it is strongly expressed in the vasculature of aggressive tumours.[1,2] The antibody L19-SIP has been generated to specifically target the ED-B of fibronectin.[3] Immunocytokines coupled to L19 have been demonstrated to significantly inhibit tumour growth and metastasis.[4] Research has led to the clinical introduction of L19-based targeting strategies. Clinical reports show that the use of L19 is safe and feasible in head and neck squamous cell carcinoma in human patients[5] and that 131I-L19SIP radioimmunotherapy may be successfully applied in Hodgkin lymphoma patients.[6]

Despite growing experimental and clinical experience with L19, its microvascular biodistribution characteristics are not fully understood. However, microvascular biodistribution is of major importance for an adequate understanding of clinical applications of L19 based targeting strategies as there are several physiologic barriers to the delivery of macromolecules in tumours.[7] It was the aim of our study to visualise and characterise the microvascular binding and biodistribution properties of the L19-SIP in two distinct tumour models i.e. F9 teratocarcinoma and a SF126 glioma model.

2. Material and methods

2.1. Tumour cell lines

Murine F9 teratocarcinoma cells and human SF126 glioma cells were grown in DMEM with 4.5 g/L Glucose supplemented with 10% faetal bovine serum (PAA GmbH, Linz, Austria) at 37 °C in 5% CO_2 humidified incubators following conventional protocols.

2.2. Dorsal skinfold chamber model

For the assessment of tumour microvascularisation, F9 teratocarcinoma cells were implanted into dorsal skinfold chambers in C57/B6 mice ($n = 6$ per group) and SF126 glioma cells were implanted in nude mice ($n = 4$ per group) followed by intravital microscopy.

Intravital microscopic analysis was performed on day 14 after F9-teratocarcinoma implantation (during the application of L19-SIP) and on day 7 after SF126 glioma cell implantation. Different starting points were chosen to guarantee sufficient vascularisation in both tumour models because F9 teratocarcinoma demonstrates slower growth dynamics as SF126 gliomas. Microscopy was performed during injection of L19-SIP ($t = 0$) as well as 4 h ($t = 4$h) and 24 h ($t = 24$h) after intravenous application.

The microsurgical techniques for the implantation of the dorsal skinfold chamber have been previously described in detail.[8] All the experiments were approved by the Regierungspräsidium Karlsruhe and were carried out according to the guidelines for animal care and experimentation.

2.3. Intravital-microscopy

Mice were anesthetised by intraperitoneal injection with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). Intravital fluorescence video microscopy (IFVM) was performed by epi-illumination techniques using a modified Axiotech vario microscope (Attoarc; Zeiss, Jena, Germany). Microscopic images were recorded through a charge-coupled device (CCD) video camera with an optional image intensifier for weak fluorescence (Kappa, Gleichen, Germany) and transferred to a S-VHS video system (Panasonic) for offline analysis. Offline analysis was performed using a computer assisted analysis system (CAPIMAGE; Zeintl Software Engineering, Heidelberg, Germany). Microvessels were visualised by contrast enhancement with 2% FITC-conjugated dextran (0.1 ml, intravenous; molecular weight 150,000; Sigma). Simultaneous *in vivo* application of the Cy3-labelled L19-SIP and the use of green-light epi-illumination allowed for sequential analysis of biodistribution. Microvascular permeability (P) calculated as the ratio between intra- and extravascular contrast as described previously.[8,9]

2.4. Biodistribution analysis

In order to study microvascular biodistribution of L19-SIP, we injected 100 μg of Cy3-labelled L19-SIP (SIP group) into the jugular vein of animals. Animals without implanted tumour cells in the chamber served as SIP-control group. Cy3-L19-SIP was injected intravenously in order to visualise L19-SIP biodistribution in host vasculature.

Biodistribution was studied by measuring fluorescence intensity in the vascular and interstitial compartment in a total number of 6–9 tumour vessels per animal. For the measurement of fluorescence intensity we defined 2 regions of interest (area: 250–500 μm^2) per analysed tumour vessel: (i) the vascular compartment referring the vascular wall and the close perivascular area (defined as an area of 250–500 μm^2 with a width of 3 μm located next to the analysed tumour vessel) and (ii) the interstitial compartment (area of 250–500 μm^2) located in the interstitial space with a distance of 20 μm from the analysed tumour vessel. Before injection, we measured interstitial fluorescence intensity in order to exclude autofluorescent activity.

2.5. Biodistribution analysis during antiangogenic therapy

In order to analyse the influence of antiangiogenic therapy on microvascular biodistribution of L19-SIP, SF126 glioma cells were implanted into dorsal skinfold chambers in nude mice ($n = 4$ per group). As antiangiogenic agent we used the multi-kinase inhibitor Sunitinib. This model has been described as very suitable for IFVM.[10] Treatment with Sunitinib was initiated 7 days after SF 126 glioma cell implantation. All the animals in the treatment group received daily intraperitoneal injections of Sunitinib (40 mg/kg/day) for 6 days. Control animals received daily injections of 0.9% NaCl. IFVM was performed on day 2 and 6 after the initiation of treatment. For analysis of tumour microhemodynamics we assessed microvascular red blood cell velocity (RBCV;