Antitumor Effect of Arterial Administration of a Medium-Chain Triglyceride Solution of an Angiogenesis Inhibitor, TNP-470, in Rabbits Bearing VX-2 Carcinoma

Shigeo Yanai,1,3 Hiroaki Okada,1 Kazuhiro Saito,1 Yuji Kuge,1 Masafumi Misaki,1 Yasuoki Ogawa,1 and Hajime Toguchi2

Received September 5, 1994; accepted November 28, 1994

Using rabbits bearing VX-2 carcinoma on the inner side of the leg, we examined the antitumor activity of a medium-chain triglyceride (MCT) solution of an angiogenesis inhibitor, TNP-470 (AGM-1470, 6-O-(N-chloroacetylcarbamoyl)-fumagillol), following administration into the femoral artery feeding the tumor. The MCT solution of TNP-470 (1 and 5 mg) strongly suppressed tumor growth following a single intra-arterial (i. a.) injection 2 or 3 weeks after tumor inoculation. Moreover, remarkable regression of well-developed tumors, those 4 weeks after inoculation, was obtained by i. a. injection of the MCT solution containing 20 mg of TNP-470 without any influence on body weight. The antitumor effects were potentiated by coadministration of doxorubicin or mitomycin C (MMC) in the solution or microspheres containing MMC. In a shell-less chorioallantoic membrane (CAM) assay, angiogenesis was inhibited when a droplet of the MCT solution containing 25 μg of TNP-470 was placed on the CAM for 2 days, suggesting that the prolonged antitumor effect resulted from the inhibition of tumor neovascularization by sustained drug release from the preparation. These results indicate that i. a. injection of the MCT solution of TNP-470 is promising for treating well-developed tumors.

KEY WORDS: TNP-470; angiogenesis inhibitor; medium-chain triglyceride; intra-arterial injection; rabbit VX-2 carcinoma; CAM assay.

INTRODUCTION

TNP-470 (AGM-1470, 6-O-(N-chloroacetylcarbamoyl)-fumagillol, Fig. 1) synthesized by Takeda Chem. Ind., Ltd. (1) is a new type of anticancer drug that inhibits tumor neovascularization and blocks the supply of nutrients to tumors (2, 3). In cancer therapy, chemoembolization is a useful treatment strategy which combines chemotherapy and tumor devascularization by intra-arterial (i. a.) administration of an embolizing material. This type of treatment can achieve regional elevation of drug concentration and prolonged retention of anticancer drugs at the tumor site, and results have suggested enhanced antitumor activity with less severe systemic side effects. Furthermore, drugs dissolved in Lipiodol (LPD), a lymphographic oil, showed selective accumulation and retention at the tumor site after arterial administration due to the difference in time required for the oil to be removed from normal capillaries and the tumor vasculature (4, 5). In a previous study, we found that TNP-470 microspheres (msp) prepared using a biodegradable polymer, poly (lactic / glycolic) acid (PLGA), and a TNP-470 LPD solution which could achieve selective drug targeting and retention at the tumor site caused striking tumor regression in rabbits bearing VX-2 carcinoma after a single injection into the femoral artery running to the tumor (6, 7).

In the present study, the antitumor activity of i. a. administration of TNP-470 dissolved in medium-chain triglyceride (MCT, caprylic and capric acid triglyceride), which was found to be a preferred oil base in terms of solubility, stability and release sustainability in our formulation studies (8), was evaluated in the rabbit tumor model. The antitumor activities of TNP-470 coadministered with conventional chemotherapeutic agents, doxorubicin (ADM) and mitomycin C (MMC), and PLGA msp containing MMC were also investigated.

MATERIALS AND METHODS

TNP-470 was synthesized at Takeda Chemical Ind. (Osaka, Japan), and its structure is shown in Fig. 1. ADM, MMC and PLGA (molecular ratio of lactic and glycolic acid of 75:25, weight-average molecular weight of around 14,000) were obtained from Wako Pure Chemical Ind. (Osaka, Japan). MCT (MIGLYOL 812) was obtained from Huls A.G. (Marl, Germany), and sesame oil came from Takemoto Yushi (Aichi, Japan).

Preparation of Dosage Forms

TNP-470 was dissolved in sesame oil or MCT with mild shaking at room temperature, and the solution was sterilized by filtering (0.22 μm, Dimex filter, Millipore Ind. Tokyo, Japan). ADM or MMC was suspended in the oil solution after pulverization in an earthenware mortar. MMC was microencapsulated in PLGA by an in-water drying method. Briefly, MMC was dissolved in 50% PLGA-methylene chloride. The solution was poured into a 0.15% aqueous solution of polyvinyl alcohol under stirring with a turbine-shaped mixer. The oil/water emulsion was continuously stirred for 2 hr to evaporate the methylene chloride. The hardened msp were sized using sieves with apertures of 250 and 125 μm. Themsp (125–250 μm) were rinsed with distilled water 3 times and lyophilized. The resulting msp contained 0.14 mg of MMC / 1 mg of msp. These msp (1 mg as MMC) were suspended in MCT or TNP-470 MCT solution before administration.

Tumor Inoculation

Experiments were carried out by the method reported previously with some modification (7). Transplantable anaplastic VX-2 carcinoma that originated from spontaneously transformed Shope papilloma was used. A female rabbit bearing VX-2 carcinoma and male rabbits (Kbl, JW) weighing around 2.5 to 3.0 kg were purchased from Funabashi Farm (Chiba, Japan) and Kitayama LABES (Kyoto, Japan), 1

1 DDS Research Laboratories, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 2-17-85, Juso-honmachi, Yodogawa-ku, Osaka 532, Japan.
2 Research on Research, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 2-17-85, Juso-honmachi, Yodogawa-ku, Osaka 532, Japan.
3 To whom correspondence should be addressed.
respectively. VX-2 carcinoma was minced with scissors and sieved with a wire mesh (60 mesh), and tumor cells were suspended in Hank’s solution containing 10% rabbit serum, 120 μg/ml of penicillin and 100 μg/ml of streptomycin. Male rabbits were inoculated subcutaneously with VX-2 carcinoma cell suspension (10% w/v), 0.5 ml at a position on the inside of the right leg just below the knee. The VX-2 carcinoma cell line was maintained by successive inoculation of untreated rabbits.

Antitumor Effects

Two, 3 or 4 weeks after inoculation, preparations (sesame oil solution, 1 ml; MCT solution, 0.5 ml) were injected into the femoral artery through polyethylene tubing (PE-50, Clay Adams, NJ) under pentobarbital anesthesia. After administration, the blood flow in the femoral artery was re-opened by inserting the tubing upward into the artery after being shortened to a length of about 4 cm. Since total ischemia occurs for less than 3 min during the administration procedure, it should have no effect on tumor growth. Antitumor activities were evaluated using untreated control rabbits for comparison. Tumor volume was taken to be the product of the length, width and height as measured with calipers through the skin and was expressed as a ratio to the volume just before treatment (tumor volume ratio).

Shell-less Chorioallantoic Membrane (CAM) Assay

The assay was carried out by the method reported by Y. Nozaki et al. (9). Briefly, day-3 eggs were cracked, and embryos were placed on hammocks of polyethylene sheets hanging in plastic cups and then incubated at 37°C under an atmosphere of 3% CO₂ and saturated humidity for a further 6 days. On day 9, 5 μl of oil solutions containing TNP-470 were placed on the CAM. After incubation for 2 days, responses induced by the samples were examined under a stereoscope (SMZ-10, Nikon, Tokyo, Japan).

RESULTS AND DISCUSSION

In this study, a rabbit carcinoma implanted subdermally at a position on the hind leg was utilized to evaluate antitumor activity. The ultimate goal of our experiments using rabbits and rats is the treatment of hepatic cancer (10), and although this tumor model may not be realistic for hepatic cancer, it has the advantage, especially in formulation studies, of allowing tumor size to be measured easily and continuously through the skin.

Repeated i.v. and s.c. injection of aqueous solution of TNP-470 suppressed the tumor growth in the same rabbit tumor model but did not reduce the tumor volume sufficiently, and chemoembolization with PLGA msp containing TNP-470 resulted in strong suppression of the tumor growth for about 1 week, which corresponds to the period of TNP-470 sustained release (7). In further formulation studies, we found that 1) TNP-470 is soluble in various oils and especially in MCT with a solubility of approximately 100 mg/ml at 25°C, which is an important strategic factor in the present drug targeting therapy; 2) TNP-470 is labile in aqueous solution with a degradation half life of 4–5 hr (pH = 7.0 at 37°C) and insufficiently stable in sesame oil or LPD, whereas in MCT more than 90% of the initial TNP-470 content (10 mg/ml) is preserved even when the solution is stored at 40°C for 6 months, indicating pharmaceutical superiority of the TNP-470 MCT solution; 3) the MCT solution provided sustained release of TNP-470 for a period of more than 1 week according to first-order release kinetics in contrast to the disappearance of TNP-470 from the PLGA msp during 5 days as an in vitro release test (8); and 4) the MCT solution of TNP-470 achieved selective targeting to the tumor site in rats bearing Walker 256 carcinosarcoma in the liver after administration via the hepatic artery and the tumor-specific retention of TNP-470 for more than 2 weeks (10). MCT is used clinically as a component in a lipid emulsion formulation for parenteral nutrition, indicating satisfactory biocompatibility. In the present study, we examined the antitumor activity of this promising formulation of TNP-470 in the rabbit tumor model.

Figure 2 shows the antitumor effect on tumors 2 weeks