Pharmacokinetics, tissue distribution, and in vivo antitumor effects of the antimelanoma immunotoxin ZME-gelonin

Abstract Antibody ZME-018 is directed against the gp240 glycoprotein on the surface of more than 80% of human melanoma cell lines and fresh biopsy specimens. Previous studies in our laboratory described the in vitro cytotoxicity and specificity of an immunoconjugate composed of mAb ZME-018 and the plant toxin gelonin. The present study describes the in vivo pharmacokinetics and therapeutic effects of ZME-gelonin in human xenograft/nude mouse models. Pharmacokinetic studies of $^{125}$I-labeled ZME-018 and ZME-gelonin demonstrated a shorter terminal-phase plasma half-life of the immunoconjugate than native ZME (20.6 h compared to 41.3 h). The initial volume of distribution of the ZME-gelonin was also higher compared to that of ZME alone (2.85 ml compared to 1.91 ml) suggesting an enhanced distribution of the conjugate outside the vasculature. The corresponding area under the concentration/time curve for the ZME-gelonin conjugate was 40% lower than that of ZME alone (80.8 compared to 139.6 μCi·ml$^{-1}$·min). In nude mice bearing well-developed human tumor A375 melanoma xenografts, administration of $^{125}$I-labeled ZME and ZME-gelonin resulted in tumor-to-blood ratios of 1.9 ± 0.5 and 1.5 ± 0.6 respectively by 72 h. Compared with ZME, ZME-gelonin conjugate caused an increase in the content of radiolabel in kidney, spleen and liver. Treatment of nude mice bearing well-developed (150 mm$^3$) s.c. A375-M xenografts with divided doses of ZME-gelonin, ZME, gelonin, or saline resulted in suppression of tumor growth in the immunotoxin group but virtually no retardation of tumor growth in the control groups. Using a murine model for a rapidly growing lethal metastatic human melanoma, treatment with ZME-gelonin resulted in a mean survival of 44 days, a 213% increase in mean survival time compared with the saline treatment (14.2 ± 2 day survival). Given these encouraging results, we are proceeding with further preclinical development of this immunotoxin.

Key words: Monoclonal antibodies • Immunotoxin • Immunotherapy • Pharmacokinetics

Introduction

Over the past few years, attempts have been made to utilize the tumor-localizing properties of monoclonal antibodies (mAb) for therapeutic purposes [4, 5, 10, 12]. Numerous clinical studies have focused on the administration of unlabeled antibodies or radiolabeled antibodies to attain a therapeutic effect. Although the administration of large amounts of murine antibody to patients has been shown to be safe, therapeutic studies with antibodies alone for the treatment of solid tumors have demonstrated limited clinical success [3, 7]. On the other hand, studies with radiolabeled antibodies have demonstrated some therapeutic promise [3]. In addition, clinical studies with antibody-toxin conjugates have also demonstrated some clinical therapeutic efficacy [1].

Immunotoxins are extremely cytotoxic proteins constructed by covalently linking mAb to bacterial toxins such as Pseudomonas exotoxin [14] or to plant toxins such as the A chain of ricin or gelonin [9, 15–18, 23, 26, 27]. The plant toxin gelonin is a single-chain protein (28 kDa) that inhibits protein synthesis by causing enzymatic damage to 28S ribosomal RNA [23]. Although gelonin is similar in mechanism of action to ricin A, studies in our laboratory have demonstrated only a 32% sequence homology of gelonin with ricin A [21]. Gelonin toxin alone is non-toxic to dividing cells since...
it lacks a cell-binding component [24]. A monoclonal antibody coupled to gelonin provides both a specific tumor-cell-binding component and a vehicle for efficient delivery of the toxin to the intracellular compartment.

The murine mAb ZME-018 is directed to the high-molecular-weight proteoglycan gp240, which is present on the surface of more than 80% of human melanoma cell lines and fresh biopsy specimens [30]. This antibody and a closely related antibody 9.2.27 bind to distinct epitopes on the same melanoma-associated cell-surface antigen. Both ZME-018 and 9.2.27 have been studied extensively and ZME-018 has been found to localize well within melanoma lesions after intravenous administration to cancer patients [13].

We have previously conducted extensive in vitro studies on a ZME-gelonin immunoconjugate [20]. In the present study, we have examined the in vivo tissue distribution and pharmacokinetics of ZME-gelonin immunoconjugate. We have also examined the therapeutic effects of ZME-gelonin conjugate against well-established human melanoma (A375-M) xenografts in nude mice and the survival of mice treated with ZME-gelonin in a highly metastatic AAB-527 melanoma model.

Materials and methods

Cell lines

The cultured human melanoma cell line A375-M was kindly provided by Dr. I. J. Fidler of MD Anderson Cancer Center (Houston, TX). The cells were grown in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (Hyclone Products, Logan, Utah) 20 mM glutamine, 50 μg/ml gentamicin, non-essential amino acids and 100 mM sodium pyruvate (Cellgro Products). The cells were passaged twice a week, maintained at 37 °C and tested for Mycoplasma routinely.

Monoclonal antibodies

The ZME-018 [anti-(240-kDa glycoprotein)] murine mAb was kindly provided by Dr. Richard Bartholomew, Hybritech Inc. (San Diego, CA). Control murine mAb 15A8 [anti-breast carcinoma] was supplied by Dr. Renato Dubœccio and Dr. Ross Allen of The Salk Institute (San Diego, CA).

Purification of gelonin toxin

Natural gelonin was purified from the seeds of Gelonium multiflorum. The purification and characterization of this plant toxin were previously described by our group [20] and by others [24] in detail.

Coupling of ZME-018 to natural gelonin

The coupling, purification and in vitro testing of mAb ZME-018 and the plant toxin gelonin have also been described in detail previously [21]. The immunoreactivity of the purified ZME-gelonin immunotoxin was examined by enzyme-linked immunosorbent assay (ELISA) against antigen-positive A375-M (melanoma) and antigen-negative T-24 (bladder carcinoma) cells. The in vitro efficacy of ZME-018 gelonin immunotoxin was also examined against antigen-positive A375-M (melanoma) and antigen-negative T-24 (bladder carcinoma) cells using a 72-h cytotoxicity assay also previously described [20].

Antibody and antibody-toxin labeling using P-iodobenzoate

One drawback in the use of 125I- or 131I-labeled protein in vivo is the potential for rapid and extensive dehalogenation. A novel procedure for radioiodination has been described and utilized for monoclonal antibodies which incorporates iodine into protein via a metabolically stable linkage [29]. This method conjugates N-succinimidyl p-iodobenzoate to the mAb. Briefly, 37.5 μl 1% AcOH/MeOH, 10 μl 1-mg/ml solution of N-chlorosuccinimide in MeOH and 10 μl phosphate-buffered saline (PBS) were sequentially added to a reaction vial fitted with a rubber septum containing 12.5 μg N-succinimidyl 4-tri-n-butylstannyl benzoate (Neorx Corp., Seattle, WA.) in 12.5 μl AcOH/MeOH. A 1-mCi sample of 125I (Dupont) was added to the reaction solution and, after 5 min, the reaction was quenched by addition of 10 μl 0.1 M NaHSO3. The MeOH solvent was evaporated under a N2 stream for 10 min. A 500-μg sample of mAb in 100 μl PBS was mixed with 100 μl 0.5 M borate buffer (pH 9.3) and then added to the reaction vial. The conjugation was allowed to proceed for 5 min at room temperature. Radiolabeled mAb was separated from unreacted radiiodine by chromatography on a Sephadex G-25 (PD-10) column (Pharmacia LKB Biotechnology, Piscataway, N.J.). The radiochemical yield was 40%-60%. Incorporation of radiolabel into mAb measured by trichloroacetic acid precipitation was greater than 90%. The specific activity of radiolabeled mAb was in the range 0.2-0.3 μCi/μg.

Immunoreactivity assay

The immunoreactivity of radiolabeled mAb and immunotoxin was evaluated using the Lindmo method [11]. Briefly, melanoma cells (2 x 106 A375-M) were incubated with various concentrations of 125I-labeled antibody or immunotoxin for 1 h at 4 °C. The cells were washed with PBS containing 1% bovine serum albumin, lysed with 2% Nonidet N-40 (Sigma, St. Louis, Mo.) and counted in a gamma counter (Packard model 5360). The immunoreactivity values ranged from 40% to 60% for both ZME-gelonin immunotoxin and ZME-018 mAb.

Animal model studies

Tissue distribution study

Athymic (nu/nu) mice, 4-6 weeks old, were obtained from Harlan Sprague Dawley (Indianapolis, Ind.). The animals were maintained under specific-pathogen-free conditions and were used at 6-8 weeks of age. Animals were injected s.c. (right flank) with 2 x 106 log-phase A375-M melanoma cells and tumors were allowed to establish for 3 weeks. Monoclonal antibodies and immunotoxins were labeled with 125I for 24 h prior to injection at a specific activity of 0.3 μCi/μg protein. After evaluation of the immunoreactivities of the antibody and immunotoxins (mAb ZME-018, ZME-gelonin immunotoxin...