Cancer Chemother Pharmacol (2002) 50: 360–366
DOI 10.1007/s00280-002-0505-8

ORIGINAL ARTICLE

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

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

Received: 23 May 2002 / Accepted: 8 July 2002 / Published online: 20 September 2002

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: $T_{1/2}\beta$ 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

R. Noy · Z. Ben-Zvi · M. Elezra · R. Agbaria (✉)
Department of Clinical Pharmacology,
Faculty of Health Sciences,
Ben-Gurion University of the Negev,
Beer-Sheva 84105, Israel
E-mail: riad@bgumail.bgu.ac.il
Tel.: +972-8-6477372
Fax: +972-8-6477625

F. Candotti
National Human Genome Research Institute,
Genetics and Molecular Biology Branch,
NIH, Bethesda, MD, USA

H. Ford Jr · V.E. Marquez · D.G. Johns
Laboratory of Medicinal Chemistry,
Center for Cancer Research,
National Cancer Institute,
Frederick, MD, USA

J.C. Morris
Metabolism Branch,
Center for Cancer Research, NCI,
National Institutes of Health,
Bethesda, MD 20892, USA

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 antiherpetic 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; (1*R*,2*S*,4*S*,5*S*)-1-(hydroxymethyl)-2-hydroxy-4-(5-methyl-2,4(1*H*,3*H*)-dioxopyrimidin-1-yl) bicyclo[3.1.0]hexane; Fig. 1], has shown particularly striking antiherpetic 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 tumor cells 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.

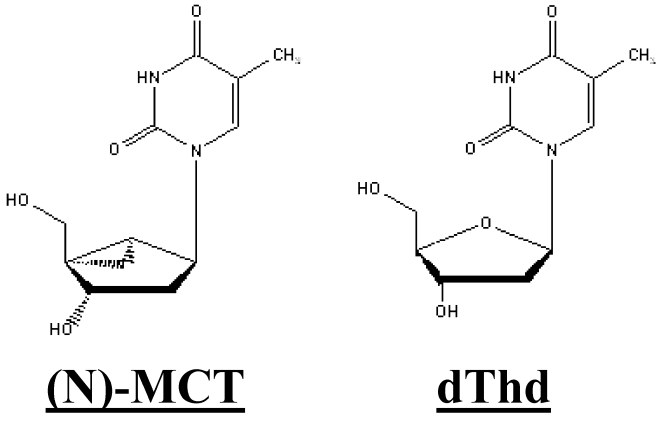

Fig. 1. Chemical structures of *N*-methanocarbathymidine [(N)-MCT] and thymidine (dThd)

Materials and methods

Chemicals

(N)-MCT was synthesized as described by Marquez et al. [21]. [Methyl-^{3}H]-(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 m*M* L-glutamine. Cells were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. 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 25×10^5 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 [^{3}H]-(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 [^{3}H]-(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

heated for 3 min at 95°C. The mixture was then cleared by centrifugation for 10 min at 12,000 *g*. The supernatant was collected, evaporated under nitrogen and the residue was reconstituted in 150 µl deionized water. The (N)-MCT metabolites were analyzed by reverse-phase HPLC using a Beckman Ultrasphere ODS column (250×4.6 mm, 5-µm particle size) eluted isocratically with 5% methanol in distilled water at a flow rate of 2 ml/min. Fractions were collected at 1-min intervals and their radioactivity was determined. The retention time of (N)-MCT was 23 min. Under these HPLC conditions, the limit of detection for (N)-MCT was 10 ng/ml. (N)-MCT was separated with high resolution from endogenous ribonucleosides and deoxribonucleosides as demonstrated with authentic standards.

Pharmacokinetic analysis

The data for (N)-MCT concentration in plasma were analyzed using a two-compartment open model, and drug half-life ($T_{1/2}$), clearance (CL), volume of distribution (Vd), area under the concentration-time curve (AUC) and mean residence time (MRT) were calculated using ModKine software (Biosoft, Cambridge, UK).

Statistical analysis

Student's *t*-test was used for statistical analysis and differences were considered significant for P values ≤ 0.05.

Results

Pharmacokinetics of (N)-MCT

Mice with bilaterally implanted subcutaneous flank tumors were used to evaluate the plasma pharmacokinetics of our novel thymidine analog. The time-concentration curves in plasma of (N)-MCT, administered as a single (100 mg/kg) i.p. injection showed a biexponential decline (Fig. 2). (N)-MCT reached a peak concentration in plasma (Cmax) of approximately 120 µg/ml 30 min after drug administration. The AUC for (N)-MCT was 147 µg·h/ml, while the $T_{1/2}\beta$ and plasma clearance (CL) were 4.7 h and 0.690 l/h per kg, respectively. Other relevant pharmacokinetic parameters of (N)-MCT are listed in Table 1.

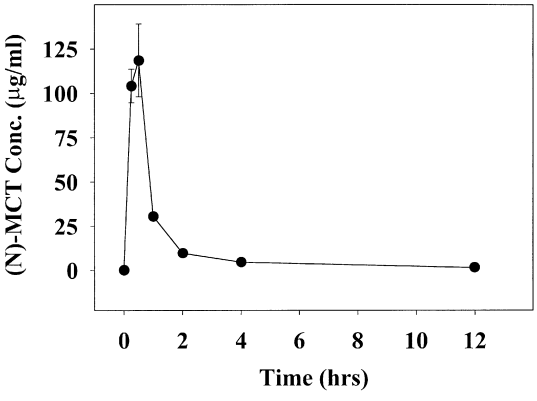

Fig. 2. Plasma concentration-time curves of (N)-MCT in mice bearing MC38 and MC38/HSV-tk tumors. Mice bearing HSV-tk-transduced and nontransduced tumors were injected i.p. with [^{3}H](N)-MCT, 100 mg/kg, 400 µCi. At the time-points indicated, animals were killed, blood was collected and plasma radioactivity was determined as described in Materials and methods. Values are means ± SEM ($n = 6$)

Tissue distribution of (N)-MCT

Tissue levels of (N)-MCT were measured at different times after a single i.p. dose of (N)-MCT (100 mg/kg) administered to mice bearing nontransduced and HSV-tk-expressing tumors. The data are displayed in Fig. 3. The levels of (N)-MCT were determined by measuring the total radioactivity in tissue extracts. In all organs studied, the concentrations of (N)-MCT reached peak levels (Cmax) between 15 and 30 min (Tmax) after administration of the drug, and then rapidly declined within the first 2 h (Table 2, Fig. 3), followed by a slower decline over 24 h. The highest concentrations of (N)-MCT were found in kidney, spleen and liver, while the lowest concentrations were found in testes and brain. The highest AUC levels for (N)-MCT in the various tissues were found (in descending order) in the kidney, large intestine, and small intestine (Table 2).

Distribution of (N)-MCT in tumors

The levels of total (N)-MCT radioactivity were determined in nontransduced and HSV-tk-transduced tumors from mice at different times after drug administration. As shown in Fig. 4, the levels of (N)-MCT in HSV-tk-transduced tumors were significantly higher than those in nontransduced tumors between 4 and 24 h after drug administration. The Cmax values for total (N)-MCT radioactivity in nontransduced and HSV-tk-transduced tumors were similar (approximately 55 µg/g tumor), and were achieved within 15 min after drug injection. In contrast, the AUC values for (N)-MCT in the HSV-tk tumors were significantly higher (nearly twofold) than in the nontransduced tumors (Table 3).

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

To determine whether (N)-MCT had significant metabolism in the vascular compartment, plasma and urine samples were collected from mice at different times after drug injection. Methanolic extracts of samples were prepared and analyzed by reverse-phase HPLC as

Table 1. Plasma pharmacokinetic parameters for (N)-MCT in tumor-bearing mice. Values are means ± SEM ($n = 6$)

$T_{1/2}\alpha$ (h)	0.20 ± 0.01
$T_{1/2}\beta$ (h)	4.71 ± 0.62
CL (l/h/kg)	0.69 ± 0.17
Vd (l/kg)	0.32 ± 0.1
AUC_{0-8} (µg·h/ml)	147 ± 12
MRT (h)	7.4 ± 1.1

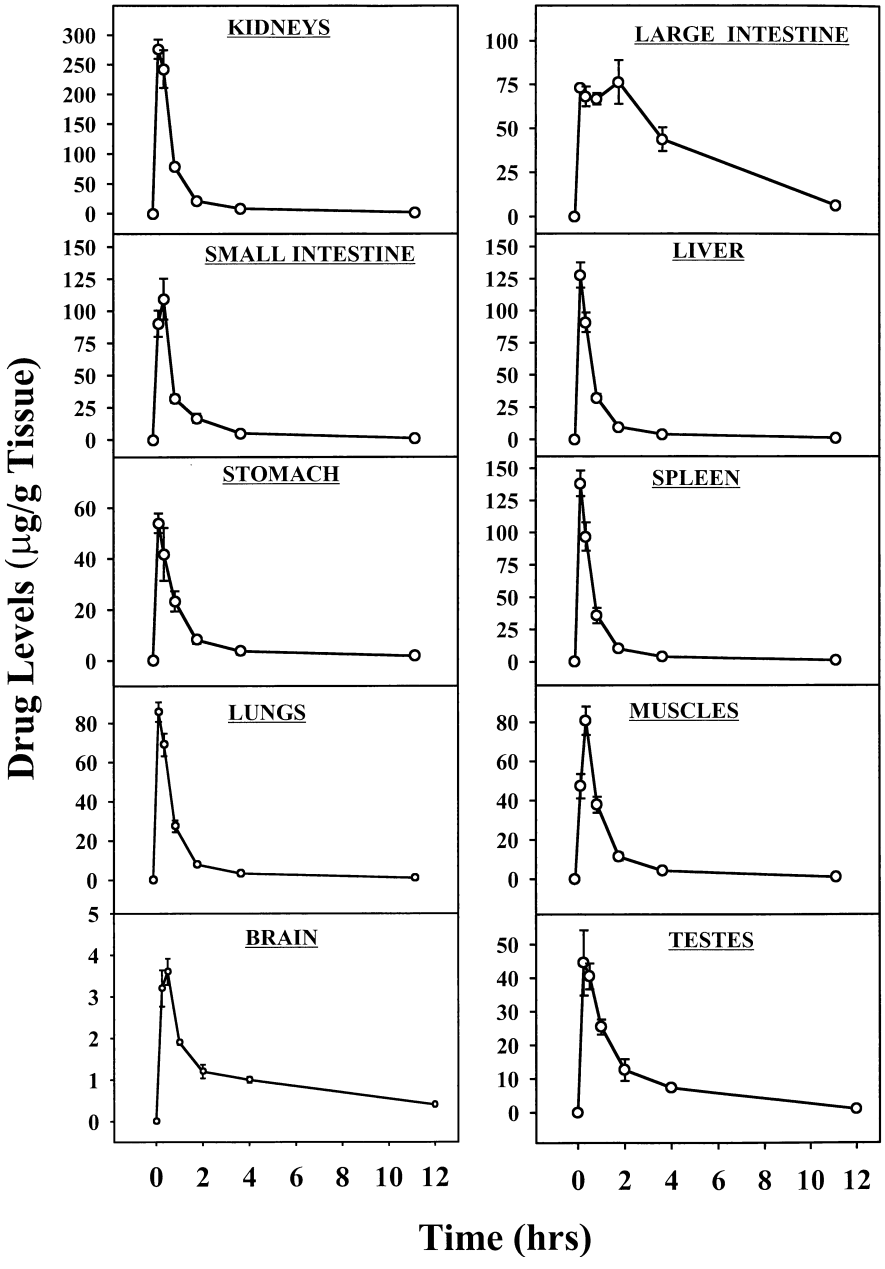

Fig. 3. Distribution of (N)-MCT in tissues of mice bearing MC38 and MC38/HSV-tk tumors after a single i.p. injection of 100 mg/kg of (N)-MCT and determined as described under Materials and methods. Values are means ± SEM ($n = 6$)

Table 2. Cmax, Tmax and AUC of (N)-MCT in murine tissues. Values are means ± SEM ($n = 6$)

Tissue	Cmax (μg/g tissue)	Tmax (h)	AUC (μg·h/g)
Kidneys	276 ± 15	0.25	327 ± 14
Spleen	138 ± 9	0.25	151 ± 3
Liver	128 ± 9	0.25	142 ± 5
Small intestine	110 ± 15	0.50	157 ± 12
Heart	86 ± 9	0.50	124 ± 6
Lung	86 ± 5	0.25	114 ± 2
Muscle	81 ± 7	0.50	128 ± 3
Large intestine	73 ± 1	0.25	467 ± 42
Stomach	54 ± 4	0.25	94 ± 10
Testes	45 ± 9	0.25	119 ± 3
Brain	4 ± 0.3	0.50	17 ± 1

described above. The profiles of ^{3}H-radioactivity from HPLC analysis of plasma or urine collected from (N)-MCT-treated mice indicated that all the radioactivity was accounted for by the parent drug (N)-MCT (Fig. 5) indicating that little or no metabolism occurred in the circulation.

Discussion

The present study was undertaken with the following objectives: (1) to determine the pharmacokinetic parameters following i.p. administration of (N)-MCT and (2) to measure tumor and organ distribution of (N)-MCT in an animal model.

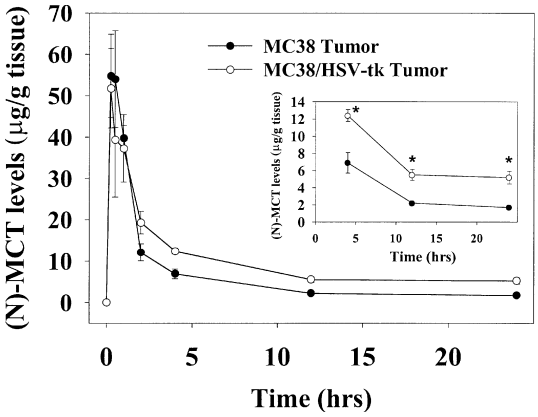

Fig. 4. Distribution of (N)-MCT in MC38 and MC38/HSV-tk tumors in mice. MC38 and MC38/HSV-tk tumors were implanted in mice as described in Materials and methods. Drug levels were determined after a single i.p. injection of (N)-MCT, 100 mg/kg, 400 μCi. Values are means ± SEM ($n = 6$). *$P < 0.05$ versus nontransduced MC38 tumors

Recently, we have demonstrated that equivalent doses of (N)-MCT and GCV in mice can significantly inhibit tumor growth of subcutaneously implanted MC38 murine colon cancer cells transduced with the HSV-tk gene [24]. (N)-MCT and GCV have no effect, however, on tumor growth of wildtype tumors. Furthermore, we have found that (N)-MCT undergoes significant phosphorylation only in HSV-tk-expressing tumors, and that its triphosphate metabolite is generated in high yield [24]. This would suggest that the inhibition of tumor growth in vivo of HSV-tk-expressing MC38 tumors, like the inhibition of cell proliferation in vitro, is due to the conversion to (N)-MCT-TP, the active metabolite of (N)-MCT, which competes with dTTP for incorporation into DNA, resulting in inhibition of DNA synthesis and cell death [24], an overall mechanism which is similar to that of GCV-TP, the active metabolite of GCV [1, 2, 17].

Table 3. Cmax and Tmax of (N)-MCT in murine HSV-tk-transduced and nontransduced tumors. Values are means ± SEM ($n = 6$)

Tumor	Cmax (μg/g tissue)	Tmax (h)	AUC (μg·h/g)
MC38	55 ± 10	0.25	134 ± 15
MC38/HSV-tk	52 ± 10	0.25	219 ± 39

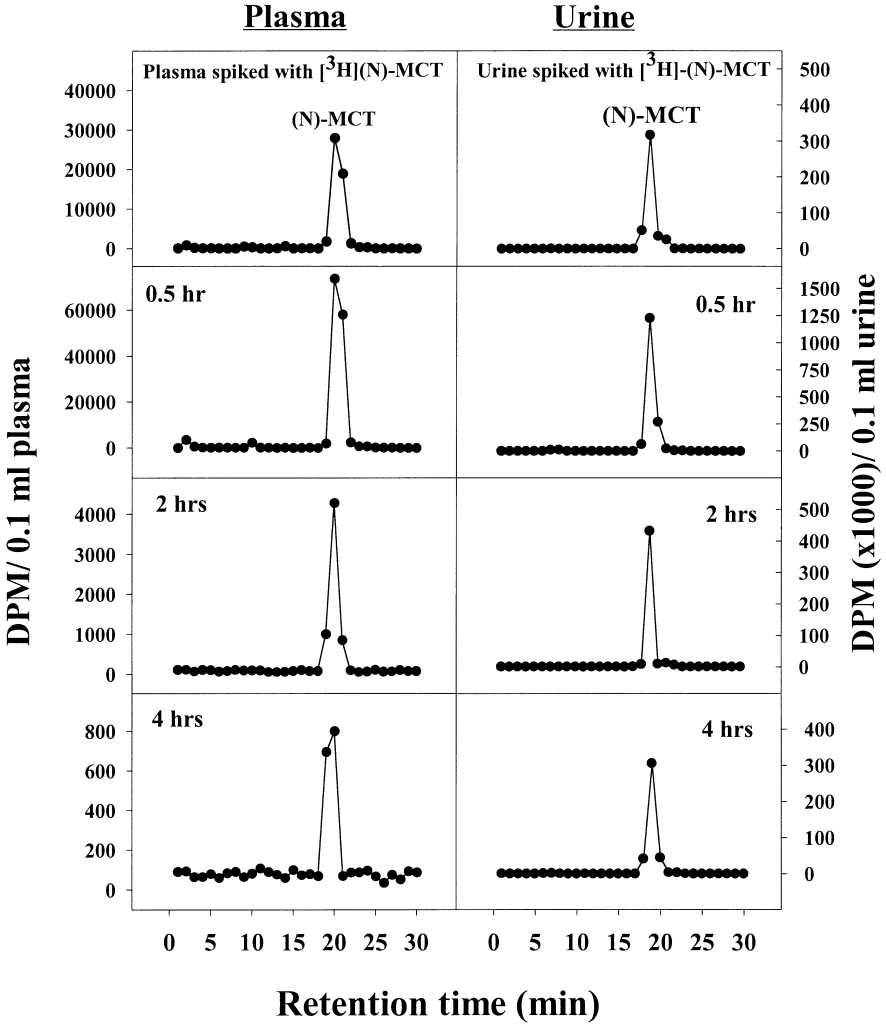

Fig. 5. Reverse-phase HPLC chromatogram of (N)-MCT in mouse plasma and urine. Mice were injected i.p. with (N)-MCT, 100 mg/kg, 400 μCi. At the indicated times animals were killed, blood and urine were collected and methanolic extracts were prepared and analyzed by reverse-phase HPLC, as described in Materials and methods

The plasma pharmacokinetic profile of (N)-MCT administered i.p. fits a two-compartment open model with a biphasic decline of its concentration in plasma as the parent drug. This is similar to the pharmacokinetics reported for GCV in humans and primates [11, 28, 29, 30]. Reverse-phase HPLC analysis of extracts of plasma and urine obtained from mice treated with [^{3}H]-MCT revealed that >99% of the tritium in plasma and urine was from unchanged (N)-MCT. Studies have shown that the presence of a tumor in the host may induce structural and functional changes that affect drug distribution and metabolism, such as changes of metabolic capacity of the liver (microsomal and cytosolic enzymes) and other factors [27, 34]. Other studies have indicated that the presence of toxic factors in the blood of tumor-bearing animals is possibly responsible for modifications in the host metabolic functions [8]. However, our experiments demonstrated that (N)-MCT does not undergo any biotransformation and is secreted unchanged in the urine of mice bearing tumors.

Detectable levels of (N)-MCT in the HSV-tk-expressing tumors were sustained for 24 h after drug administration. The mean concentrations of (N)-MCT measured between 12 and 24 h after dosing in HSV-tk tumors were significantly higher than the corresponding levels in nontransduced tumors. This difference may be attributed to more efficient cellular trapping of the phosphate metabolites of (N)-MCT, which constituted the majority of the radioactivity, and to the high level of incorporation of the phosphorylated drug into cellular DNA of HSV-tk-transduced cells, as we have recently reported [24]. Furthermore, we have shown that (N)-MCT undergoes phosphorylation only in HSV-tk tumors in mice bearing tumors, and the triphosphate metabolite is generated in high yield [24]. These results are consistent with the observation of Haberkorn et al. [14] that the rate of uptake of GCV by HSV-tk tumor cells in vitro is greater than by nontransfected tumor cells. Also, greater uptake and higher concentrations of acyclovir have been observed in tissues of mice infected with herpes virus, compared with those of uninfected mice [4].

The study of the distribution of (N)-MCT in normal tissues indicates that the drug was highly excluded from the brain and testes in which the lowest levels of (N)-MCT were found, whereas liver and kidneys showed the highest levels. The low levels of (N)-MCT found in normal brain tissue would be expected, in part, due to the effect of the blood-brain barrier. Similarly, low levels of GCV have been detected in the brain of human subjects following intravenous injection [20]. Despite these low levels, regression of HSV-tk-transduced brain tumors in response to GCV has been seen in clinical trials [6, 7, 18, 25, 32], indicating that sufficient GCV penetrates the blood-brain barrier to achieve adequate therapeutic levels. Other studies have found evidence that GCV uptake by tumor and surrounding tissue in the brain is significantly higher than in normal brain tissue, possibly reflecting the "leaky" properties of the blood-brain tumor barrier [18]. As is with GCV, modifiers of vascular barriers of the CNS such as RMP-7 [3, 9, 10, 18, 35, 36] may be useful in practice for increasing (N)-MCT permeability into brain tumors.

In summary, these findings demonstrate that (N)-MCT, a new HSV-tk-activated prodrug, is rapidly absorbed and distributed in murine tissues after a single i.p. administration. It is efficiently phosphorylated only in tumor cells transduced with the HSV-tk gene. Taken together, these results will be of value in determining the most efficient use of (N)-MCT in "suicide" gene therapy approaches for the treatment of cancer.

References

1. Balzarini J, Bohman C, De-Clercq E (1993) Differential mechanism of cytostatic effect of (E)-5-(2-bromovinyl)-2′-deoxyuridine, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, and other antiherpetic drugs on tumor cells transfected by the thymidine kinase gene of herpes simplex virus type 1 or type 2. J Biol Chem 268:6332
2. Balzarini J, Bohman C, Walker RT, De-Clercq E (1994) Comparative cytostatic activity of different antiherpetic drugs against herpes simplex virus thymidine kinase gene-transfected tumor cells. Mol Pharmacol 45:1253
3. Bartus RT, Elliott PJ, Dean RL, Hayward NJ, Nagle TL, Huff MR, Snodgrass PA, Blunt DG (1996) Controlled modulation of BBB permeability using the bradykinin agonist, RMP-7. Exp Neurol 142:14
4. Biron KK, Noblin JE, de Miranda P, Elion GB (1982) Uptake, distribution, and anabolism of acyclovir in herpes simplex virus-infected mice. Antimicrob Agents Chemother 21:44
5. Blaese RM, Mullen CA, Ramsey WJ (1993) Strategies for gene therapy. Pathol Biol 41:672
6. Chen SH, Shine HD, Goodman JC, Grossman RG, Woo SLC (1994) Gene therapy for brain tumors: regression of experimental gliomas by adenovirus-mediated gene transfer in vivo. Proc Natl Acad Sci U S A 91:3054
7. Culver KW, Ram Z, Wallbridge S, Ishii H, Oldfield EF, Blaese RM (1992) In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science 256:1550
8. Donelli MG, D'Incalci M, Garattini S (1984) Pharmacokinetic studies of anticancer drugs in tumor-bearing animals. Cancer Treat Rep 68:381
9. Elliott PJ, Hayward NJ, Huff MR, Nagle TL, Black KL, Bartus RT (1996) Unlocking the blood-brain barrier: a role for RMP-7 in brain tumor therapy. Exp Neurol 141:214
10. Emerich DF, Dean RL, Snodgrass P, Lafreniere D, Agostino M, Wiens T, Xiong H, Hasler B, Marsh J, Pink M, Kim BS, Perdomo B, Bartus RT (2001) Bradykinin modulation of tumor vasculature: II. Activation of nitric oxide and phospholipase A2/prostaglandin signaling pathways synergistically modifies vascular physiology and morphology to enhance delivery of chemotherapeutic agents to tumors. J Pharmacol Exp Ther 296:632
11. Fletcher C, Sawchuk R, Chinnock B, De Miranda P, Balfour HH Jr (1986) Human pharmacokinetics of the antiviral drug DHPG. Clin Pharmacol Ther 40:281
12. Freeman SM, Whartenby KA, Freeman JL, Abboud CN, Marrogi AJ (1996) In situ use of suicide genes for cancer therapy. Semin Oncol 23:31
13. Gomez-Navarro J, Curiel DT, Douglas JT (1999) Gene therapy for cancer. Eur J Cancer 35:2039
14. Haberkorn U, Khazaie K, Morr I, Altmann A, Müller M, Van-Kaick G (1998) Ganciclovir uptake in human mammary carcinoma cells expressing herpes simplex virus thymidine kinase. Nucl Med Biol 25:367

15. Hamzehb FM, Lietman PS (1991) Intracellular accumulation of subgenomic noninfectious human cytomegalovirus DNA in infected cells in the presence of ganciclovir. Antimicrob Agents Chemother 35:1818
16. Haynes P, Lambert TR, Mitchell ID (1996) Comparative in-vivo genotoxicity of antiviral nucleoside analogues; penciclovir, acyclovir, ganciclovir and xanthine analogue, caffeine, in mouse bone marrow micronucleus assay. Mutat Res 369:65
17. Ilsley DD, Lee SK, Miller WH, Kuchta RD (1995) Acyclic guanosine analogs inhibit DNA polymerase α, β, and ϵ with very different potencies and have unique mechanism of action. Biochemistry 34:2504
18. LeMay DR, Kittaka M, Gordon EM, Gray B, Stins MF, Mccomb JG, Jovanovic S, Tabrizi P, Weiss MH, Bartus RT, Anderson WF, Zlokovic BV (1998) Intravenous RMP-7 increases delivery of ganciclovir into rat brain tumors and enhances the effects of herpes simplex virus thymidine kinase gene therapy. Hum Gene Ther 9:989
19. Mar EC, Chiou JF, Cheng YC, Huang ES (1985) Inhibition of cellular DNA polymerase alpha and human cytomegalovirus-induced DNA polymerase by the triphosphates of 9-(2-hydroxyethoxymethyl)guanine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine. J Virol 53:776
20. Markham A, Faulds D (1994) Ganciclovir. An update of its therapeutic use in cytomegalovirus infection. Drugs 48:455
21. Marquez VE, Siddiqui MA, Ezzitouni A, Russ P, Wang J, Wagner W, Matteucci MD (1996) Nucleosides with a twist. Can fixed forms of sugar ring pucker influence biological activity in nucleosides and oligonucleotides? J Med Chem 39:3739
22. Moolten FL, Wells JM (1990) Curability of tumors bearing herpes thymidine kinase genes transferred by retroviral vectors. J Natl Cancer Inst 82:297
23. Morris JC, Agbaria R, Higginbotham J, Wildner O, Ramsey WJ, Straus SE, Blaese RM (1997) The antiviral nucleoside penciclovir exhibits comparable cytotoxicity to ganciclovir in cell lines expressing the HSV-tk gene. In: Molecular and Cellular Biology of Gene Therapy. Keystone Symposia, Silverthorne, p 67
24. Noy R, Ben-Zvi Z, Manor E, Candotti F, Morris JC, Ford H Jr, Marquez VE, Johns DG, Agbaria R (2002) Antitumor activity and metabolic activation of (N)-methanocarbathymidine, a novel thymidine analog with a pseudosugar rigidly fixed in the northern conformation, in murine colon cancer cells expressing herpes simplex thymidine kinase. Mol Cancer Ther 1:585
25. Ram Z, Culver KW, Oshero EM, Viola JJ, Devroom HD, Otto E, Long Z, Chaing Y, Mcgarrity G, Muul LM, Katz D, Blaese RM, Oldfield EH (1997) Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. Nat Med 3:1354
26. Restifo NP, Esquivel F, Asher AL, Stotter H, Barth RJ, Bennink JR, Mule JJ, Yewdell JW, Rosenberg SA (1991) Defective presentation of endogenous antigens by a murine sarcoma. Implications for the failure of an anti-tumor immune response. J Immunol 147:1453
27. Rosso R, Donelli MG, Franchi G, Garattini S (1971) Impairment of drug metabolism in tumor-bearing animals. Eur J Cancer 7:565
28. Serabe BM, Murry DJ, Dauser R, Nuchtern J, Durfee J, McGuffey L, Berg S, Blaney SM (1999) Plasma and CSF pharmacokinetics of ganciclovir in nonhuman primates. Cancer Chemother Pharmacol 43:415
29. Sommadossi JP, Carlisle R (1987) Toxicity of 3′-azido-3′-deoxythymidine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine for normal human hematopoietic progenitor cells in vitro. Antimicrob Agents Chemother 31:452
30. Sommadossi JP, Bevan R, Ling T, Lee F, Mastre B, Chaplin MD, Nerenberg C, Koretz S, Buhles WC Jr (1988) Clinical pharmacokinetics of ganciclovir in patients with normal and impaired renal function. Rev Infect Dis 10:S507
31. Thurst R, Schacker M, Wutzler P (1996) Cytogenetic genotoxicity of the antiherpes virostatics in Chinese hamster V79-E cells. I. Purine nucleoside analogues. Antiviral Res 31:105
32. Vincent AJ, Vogel SAR, Someren GV, Esandi MC, Noteboom JL, Avezaat CJ, Vecht C, Bekkum DW, Valerio D, Bout A, Hoogerbrugge PM (1996) Herpes simplex virus thymidine kinase gene therapy for rat malignant brain tumors. Hum Gene Ther 7:197
33. Wildner O (1999) In situ use of suicide genes for therapy of brain tumours. Ann Med 31:421
34. Wilson JT (1968) An investigation of the decrease in the metabolism of hexobarbital, aminopyrine and p-nitrobenzoic acid by liver from rats bearing a pituitary mammotropic tumor. J Pharmacol Exp Ther 160:179
35. Zlokovic BV, Apuzzo ML (1997) Cellular and molecular neurosurgery: pathways from concept to reality – part I: target disorders and concept approaches to gene therapy of the central nervous system. Neurosurgery 40:789
36. Zlokovic BV, Apuzzo ML (1997) Cellular and molecular neurosurgery: pathways from concept to reality – part II: vector systems and delivery methodologies for gene therapy of the central nervous system. Neurosurgery 40:805