The Combretastatin Derivative (Cderiv), a Vascular Disrupting Agent, Enables Polymeric Nanomicelles to Accumulate in Microtumors

KATSUYOSHI HORI,[1] MASAMICHI NISHIHARA,[2] KOUICHI SHIRAISHI,[2] MASAYUKI YOKOYAMA[2,3]

[1]Division of Cancer Control, Department of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai, 980-0785 Miyagi, Japan

[2]Kanagawa Academy of Science and Technology, 3-2-1 Sakado, Takatsu-ku, Kawasaki, 213-0012 Kanagawa, Japan

[3]Medical Engineering Laboratory, Research Center for Medical Science, Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, 105-8461 Tokyo, Japan

Received 4 June 2009; revised 29 October 2009; accepted 3 November 2009

Published online 28 December 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22038

ABSTRACT: A previous study found almost no leakage of polymeric nanomicelles from vessels in microtumors. If such vessels become leaky, sufficient nanomedicines may be delivered to microtumors and large tumors. To create leaky vessels, a combretastatin derivative (Cderiv), a vascular disrupting agent, was used. Via vital microscopy with fluorescein isothiocyanate (FITC)-labeled nanomicelles, the effect of Cderiv pretreatment on changes in micelle extravasation was investigated. Whether such treatment would prolong microtumor retention of micelles was also examined. FITC-albumin was used for comparison. The degree of extravasation from intact vessels in microtumors (rat sarcoma LY80) was extremely low and comparable to that from normal vessels. Cderiv pretreatment (1 or 3 days before administration of FITC-labeled compounds) markedly enhanced extravasation of such nanomicelles and albumin from vessels that survived treatment and had restored blood flow. A high concentration of extravasated macromolecules remained even 24 h later in tissue areas whose microcirculatory function had collapsed. Tumors receiving 10 Gy irradiation 3 days before the macromolecules evidenced gradual removal of extravasated macromolecules, which did not accumulate in those areas, despite extravasation from tumor vessels. Our results strongly suggest that pretreatment with Cderiv is quite effective for maintaining microtumor concentrations of nanomicelles and albumin associated with anticancer or diagnostic drugs. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2914–2925, 2010

Keywords: cancer; drug targeting; macromolecular drug delivery; micelle; polymeric drug carrier; albumin; combretastatin; irradiation; EPR effect; vital microscopy

INTRODUCTION

In recent years, nanoparticles incorporating anticancer drugs have been developed as a technique for achieving selective drug delivery to solid tumors.[1–9] This therapeutic strategy is based on the hypothesis that particles larger than a specific size selectively extravasate from tumor vessels, because tumor vessels have wider intercellular gaps than normal vessels.[1,4,7]

Recently, however, by means of vital microscopic analysis with fluorescein isothiocyanate (FITC)-labeled polymeric micelles, the following results concerning extravasation and retention of micelles in tumors were obtained:[10] (i) polymeric micelles readily leaked from vessels at the interface between normal and tumor tissues and at the interface between tumor tissues and necrotic areas, with the latter interface showing markedly enhanced extravasation; (ii) polymeric micelles accumulated at a high concentration in necrotic tumors with circulatory dysfunction; and (iii) polymeric micelles barely extravasated from vessels in microtumors <2–3 mm in diameter that had no necrotic areas, and these micelles did not accumulate in such microtumors.

Findings (i) and (ii) are important phenomena for inducing the enhanced permeability and retention (EPR) effect[11–13] discovered by Maeda and Matsumura. Finding (iii), however, indicates that

Correspondence to: Katsuyoshi Hori (Telephone: 81-22-717-8532; Fax: 81-22-717-8533; E-mail: k-hori@idac.tohoku.ac.jp)

Journal of Pharmaceutical Sciences, Vol. 99, 2914–2925 (2010)

the EPR effect is not often seen in microtumors. This result suggests that microscopic metastatic foci may escape from the attack of nanomedicines that have encapsulated anticancer drugs. This extremely important problem influences survival of individuals who have cancer cells that survive in micrometastatic foci. Thus, to enhance the therapeutic ability of such drugs, the EPR effect must function not only in large tumors but also in microtumors.

To induce necrotic areas and leaky blood vessels in microtumors, a combretastatin derivative (Cderiv), (*Z*)-*N*-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl] phenyl]-L-serinamide hydrochloride (formerly known as AC7700[14]) was used, because Cderiv selectively interrupts tumor blood flow, disrupts tumor vessels, and thereby causes necrosis of tumor tissue.[15–18] Such effects of Cderiv were observed in carcinogen-induced primary tumors[19] and transplanted tumors.[16,20] They were seen not only in subcutaneous tumors but also in tumors growing in internal organs, in lymph node metastases, and in microtumors <1 mm in diameter.[20] In addition, X-irradiation was used in the present study because it enhances the permeability of blood vessels while simultaneously damaging tumor tissues.[21] If the EPR effect of nanomedicines were enhanced by pretreatment with Cderiv or X-irradiation, advancements in cancer therapy and diagnosis may follow.

In the present study, we used FITC-labeled polymeric micelles and analyzed how extravasation and intratumor retention of such nanoparticles changed after pretreatment with Cderiv or X-irradiation. FITC-albumin was used as a comparison. The aim of the present study was to show that the EPR effect can effectively be induced in microtumors by causing the collapse of tumor microcirculatory function via pretreatment with Cderiv.

MATERIALS AND METHODS

Rats and Tumors

Male Donryu rats (Crj-Donryu; Nippon Charles-River, Yokohama, Japan), 7–8 weeks old and with an average weight of 220–250 g, were used for all experiments. Rats were bred and maintained in accordance with The Law (No. 105) and Notification (No. 6) issued by the Japanese Government. Specifically, they were comfortably housed in a ventilated, temperature-controlled ($24 \pm 1^{\circ}C$), specific pathogen-free environment on a bed of wood shavings, with food and water freely available and a 12-h light–dark cycle. Rats that were equipped with transparent chambers for vital microscopic observations (see below) were caged singly (cage volume, $30 \times 40 \times 25\,cm^3$).

A variant of Yoshida sarcoma, LY80, was used in the present experiments. This cell line, which can grow in ascites and in solid form, was maintained by successive i.p. transplantation. The transplantation rate of LY80 to Donryu rats was ~100%, without spontaneous regression.

All experimental protocols were reviewed by the Committee on the Ethics of Animal Experiments of Tohoku University and were carried out according to the Guidelines for Animal Experiments issued by Tohoku University.

Anesthesia

Both pentobarbital sodium salt (Tokyo Kasei Kogyo Co., Tokyo, Japan) and enflurane (Abbott Laboratories, North Chicago, IL), given simultaneously, were used for anesthesia. Pentobarbital solution (50 mg/mL) was administered i.m., 10 min before the experiment, at a dose of 30 mg/kg, and supplemental doses (15 mg/kg i.m.) were given at 90-min intervals to maintain immobilization. Enflurane concentration was maintained at 1% in the inhaled gas, which was administered at a rate of 1 L/min by means of an anesthetic apparatus for small laboratory animals.[10]

Implantation of Transparent Chambers and Tumor Transplantation

For *in vivo* analysis of extravasation of FITC-labeled compounds, transparent chambers were implanted, under aseptic conditions, in dorsal skin flaps of rats.[22] For tumor transplantation, a small fragment (~$0.1\,mm^3$) of solid tumor from a donor rat was transplanted onto the normal tissue in a transparent chamber while the chamber was being implanted in the dorsal skin flap. This transparent chamber enables observation of changes in the tumor vascular system, from angiogenesis to necrosis (for ~2 weeks).

Microtumors

The term "microtumors" in the present study was defined as tumors <3 mm in diameter growing in the transparent chamber. Experiments on microtumors were usually performed 7–9 days after tumor transplantation.

FITC-Labeled Polymeric Micelles and FITC-Albumin

To observe macromolecular behavior *in vivo*, FITC-labeled polymeric micelles (Mw of the block copolymer 15,259) and FITC-albumin (Mw 69,680) (Sigma–Aldrich Japan K.K., Tokyo, Japan) were used. FITC-labeled micelles were synthesized by the Kanagawa Academy of Science and Technology. The characteristics of this polymeric micelle preparation were described in detail elsewhere.[10] In brief, the micelle consists of the poly(ethylene glycol)-*b*-poly (benzyl-L-aspartate) block copolymer, with 16.9% of