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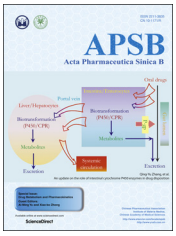

ORIGINAL ARTICLE

Tissue distribution and tumor uptake of folate receptor–targeted epothilone folate conjugate, BMS-753493, in CD2F1 mice after systemic administration

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Abstract To assess targeting of an epothilone folate conjugate (BMS-753493) to the folate receptor (FR)-overexpressed tumor in mice bearing both FR+ and FR– tumors, a series of experiments were conducted by quantitative whole-body autoradiography (QWBA) and LC–MS/MS following i.v. administration of BMS-753493 or its active moiety, BMS-748285 in mice bearing FR+ (98M109) and FR– (M109) tumors. QWBA showed [^{3}H]BMS-753493–derived radioactivity was extensively distributed to various tissues. The FR over-expressing 98M109 tumors showed consistently higher level of radioactivity than FR-negative tumors (*i.e.*, M109 tumors) up to 48 h post dose of [^{3}H]BMS-753493, despite the magnitude of difference between the tumors is relatively small (generally 3～5-fold). The radioactivity level in 98M109 tumors was 2～12-fold of normal tissues except intestine/content at 48 h post dose. No selective radioactivity uptake into 98M109 tumors over M109 or normal tissues was observed after i.v. administration of the active epothilone, [^{3}H]BMS-748285. LC–MS/MS measurements demonstrated that the concentrations of BMS-748285, presumably from hydrolysis of the folate conjugate, in 98M109 tumors were greater than those in M109 tumors after i.v. administration of BMS-753493 (2–3-fold) whereas no differential uptake in the tumors following BMS-748285 administration. Those data were consistent with radioactivity determinations. Those results demonstrated that the folate conjugation in BMS-753493 enabled moderately preferential distribution of the active epothilone to FR over-expressing 98M109 tumors, thereby supporting targeted delivery of cytotoxics through the folate receptor.

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1. Introduction

Folic acid (folate, vitamin B9) is an essential nutrient that is involved in many metabolic pathways, including amino acid interconversions and nucleotide synthesis in normal and proliferating cells[1]. Folate receptor (FR), a family of glycosylphosphatidylinositol-linked proteins, captures folate and other ligands from the extracellular milieu and transports them inside the cell *via* a non-destructive, recycling endosomal pathway. Two membrane folate receptors (FRα and FRβ) and two soluble receptors (FRγ and FRδ) have been identified. Both FRα and FRβ are functional for high-affinity folate binding, although they can display some differences in their binding affinity for reduced folate cofactors or antifolates[2,3]. Surprisingly, FR is expressed on few cell types. FRα is detected on the apical surfaces of several epithelial cells where it is inaccessible to parenterally administered folate conjugates. FRβ, in contrast, is expressed on activated macrophages. FRγ and FRδ have been difficult to detect in human tissues[3–6].

Elevated expression of FRα occurs in many human malignancies, as in the case of ovarian, endometrial, renal, lung and breast carcinomas, especially when associated with aggressively growing cancers[2]. Non-mucinous ovarian cancer, which represents the majority of ovarian cancers, was the first tumor type to be associated with FR "over-expression"[7,8]. Importantly, conjugation of molecules to folic acid does not normally interfere with the high affinity ($K_d < 1$ nmol/L) of folate for its receptor nor with its endocytosis into cells[9]. Folate conjugates undergo internalization *via* receptor-mediated endocytosis, delivering the folate-linked drugs with high affinity into the cell interior, and with a proper linker, the active drug can be released under the acidic condition in the endosome[10,11]. Taken together, FR-targeted strategies could have a significant impact on cancer treatment for patients diagnosed with FR-positive (FR+) disease.

BMS-753493 is a novel epothilone folate conjugate which is designed to target FR over-expressing tumors. As shown in Fig. 1, a molecule of BMS-753493 contains an active epothilone, BMS-748285, a folate moiety, and a peptide linker with a disulfide bond tethering the epothilone piece. The mechanism of action of the epothilone folate conjugate is presumably through tight binding of the folate moiety to FRα receptor on the target cell surface, followed by endocytosis of the receptor–ligand complex, and subsequent cleavage of the linker to release the active epothilone. BMS-753493 demonstrated antitumor activity in various experimental mouse xenograft models where folate receptor was over-expressed[12]. By targeting the folate receptor, BMS-753493 is expected to provide preferential distribution of BMS-748285 into the FR+ tumors in comparison with FR negative (FR–) tumors or normal tissues, thereby improving the therapeutic index of cancer treatment. However, tissue distribution and tumor concentration of BMS-753493 and BMS-748285 relative to plasma and other normal tissue concentrations are unknown.

The goal of this study was to investigate the distribution of radioactivity after a single i.v. injection of [^{3}H]BMS-753493 and [^{3}H]BMS-748285 at molar equivalent doses to CD2F1 mice bearing bilateral subcutaneous murine lung tumors 98M109 (FR+) and M109 (FR–) at left or right flank of animal using quantitative whole body autoradiography (QWBA). Further experiments were performed to measure BMS-748285 levels in plasma, tumors, and representative normal tissue samples using LC–MS/MS assays after administration of unlabeled BMS-753493 and BMS-748285.

2. Materials and methods

2.1. Chemicals

[^{3}H]BMS-753493 (20.5Ci/mmol) and [^{3}H]BMS-748285 (21.3Ci/mmol) were supplied by Radiochemistry Group of Department of Chemical Synthesis, Bristol-Myers Squibb (Princeton, NJ, USA). Radiochemical purity of the compounds was determined to be > 99% by radio-HPLC. The chemical structures of BMS-753493 and BMS-748285 are shown in Fig. 1. Unlabeled BMS-753493 and BMS-748285 were supplied by the Process Research and Development, Bristol-Myers Squibb (New Brunswick, NJ, USA). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless stated in the text.

2.2. Cell culture

Folate receptor-positive Madison 109 (98M109) lung carcinoma cells were cultured in folate-deficient RPMI 1640 medium with 10% fetus bovine serum at 37 °C in a 5% CO_2 humidified atmosphere. Folate receptor negative Madison 109 (M109 cells) were cultured in normal RPMI 1640 medium under similar conditions before inoculation.

2.3. Animal models and tumors

Six- to eight-week old CD2F1 female mice were used in these studies. Mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN, USA) and maintained in a specific pathogen free facility at Bristol-Myers Squibb Research and Development with food and water *ad libitum*. All animal experiments were done under a protocol approved by the Bristol-Myers Squibb Animal Care and Use Committee.

A tumor brei (2%, *w/v*) of M109 and 98M109 tumor cells were inoculated in the subcutaneous space of the mouse right and left flank of CD2F1 mice, respectively. High expression of FRα in 98M109 cells has been detected and confirmed (unpublished data). The xenografts were allowed to grow for two weeks after inoculation before being used in these distribution experiments. The approximate size of each tumor was around 100 mg at the time of dosing.

2.4. Tissue distribution study of radiolabeled BMS-753493 and BMS-748285

[^{3}H]BMS-753493 or [^{3}H]BMS-748285 was administered intravenously though tail vein injection at a dose level of 2.2 nmol/kg (4 and 1.2 mg/kg for BMS-753493 and BMS-748285, respectively; 10 mCi/kg) into M109 and 98M109 tumor-bearing mice for biodistribution experiments ($n=4$ in each treatment). The

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