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Sensitive high-performance liquid chromatographic assay for motexafin gadolinium and motexafin lutetium in human plasma☆

Robert A. Parise[a], Dale R. Miles[b], Merrill J. Egorin[a,c,d,*]

[a]*Program of Molecular Therapeutics and Drug Discovery, University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213, USA*
[b]*Pharmacyclics, Sunnyvale, California, CA 94086, USA*
[c]*Division of Hematology/Oncology, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA*
[d]*Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA*

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Abstract

We present new HPLC methods for the quantitation in human plasma of two investigative metallotexaphyrin agents, motexafin gadolinium (Gd-Tex) and motexafin lutetium (Lu-Tex). Each assay uses: the other texaphyrin analogue as an internal standard; protein precipitation with acetonitrile:methanol (50:50, v/v); an ODS reversed-phase column; an isocratic mobile phase of 100 m*M* ammonium acetate, pH 4.3:acetonitrile:methanol (59:21:20, v/v/v); and absorbance detection at 470 nm. The Gd-Tex assay has a lower limit of quantitation (LLOQ) of 0.01 μ*M* and is linear between 0.01and 30 μ*M*. The Lu-Tex assay has an LLOQ of 0.1 μ*M* and is linear between 0.1 and 30 μ*M*. The assays are suited for in vivo preclinical studies and clinical trials because they require minimal amounts of plasma, are sensitive, and involve a 30-min run time. These assays are important tools for evaluating the potential of Gd-Tex and Lu-Tex as a radiation enhancer and photosensitizer, respectively. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Motexafin gadolinium; Motexafin lutetium; Texaphyrin; Lanthanide

1. Introduction

Texaphyrins [1–9] are aromatic pentadentate ligands that belong to the general class of compounds referred to as expanded porphyrins [6]. The central core of a texaphyrin can form highly stable complexes with lanthanide series cations such as Gd (III) and Lu (III) [10]. Two metallotexaphyrins, motexafin gadolinium (Gd-Tex) and motexafin lutetium (Lu-Tex) (Fig. 1), are the objects of active clinical development programs [11–30]. A phase III, randomized trial in patients with brain metastases is currently evaluating Gd-Tex (Fig. 1) as an MRI-detectable radiation enhancer [10–18]. Lu-Tex (Fig. 1), which localizes in both tumor tissue and atherosclerotic plaque, is being evaluated in the clinic as a photosensitizer for the photodynamic treatment of tumors, atherosclerosis, and age-related macular degeneration [19–30]. Some preclinical and clinical studies of both Gd-Tex and Lu-Tex have included pharmacokinetic components. However, the analyti-

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*Corresponding author. Tel.: +1-412-624-9272; fax: 1-412-648-9856.

E-mail address: egorinmj@msx.upmc.edu (M.J. Egorin).

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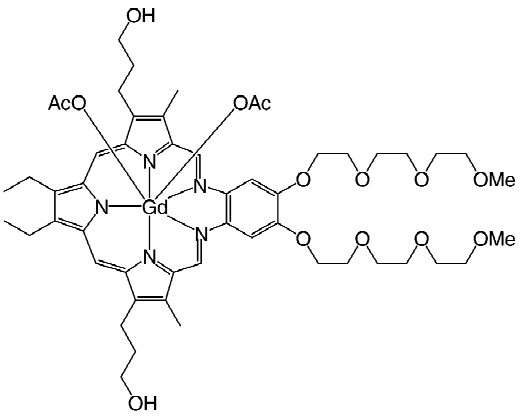
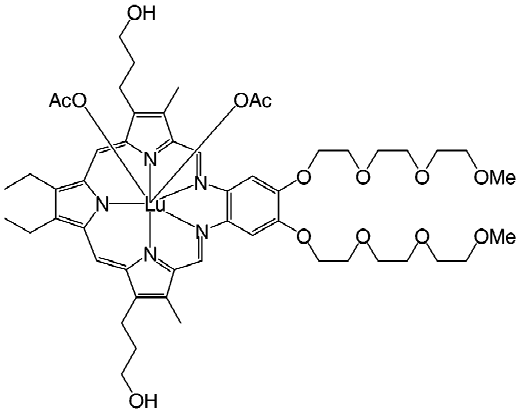


Motexafin gadolinium



Motexafin lutetium

Fig. 1. The structures of motexafin gadolinium (Gd-Tex) (A) and motexafin lutetium (Lu-Tex) (B).

cal methods for quantifying these materials in various biological matrices used radiolabelled compound [^{14}C and/or ^{153}Gd] [10,22], total fluorescence [20,24], or inductively coupled plasma spectrometry (ICP) [11,15]. Other studies attempted to assess tissue content of Gd-Tex by evaluating enhancement of magnetic resonance images [10,16,17,22]. Although these methods provided important pharmacokinetic information, they were not specific for Lu-Tex or Gd-Tex.

An HPLC assay specific for Gd-Tex or Lu-Tex would provide important information needed for a complete understanding of the pharmacokinetics of these two drugs, and would complement ICP and fluorescence spectrophotometry data. A comparison of the amount of total metal (measured by ICP) with intact parent compound (measured by HPLC) in plasma and/or tissues may indicate the extent of potential degradation/metabolism of Gd-Tex or Lu-Tex with time. The amount of fluorophore (measured by fluorescence spectrophotometry) in plasma is indicative of the total amount of photoactive drug, and would correlate with intact parent compound as long as any degradants/metabolites that may be formed do not possess a metallotexaphyrin structure. By using assays that measure different features of a drug, potential misinterpretations of pharmacokinetic data can be avoided as illustrated in early studies of cisplatin [31–36]. In those studies, the plasma and tissue half-lives of platinum were much longer than that of active parent compound. Subsequent studies that measured both total plasma platinum and active parent compound in plasma ultrafiltrate provided a more complete and accurate description of cisplatin pharmacokinetics [36].

Although HPLC methods have been developed for the quantitation of metallotexaphyrins in non-biological solutions [22,37] the expanded clinical evaluation of both Gd-Tex and Lu-Tex has created a need for assays that will allow quantitation of parent compound, and possibly other relevant metabolites, in biological matrices. Therefore, we have developed HPLC methods for quantifying Gd-Tex and Lu-Tex in human plasma. Both assays have characteristics that make them very suitable for use in preclinical in vivo studies and in clinical trials. These assays require minimal amounts of plasma, use a relatively simple sample preparation procedure, have manageable run times, are sensitive and reproducible, are not susceptible to gadolinium DTPA interference, and use instrumentation that is widely available.

2. Experimental

2.1. Materials

Gd-Tex (lot #SB801) and Lu-Tex (lot #SA701) were graciously provided by Pharmacyclics (Sunnyvale, CA, USA). Bis-*N*-methyl-amide of gadolinium DTPA was clinical grade material (Omniscan®) (Sterling-Winthrop, New York, NY, USA). Acetoni-

trile, methanol, glacial acetic acid, and ammonium acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Control human plasma was obtained from the Central Blood Blank, Pittsburgh, PA, USA. Medical grade nitrogen was purchased from the Praxair (Pittsburgh, PA, USA).

2.2. Procedure

2.2.1. Sample preparation

Triplicate 250-μl samples of human plasma, containing 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 μ*M* Gd-Tex or Lu-Tex, or patient specimens, were placed into 1.5-ml microcentrifuge tubes. For the Gd-Tex assay, 10 μl of a 25 μ*M* Lu-Tex solution in 25 m*M* ammonium acetate, pH 5.2:methanol (60:40, v/v) were added to each tube, and the tubes were mixed. For the Lu-Tex assay, a 25 μ*M* solution of Gd-Tex replaced Lu-Tex as the internal standard. After addition of internal standard, 1 ml of acetonitrile:methanol (50:50, v/v) was added to each tube, and the mixtures were vortexed and centrifuged at 12 000×*g* for 10 min. The resulting supernatants were transferred to 12×75 mm, borosilicate glass tubes and dried under nitrogen at 40°C. Dried residues were reconstituted in 130 μl of the mobile phase described below and placed into a sonicating water bath for 10 min. The reconstituted residues were subsequently transferred to 2-ml microcentrifuge tubes and centrifuged at 12 000×*g* for 4 min. The resulting supernatants were transferred to autosampler vials, and 100-μl aliquots were injected into the HPLC system.

2.2.2. HPLC system

The HPLC system consisted of a Hewlett–Packard model 1050 autosampler (Hewlett–Packard, Palo Alto, CA, USA), a Waters (Waters Associates, Milford, MA, USA) model 6000 pump fitted with a Brownlee RP 18 NewGuard (7-μm, 15×3.2 mm) guard column (PerkinElmer, Norwalk, CT) and a Zorbax Eclipse XDB-C 18 (3.5-μm, 3.0×150 mm) analytical column (Hewlett–Packard, Wilmington, DL, USA). A LC-22A (Bioanalytical Systems, W. Lafayette, IN, USA) column heater was used to maintain the column temperature at 55°C. The isocratic mobile phase, consisting of 100 m*M* ammonium acetate (adjusted to pH 4.3 with glacial acetic acid):acetonitrile:methanol (59:21:20, v/v/v), was pumped at 0.6 ml/min. Column eluate was monitored at 470 nm with a Waters 2487 dual-wavelength detector, and the detector signal was recorded and integrated with Chrom Perfect software (Justice Innovations Chromatography Data Systems, Mountain View, CA, USA) installed on a Gateway 2000 (N. Sioux City, SD, USA) Pentium 166 MHz computer. The I.S. ratio was calculated for each standard by dividing the analyte peak area by the peak area of the internal standard. Standard curves of Gd-Tex or Lu-Tex were constructed by plotting the I.S. ratio versus the known concentration of analyte in each sample. Standard curves were fit by linear regression without weighting, followed by back-calculation of concentrations.

Prior to analyzing samples from patients receiving Gd-Tex or Lu-Tex, the method described was applied to pretreatment serum samples from ten patients who were treated with chemotherapy at the University of Pittsburgh Cancer Institute and who did not receive either texaphyrin. This was done to evaluate further the potential for endogenous or other materials that might interfere with the assay.

To demonstrate the applicability of this HPLC method to clinical samples, it was used to analyze plasma obtained from a patient with pancreatic cancer who was participating in a phase I study of thrice-weekly Gd-Tex combined with daily radiation therapy. On the first day of therapy, this patient received a 15-min i.v. infusion of Gd-Tex at a dose of 2.9 mg/kg. Blood samples were collected before delivery of Gd-Tex, at the end of the infusion, and at 5, 30, and 45 min, 1, 3, 4, 6, 24, and 48 h after the end of the infusion. Each sample was centrifuged at 1000×*g* for 10 min, and the resulting plasma layer was stored at −70°C until analyzed with the procedure described above. Gd-Tex concentrations were calculated from the I.S. ratio measured for each sample and a linear function derived from the standard curve that related the I.S. ratio to Gd-Tex concentration.

3. Results

With the sample processing and chromatographic conditions described, Gd-Tex and Lu-Tex were well resolved from each other, with retention times of approximately 16 and 18 min, respectively (Fig. 2).

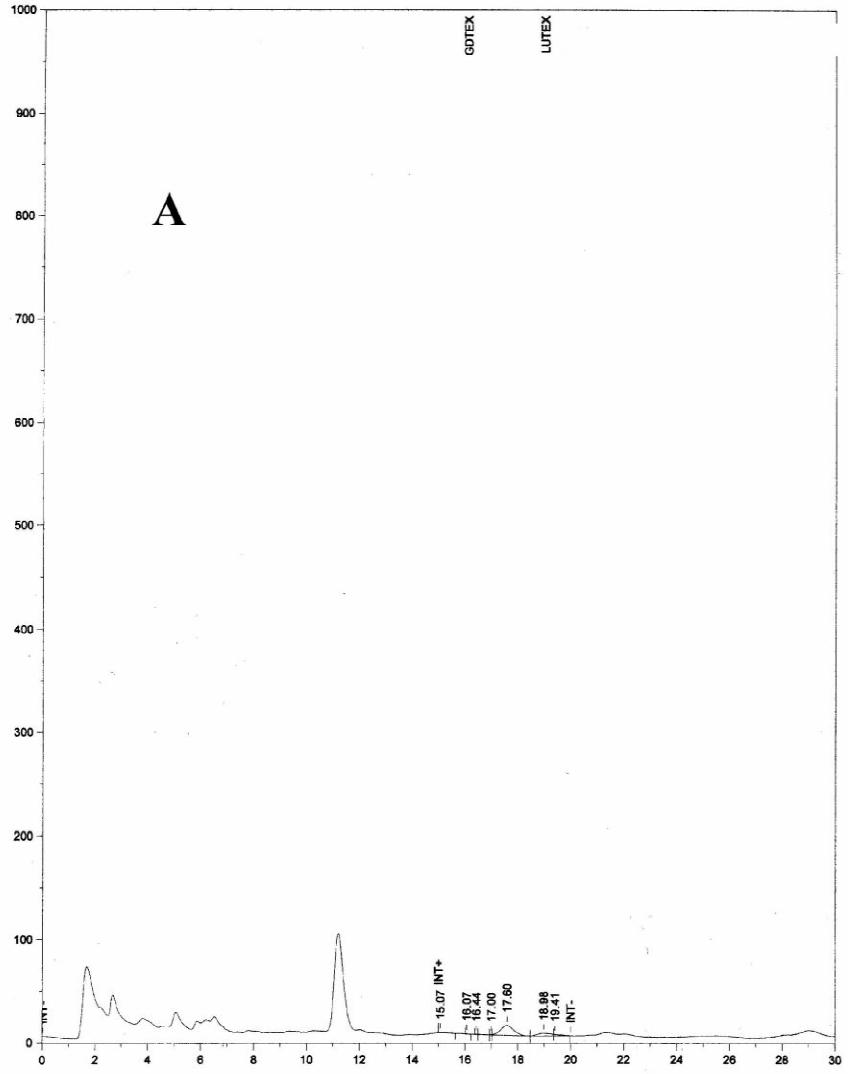


Fig. 2. Chromatograms of (A) control plasma, (B) a plasma standard containing 1 μ*M* Gd-Tex and Lu-Tex, and (C) plasma obtained from a patient at 60 min after completion of a 15-min Gd-Tex infusion at the dose of 2.9 mg/kg.

There were no endogenous materials in plasma that interfered with the determination of either compound. Both Gd-Tex and Lu-Tex were stable in plasma, undergoing less than 1% decomposition when incubated for 5 h at 22°C. Both Gd-Tex and Lu-Tex were also stable in deproteinized plasma, as the deproteinization procedure and subsequent sample handling produced a recovery of >90% when compared to a direct injection of either Gd-Tex or Lu-Tex in mobile phase. The assay was also suitable for use with an autosampler because there was no decay in solutions of either Gd-Tex or Lu-Tex when stored in mobile phase for up to 24 h at room temperature. The lower limit of quantitation for Gd-Tex was 0.01 μ*M* [38], and the assay proved linear over the concentration range of 0.01–30 μ*M*. The correlation coefficients for three successive, Gd-Tex triplicate standard curves were 0.995, 0.997, and 0.995. With back-calculation, calculated values varied from 8.3–20% and 4.4–6.7% of the theoretical

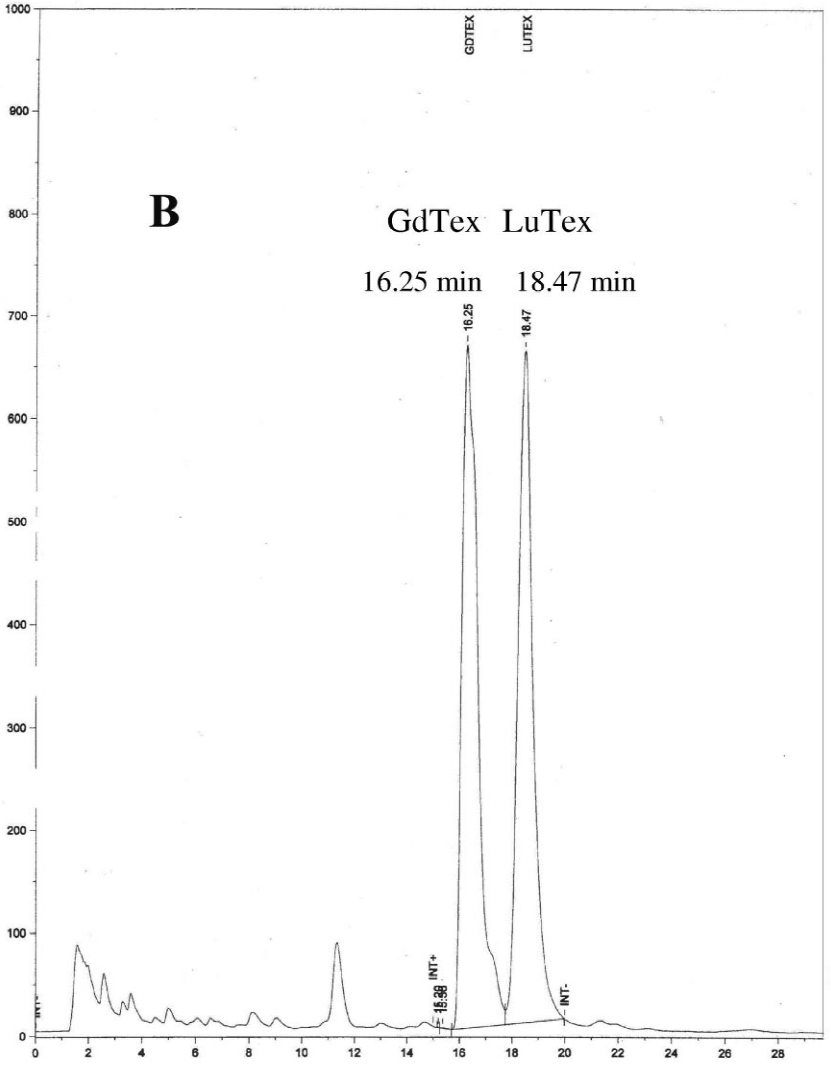


Fig. 2. (*continued*)

value at 0.01 and 0.03 μ*M*, respectively to 0.4–5.5% at 30 μ*M*. The within-day variation in triplicate samples was always <15% at every concentration studied. Between-day variation in three successive triplicate standard curves was also minimal. Coefficients of variation of 8.3, 8.6, 3.4, and 5.9% were calculated for Gd-Tex:internal standard ratios associated with Gd-Tex concentrations of 0.01, 0.1, 1, and 10 μ*M*. The day-to-day reproducibility for other concentrations was similar, and the coefficient of variation of the slopes associated with repeated standard curves was 7%. One m*M* gadolinium DTPA (the estimated plasma concentration produced when gadolinium DTPA is used as a contrast agent in MRI studies) had no effect on Gd-Tex standard curves.

The lower limit of quantitation for Lu-Tex was 0.1 μ*M* [38], and the assay proved linear over the concentration range of 0.01–30 μ*M*. The correlation coefficients for three successive, Lu-Tex triplicate standard curves were 0.997, 0.997, and 0.998. The within-day variation in the triplicate samples was always <11% at every concentration studied. Between-day variation in three successive triplicate standard curves was also minimal. Coefficients of

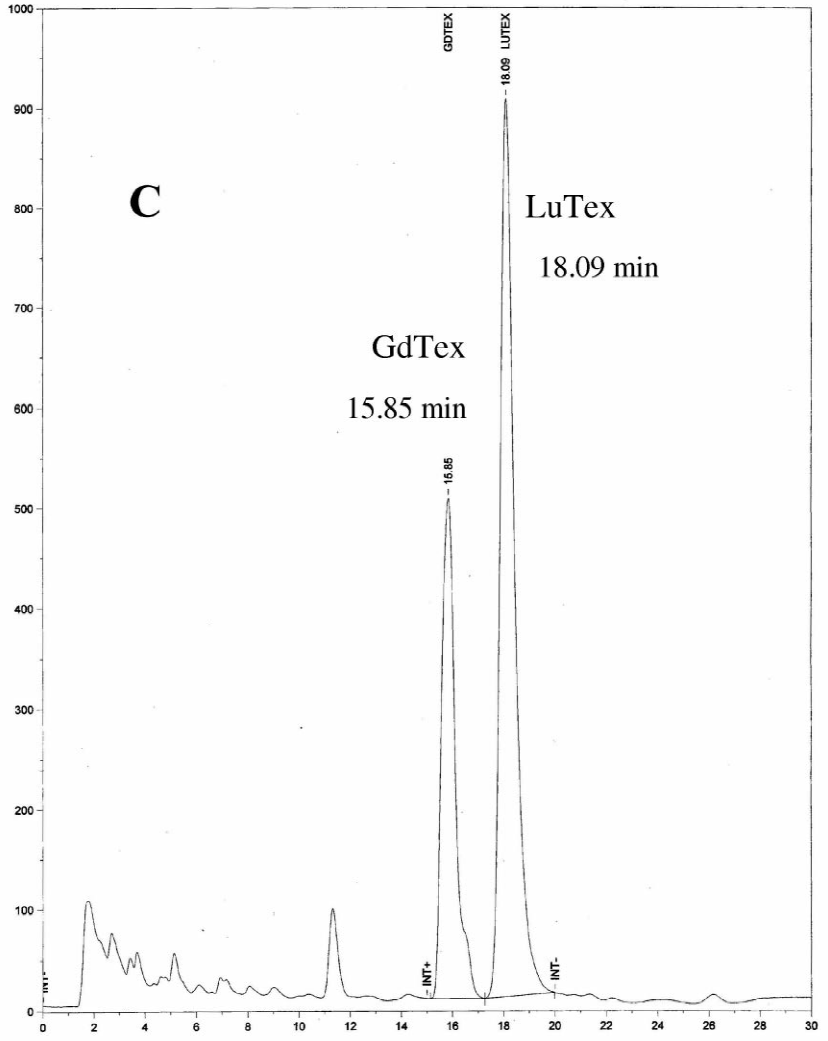


Fig. 2. (*continued*)

variation of 19, 7.2, and 5.3% were calculated for LuTex:internal standard ratios associated with LuTex concentrations of 0.1, 1, and 10 μ*M*. The day-to-day reproducibility was similar for other concentrations, and the coefficient of variation of the slopes associated with repeated standard curves was 2.9%.

Gd-Tex was easily detected in each patient sample (Fig. 3). As expected, plasma concentrations of Gd-Tex peaked at the end of the 15-min infusion and then declined with time. Gd-Tex was still easily detectable at 48 h after treatment. It was impossible to study times later than this because the thrice-weekly treatment schedule meant that a subsequent dose of Gd-Tex was administered at 48 h. Similarly, the absence of a clinically approved protocol for use of Lu-Tex at our institution precluded the demonstration of the clinical applicability of the assay for that compound.

4. Discussion

The tumor-localizing properties of porphyrin compounds have led to efforts to develop porphyrin-like

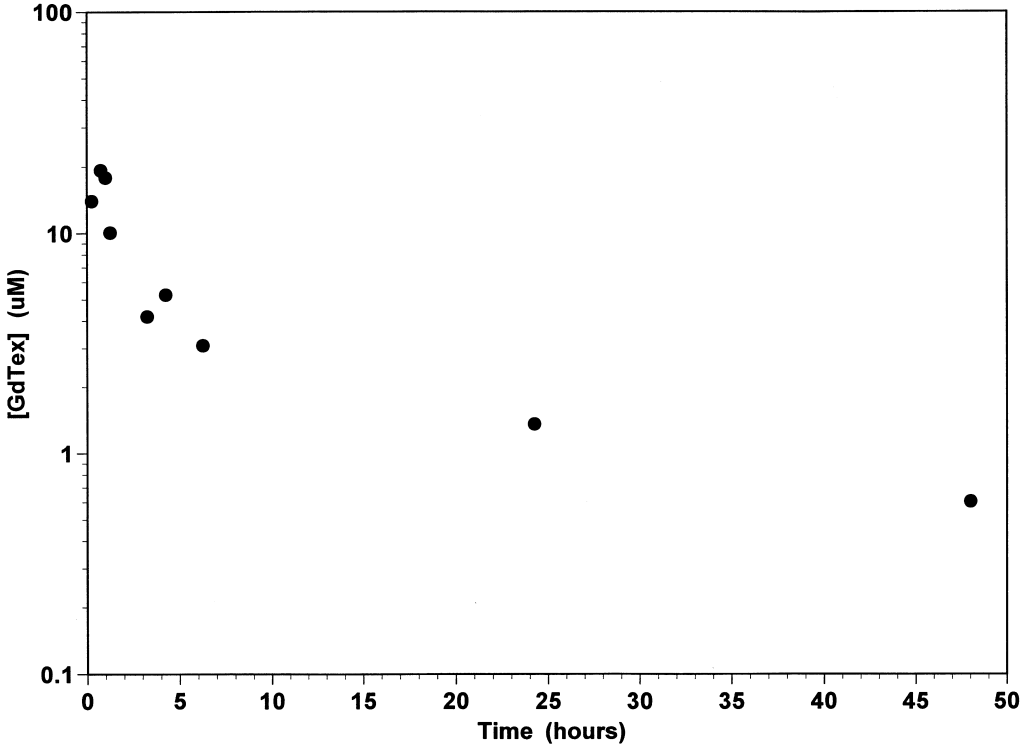


Fig. 3. Time course of Gd-Tex in plasma of a patient after his first 15-min infusion of a 2.9 mg/kg dose.

macrocycles as diagnostic agents, photosensitizers, and radiation sensitizing agents [11–30]. The development of paramagnetic metalloporphyrins has been hindered by their relatively high toxicity, limited tumor specificity, and chemical instability. Furthermore, the four-pyrrole ring structure of the porphyrins is too small to accommodate lanthanide series cations [10], whose chemical properties make them most suitable for use in the clinical situations mentioned above.

A novel class of 'expanded' porphyrin compounds has been synthesized and named 'texaphyrins' [1–9]. The central core of these texaphyrins consists of five nitrogens as compared to the four nitrogens of the porphyrin central core [10]. As a result, the central core of texaphyrins is approximately 20% larger than that of porphyrins and can form highly stable complexes with lanthanide series cations such as Gd (III) and Lu (III) [10]. Two metallotexaphyrins, motexafin gadolinium (Gd-Tex) and motexafin lutetium (Lu-Tex), are currently under clinical development [11–30]. Gd-Tex is being broadly evaluated as a potential MRI-detectable radiation-enhancing agent [10–18]. LuTex is being actively investigated as a photosensitizer for the treatment of cancer, cardiovascular disease, and ocular disease [19–26]. In view of each of these proposed uses, it is important to be able to characterize the pharmacokinetics and tissue distribution of both Gd-Tex and Lu-Tex. To date, studies in this regard have used non-specific measurements such as total radioactivity [10,22], total fluorescence [20,24], inductively coupled plasma spectrometry [11,15], or enhancement of magnetic resonance imaging signal [10,16,17,22].

With the enhanced and expanded development of both Gd-Tex and Lu-Tex, there has been an increased need for suitable methodology to quantify parent compound as well as potential metabolites in biological matrices. The HPLC assays presented in the current manuscript fulfill this need. Each assay is sensitive and specific. This specificity removes the clinically undesirable restriction on using gadolinium

DTPA-enhanced magnetic resonance imaging studies in patients at times close to those of Gd-Tex pharmacokinetic studies. The volume of sample required for analysis in these assays is small, sample preparation is simple, and the run times are reasonable. Because of these factors, as well as the use of an isocratic mobile phase and the availability of the necessary instrumentation, these assays can be widely implemented. They can now be applied to the numerous in vivo preclinical and clinical studies investigating the potential uses of these metallotexaphyrins.

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