Pharmacokinetics and organ distribution of N-methanocarbathymidine, a novel thymidine analog, in mice bearing tumors transduced with the herpes simplex thymidine kinase gene

Roy Noy · Zvi Ben-Zvi · Miri Elezra · Fabio Candotti
Harry Ford Jr · John C. Morris · Victor E. Marquez
David G. Johns · Riad Agbaria

Abstract Purpose: The conformationally rigid nucleoside, N-methanocarbathymidine [(N)-MCT] exerts a potent antiproliferative effect both in vitro and in vivo against murine colon cancer cells (MC38) expressing the herpes simplex virus thymidine kinase gene (MC38/HSV-tk). Metabolic studies have revealed that high levels of (N)-MCT triphosphate accumulate in transduced cells and are incorporated into DNA, resulting in cell death. The objective of the present study was to assess the pharmacokinetic profile of (N)-MCT in C57BL/6 mice bearing nontransduced MC38 and MC38/HSV-tk tumors. Methods: Male black C57BL/6 mice bearing subcutaneous tumors derived from wild-type and HSV-tk-transduced MC38 murine colon cancer cells in the left and right flank, respectively, were treated i.p. with radiolabeled (N)-MCT (100 mg/kg). Mice were killed at each of the predetermined times after drug administration. Blood, urine, tumors and various organs and tissues were obtained for measurement of drug levels. Results: Plasma and tissue concentrations of (N)-MCT peaked at 0.25–0.5 h. The major pharmacokinetic parameters calculated for (N)-MCT in plasma were: T1/2 β 4.7 h, AUC 147 µg·h/ml, CL 0.69 l/kg per h. The penetration of (N)-MCT into brain and testes was slow. Between 4 and 24 h after drug administration, the levels of (N)-MCT measured in HSV-tk-expressing tumors were significantly higher than in wildtype tumors. HPLC analysis of methanolic extracts of plasma and urine obtained at various times after drug administration revealed no (N)-MCT metabolites in the plasma, and the compound was secreted unchanged in the urine. Conclusions: After i.p. injection into mice, (N)-MCT was rapidly absorbed and distributed in all organs examined. No drug metabolites were detectable in plasma and the compound was secreted unchanged in urine. These results are essential for the future development and in postulating the most efficient use of (N)-MCT in the HSV-tk enzyme prodrug system for gene therapy approaches for the treatment of cancer.

Keywords N-Methanocarbathymidine · (N)-MCT · HSV-tk · Gene therapy · Cancer

Introduction

In the last decade, the use of enzyme-prodrug or “suicide” gene therapy for the treatment of cancer using the herpes simplex virus-1 thymidine kinase (HSV-tk)/ganciclovir (GCV) system has been extensively investigated both in vitro and in vivo [5, 12, 13, 33]. This approach confers selective chemosensitivity on the tumor cells as a result of the effective and specific intracellular conversion of a nontoxic prodrug (GCV) into a highly toxic metabolite in cells into which the HSV-tk gene has been transferred. Thus far, the anticancerous compounds that have demonstrated tumoricidal activity in HSV-tk-transduced tumor cells are the guanosine (Guo) analogs,
ganciclovir (9-[1,3-dihydroxy-2-propoxy]-methyl) guanine, GCV), acyclovir (9-[2-hydroxyethoxy] methyl)guanine, ACV) and more recently, penciclovir (9-[2-hydroxy-1-(hydroxymethyl)-ethoxymethyl]guanine, PCV) [2, 23]. Among these, GCV is the most widely used prodrug for this approach. In HSV-tk-transduced cells, GCV is rapidly phosphorylated to GCV monophosphate by the viral-tk enzyme. The resultant monophosphate is further phosphorylated to the di- and triphosphates by cellular kinases. GCV triphosphate (GCV-TP), the ultimate metabolite, inhibits cellular DNA polymerases and DNA synthesis leading to cell death [1, 2, 15, 16, 19, 31].

One of the major limitations in “suicide” gene therapy is the relatively limited number of prodrugs available. Recently, Marquez et al. [21] synthesized a new class of conformationally locked nucleoside analogs based on a rigid bicyclo[3.1.0]hexane template. Depending on the relative position of the base and hydroxymethyl group on this template, the resulting nucleosides have fixed conformations in either the North or South hemisphere of the pseudorotational cycle. Several of these compounds show potent antiviral activity against herpes viruses including herpes simplex 1 (HSV-1) and 2 (HSV-2) and human cytomegalovirus (HCMV). One of these compounds, N-methanocarbathymidine [(N)-MCT; (1R,2S,4S,5S)-1-(hydroxymethyl)-2-hydroxy-4-(5-methyl-2,4(1H,3H)-dioxopyrimidin-1-yl)bicyclo[3.1.0]hexane; Fig. 1], has shown particularly striking antiviral activity in plaque-reduction assays. Studies in our laboratory have shown that (N)-MCT effectively inhibits the proliferation of HSV-tk-transduced tumor cells in vitro and in vivo with no effects on tumor cells lacking the HSV-tk gene [24]. Metabolic studies have revealed that high levels of (N)-MCT triphosphate [(N)-MCT-TP] accumulate in the HSV-tk-expressing cells and this compound is incorporated into DNA, resulting in cell death [24]. While the antitumor effects of (N)-MCT on HSV-tk-transduced tumors in vitro and in vivo have been studied, there is little information on the pharmacokinetics and metabolic activation of (N)-MCT in mice bearing tumors. Such data are important for a better understanding of the efficacy and toxicity of (N)-MCT in these animal models. The principal objectives of this study were to assess the pharmacokinetics, and tumor and organ distribution of (N)-MCT and its metabolites in mice bearing MC38 and MC38/HSV-tk tumors.

Materials and methods

Chemicals

(N)-MCT was synthesized as described by Marquez et al. [21]. [Methyl-3H]-(N)-MCT (1.7 Ci/mmol) was obtained from Moravek Biochemicals (Brea, Calif.). Other nucleoside and nucleotide standards were purchased from Sigma Chemical Company (St. Louis, Mo.). Soluene-350 was purchased from Packard BioScience Company (Groningen, The Netherlands). All other chemicals and reagents were of the highest quality obtainable.

Tumor cell line

The 3-methylcholanthrene-induced murine colon adenocarcinoma (MC38) cell line was a gift from S.A. Rosenberg (NCI, NIH), and was derived from C57BL/6 mice as described by Restifo et al. [26]. All cell lines were grown in DMEM supplemented with 10% heat-inactivated fetal calf plasma, 50 IU/ml penicillin, 50 μg/ml streptomycin and 2 mM l-glutamine. Cells were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO2. Murine colon adenocarcinoma cells transduced with the HSV-tk gene (MC38/HSV-tk) were generated by transduction of the parental cell lines with supernatant from PA317-STK cells [22] and subsequent G418 selection (Geneticin; Life Technologies, Grand Island, N.Y.; 1 mg/ml for 3 weeks), followed by subcloning by limiting dilution.

Subcutaneous tumor implantation

All animal care and experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of the Ben-Gurion University of the Negev. Male black mice C57BL/6, at 6–8 weeks of age, were subcutaneously inoculated with 25·103 cells of wildtype MC38 and MC38/HSV-tk into the left and right flank, respectively. The tumors were allowed to grow for 10 days. The mice were then treated intraperitoneally (i.p.) with [3H]-(N)-MCT, 100 mg/kg, containing 400 μCi of labeled drug. Six mice were killed at each of the predetermined time-points (0.25, 0.5, 1, 2, 4, 12, and 24 h) following drug administration. Blood, urine, and the following organs and tissues including brain, heart, lungs, spleen, liver, stomach, small intestine, large intestine, kidney, testes, striated muscle, MC38 tumor and MC38/HSV-tk tumor were obtained and stored at −70°C until drug analyses were performed.

Quantification of (N)-MCT in tissues

To determine total (N)-MCT levels in murine tissues, 100–250 mg of each tissue was dissected and 1 ml Soluene 350 was added. Samples were shaken at 37°C until complete solubilization of tissue was achieved. The radioactivity was determined by liquid scintillation counting (TRI-CARB 2100TR, Packard). The level of (N)-MCT in each tissue was then calculated by using the specific activity of the [3H]-(N)-MCT administered.

Reverse-phase HPLC analysis of (N)-MCT in plasma and urine

To assess (N)-MCT metabolites in plasma and urine samples, 100 μl plasma or urine was mixed with 150 μl 100% methanol and