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Dose-dependent pharmacokinetics of rapamycin-28-*N,N*-dimethylglycinate in the mouse

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Abstract. Rapamycin-28-*N,N*-dimethylglycinate methanesulfonate salt (RG), synthesized as a potential water-soluble prodrug to facilitate parenteral administration of the antineoplastic macrolide rapamycin (RA), is active against intracranially implanted human glioma in mice. Preclinical pharmacokinetic studies to evaluate the prodrug were conducted in male $CD2F_1$ mice treated with 10, 25, 50 and 100 mg/kg doses of RG by rapid i.v. injection. The plasma concentration of RG decayed in a distinctly triphasic manner following treatment with the 100 mg/kg dose; however, prodrug disposition was apparent biexponential at each of the lower doses. RG exhibited dose-dependent pharmacokinetics, characterized by an increase in the total plasma clearance from 12.5 to 39.3 $ml \cdot min^{-1} \cdot kg^{-1}$ for dosage escalations in the range 10–50 mg/kg, while clearance values at doses of 50 and 100 mg/kg were similar. The terminal rate constants decreased linearly as the dose was increased from 10 to 100 mg/kg, eliciting an apparent prolongation of the biological half-life from 2.1 to 4.8 h. There was also a sequential increase in the steady state apparent volume of distribution from 1.73 to 8.75 l/kg. These observations are consistent with saturable binding of RG to plasma proteins while binding to tissue remains linear. Nevertheless, conversion to RA appeared to represent a prominent route of RG elimination. The molar plasma concentration of RA exceeded that of the prodrug within 30–90 min after i.v. treatment and declined very slowly thereafter, with plasma levels sustained between 0.1 and 10 μM for 48 h at each of the doses evaluated. Thus, RG effectively served as a slow release delivery system for RA, implying the possibility of maintaining therapeutic plasma levels of the drug from a more convenient dosing regimen than a continuous infusion schedule. The present findings, coupled with the demonstrated in vivo activity of RG against human brain tumor models, warrant its continued development as a much needed chemotherapeutic agent for the treatment of brain neoplasms.

The laboratory work was conducted at The Ohio State University, Comprehensive Cancer Center under the support of contracts N01-CM-97619 and N01-CA-16058 from the National Cancer Institute, NIH, DHHS. Preliminary results were presented at the 81st Annual Meeting of the American Association for Cancer Research, May, 1990, Washington, DC

Abbreviations: AUC, area under plasma concentration-time curve between time zero and infinity; CL, total plasma clearance; λz, terminal phase elimination rate constant; MRT, mean residence time; $t_{1/2,z}$, half-life of terminal disposition phase; V_z, total body apparent volume of distribution; V_1, central compartment apparent volume of distribution; V_{ss}, apparent volume of distribution at steady state

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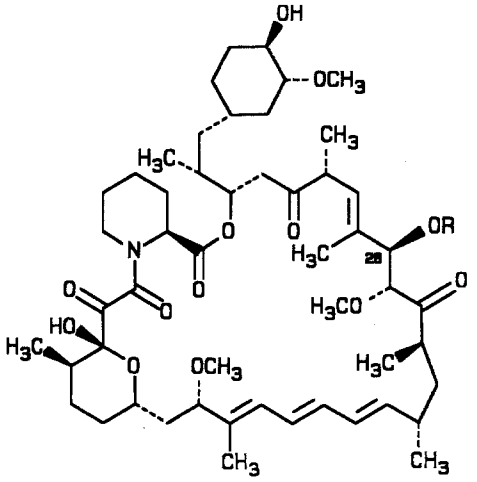
Introduction

Rapamycin (RA) was identified as the antifungal constituent in organic extracts of the bacterium *Streptomyces hygroscopicus,* which is indigenous to Easter Island [1]. The chemical structure of the compound, a macrocyclic triene, is shown in Fig. 1 [2–4]. The immunosuppressive properties of RA, first observed shortly after its discovery [5], have recently generated considerable interest in its utilization as an agent to enhance the survival of solid organ transplants [6–8]. The established biochemical effects of RA [9] and the known antitumor activity of trienine, a structurally related macrolide antibiotic [10], prompted its submission to the National Cancer Institute for evaluation as an antineoplastic agent [11, 12]. In contrast to marginal activity against murine leukemia models, the compound was significantly effective against a broad spectrum of ascites and transplantable solid tumors in mice, even when administered at sites separated from the location of the tumor implant [11–13]. Therapeutically effective treatment regimens of RA appeared considerably less toxic to mice than many other anticancer agents. The mechanism of action has not been identified; however, in vitro studies with P388 cells suggest that the antitumor properties of RA result from actions directed primarily against DNA [14].



RAPAMYCIN, R = H

RG, R = $COCH_2N(CH_3)_2 \cdot CH_3SO_3H$

Fig. 1. Structures of rapamycin and the methanesulfonate salt of its 28-*N,N*-dimethylglycinyl ester (RG)

The apparent penetration of RA through the blood-brain barrier, as evidenced by activity against intracranially (i.c.) implanted tumors, including the U-251 human glioma [15], was of particular relevance toward continued development of the compound (unpublished results). However, in addition to limited solubility in water and mixed aqueous solvent systems [2], its bioavailability is poor following oral administration [1, 13, 16]. Furthermore, difficulties were encountered in the toxicological evaluation of a parenteral formulation prepared with surfactants [17, 18]. Subsequently, several water-soluble derivatives were prepared as potential prodrugs of RA to facilitate parenteral delivery [19]. Among these compounds, the 28-*N,N*-dimethylglycinyl ester solubilized as a methanesulfonate salt (RG; Fig. 1) exhibited the best in vivo efficacy against the i.c. U-251 tumor in mice, eliciting an increase in lifespan of 139–167%, referred to untreated controls when the highest nontoxic dose of 180 mg/kg was given i.p. on a daily × 9 schedule (unpublished results). The present report describes the subsequent preclinical pharmacokinetic evaluation of this derivative as a prodrug for RA.

Materials and methods

Chemicals. Rapamycin (NSC 226080) and rapamycin-28-*N,N*-dimethylglycinate methanesulfonate (NSC 606698) were provided by the Developmental Therapeutics Program, National Cancer Institute. All additional reagents and chemicals were obtained from commercial sources in appropriate grades for use without additional purification, unless specified otherwise. β-Estradiol-3-toluate-17-acetate was synthesized by the triethylamine catalyzed reaction between estradiol-17-β-acetate and freshly distilled *p*-toluoyl chloride in methylene chloride. Recrystallization from ethanol afforded a colorless solid, melting at 169–170° C, which was dissolved in *N,N*-dimethylformamide (0.23 mg/ml) and stored at 5° C. Deionized double-distilled water was filtering through a 0.2 μm Nylon-66 membrane filter (Rainin Instrument Co., Woburn, Mass.).

Apparatus. Milligram quantities of the compounds used for dosing solutions and analytical stock solutions were weighed on a Cahn 25 electrobalance (Cahn Instruments, Cerritos, Calif.). Stock solutions were prepared in class A borosilicate glass volumetric flasks. All glassware was deactivated with 3% (v/v) Surfasil in toluene (Pierce Chemical Co., Rockford, Ill.). Centrifugation was performed at 12000 *g* using an Eppendorf 5412 microcentrifuge at ambient temperature (Brinkmann Instruments, Westbury, N.Y.). An electric minute timer (GCA/Precision Scientific, Chicago, Ill.) was employed to monitor the time of drug administration and blood withdrawal.

Liquid chromatography was performed using a 421A programmable LC system controller with two 114 M pumps (Beckman Instruments, Berkeley, Calif.) and a WISP 712 automatic injector (Waters Associates, Milford, Mass.). A variable wavelength Spectroflow 783 programmable absorbance detector (ABI Analytical, Kratos Division, Ramsey, N.J.) with a 12 μl analytical flow cell (path length 8 mm) was used to monitor UV absorption at 280 nm (5 nm bandwidth). The 1 V output of the detector was provided as the signal to a 3393A recording integrator (Hewlett-Packard, Avondale, Pa), configured to report peak heights using a 0.3 min peak width, a threshold setting of 0 and baseline construction through each detected valley point.

Dosing and sample collection. Injectable solutions were formulated immediately before administration by dissolving RG in sterile water for injection, USP, adjusted to pH ~3 with phosphoric acid, to deliver the desired dose in a volume of 100 μl. Unfasted male Harlan $CD2F_1$ mice were treated with doses of 10, 25, 50 and 100 mg/kg by 1 min tail vein injection without anesthesia using a 27 gauge, 0.5 inch Yale hypodermic needle (Becton-Dickinson, Lincoln Park, N.J.). Animals were bled through the retro-orbital plexus under ether anesthesia using heparinized microhematocrit capillary tubes (Scientific Products, McGaw Park, Ill.) at 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 480 and 600 min and at 4 h intervals thereafter to 48 h post-injection. Whole blood from each mouse was individually collected in 1.5 ml polypropylene tubes (VWR Scientific, San Francisco, Calif.) and immediately centrifuged for 5 min. Two 50 μl aliquots of the separated plasma were promptly prepared for HPLC analysis. The remaining plasma was removed from blood cells, flash frozen and stored at –20° C.

HPLC analysis. A gradient elution HPLC method with specificity to permit the concurrent determination of RA and RG in plasma was developed for these studies. Plasma (50 μl) was deproteinized in a microcentrifuge tube by thorough mixing (0.5 min, vortex stirrer) with a methanolic solution of the internal standard, β-estradiol-3-toluate-17-acetate (200 μl, 1.0 μg/ml). After centrifuging for 3 min, 200 μl of the supernatant was separated from the pelleted protein, diluted with an equivalent volume of *N,N*-dimethylformamide/7 mM aqueous sulfuric acid (7:3, v/v), and vortexed. This solution was transferred to a glass insert within an autosampler vial and sealed with a Teflon-lined septum screw cap. Degradative loss of the analytes and internal standard upon standing for 12–14 h at ambient temperature was insignificant.

The prepared samples (200 μl) were loaded onto a 4 μm Nova-Pak Phenyl Radial-Pak cartridge column (5 mm×10 cm) (Waters Associates, Milford, Mass.), protected by a 0.5 μm inline filter (Rainin Instrument, Emeryville, Calif.) and a Guard-Pak precolumn module fitted with a μBondapak C_{18} insert (Waters Associates). Separations were conducted at ambient temperature using two solvent systems, composed of methanol/ammonium formate buffer (0.05 M, pH 5.0) with 1 mM sodium dodecylsulfate, degassed in an ultrasonic bath for 15 min prior to use. Eluent A contained 75% (v/v) methanol and eluent B was 90% (v/v) methanol. At a flow rate of 1.0 ml/min, the gradient program was sequentially 30% B for 5 min, 30–70% B linearly over 10 min, 70% B for 15 min, and equilibration at 30% B for 10 min. Typical retention times were 13–15 min for RA, 18–20 min for RG and 26–28 min for the internal standard.

Pharmacokinetic samples were quantified daily along with a series of nine plasma standards which contained both analytes at concentration ranges of 0.24–7.6 μM for RG and 0.055–5.3 μM for RA. The standards were made by adding varied volumes (2–10 μl) of a single acetonitrile stock solution (RA, 0.11 mM; RG, 0.16 mM) to drug-free

plasma (0.2–4.0 ml) in glass test tubes and mixing thoroughly. Each standard solution was immediately prepared for analysis to minimize degradation of the analytes in plasma. Coefficients of variation ($n = 10$) for the lowest concentration quantified in 50 µl of plasma were 6.35% for RG (0.24 µM, 0.27 µg/ml) and 7.27% for RA (0.055 µM, 50.6 ng/ml).

Standard curves were constructed by plotting the peak area ratio of each analyte to the internal standard against concentration. Unweighted linear least-squares regression was performed, without inclusion of the origin, to determine the slope, *y*-intercept and correlation coefficient of the best fit line. Analyte concentrations in unknown samples were calculated using the results of the corresponding regression analysis. Specimens with an analyte concentration exceeding the upper range of either standard curve were reassayed upon appropriate dilution with drug-free plasma. All samples were initially assayed in duplicate, and if the replicate determinations deviated from their average by more than 10%, additional analyses were performed.

Pharmacokinetic data analysis. Time points were determined as the difference between the blood collection interval midpoint and starting time of dose administration. Geometric mean plasma concentrations at each time point were calculated from the average determinations of at least three mice. Plasma concentration-time profiles were pharmacokinetically analyzed according to model-independent methods [20]. Conventions recommended by Rowland and Tucker for symbols of pharmacokinetic terms have been adopted [21] (see list of abbreviations on title page of this article). Nonlinear least-squares regression was performed using PCNONLIN (Statistical Consultants, Lexington, Ky.) with initial parameter estimates from preliminary data analysis with the stripping routine RSTRIP (MicroMath, Salt Lake City, Utah). Each plasma profile was subject to repeated nonlinear regression analyses whereby the number of exponential terms in the fitted equation and influence of the weighting factor, y_{obs}^{-n} ($0 \leq n \leq 2$), were both evaluated to identify the simplest equation that best described the data.

The appropriate equation of the general form [22]

$$C_{RG} = \sum_{i=1}^{n} (C_i/\lambda_i\tau)\,(e^{-\lambda_i t'} - e^{-\lambda_i t})$$

was fit to observed time courses for the concentration of prodrug in plasma (C_{RG}) by unweighted nonlinear regression. The value of t' is zero until the infusion of duration τ has terminated, upon which it is defined as

$$t' = t - \tau$$

where t denotes time from the initiation of treatment. The coefficients C_i are intercept values, corresponding to i.v. bolus administration of the dose, of each log-linear phase with slope $-\lambda_i$, such that $\lambda_1 > \lambda_2 > \ldots > \lambda_z$. Parameters corresponding to the terminal decay phase are designated with a subscript z by convention.

Plasma concentration-time profiles for the drug (C_{RA}) arising from RG conversion were independently fit, with a weighting factor of $1/y_{obs}$, to empirical equations of the general form

$$C_{RA} = \sum_{i=1}^{n} C_i e^{-S_i t}$$

where C_i are the *y*-intercepts of each log-linear phase with slope $-S_i$, such that $S_1 > S_2 > \ldots > S_z$. Coefficients for exponential phases defining the initial region of increasing concentration are negative whereas those characterizing the decay phases have positive values.

Results

Plasma pharmacokinetics of RG

Plasma concentration-time profiles depicting the observed geometric mean plasma levels and best-fit curves of RG in mice treated with 10, 25, 50 and 100 mg/kg doses of the prodrug by 1 min i.v. injection are shown in Fig.2. Nonlinear least-squares estimated pharmacokinetic parameters and derived values are summarized in Table 1. The concentration of RG in plasma decayed from an initial level of 14.6 µM to less than 0.25 µM during 6 h after treatment with 10 mg/kg. At the highest dose, 100 mg/kg, sensitivity of the analytical method permitted the prodrug to be monitored for more than 12 h. Each of the plasma profiles determined for doses of 10, 25 and 50 mg/kg exhibited apparent biexponential decay; however, prodrug disposition was distinctly triexponential at the 100 mg/kg dose level. Comparative analysis of the time courses and pharmacokinetic parameters suggested that this additional exponential phase developed during the initial region of the plasma profile and was not associated with elucidation of a slow disposition phase undetected at the lower doses.

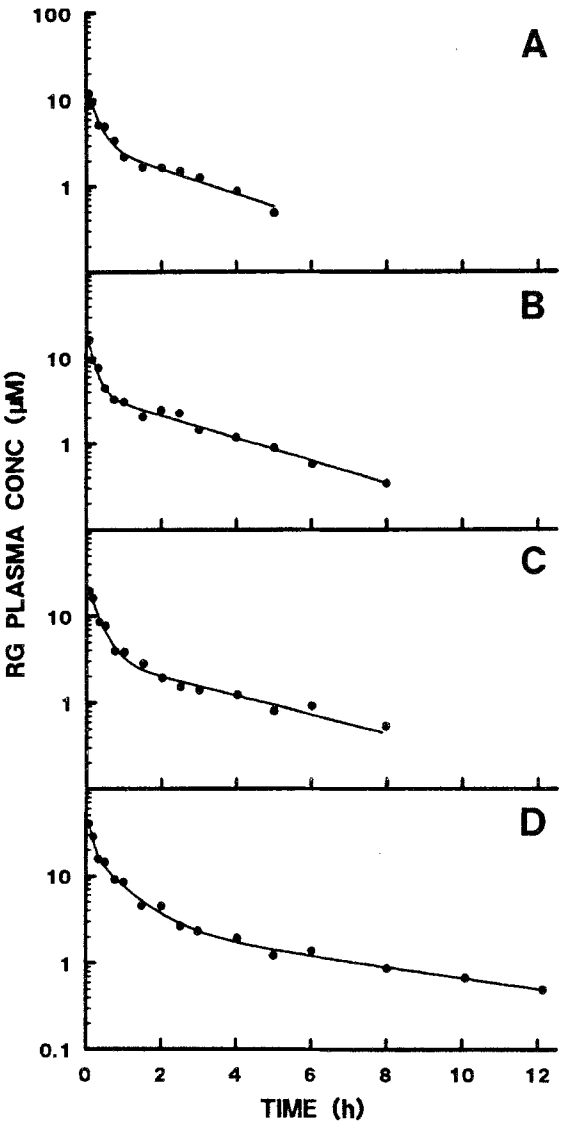


Fig. 2A–D. Plasma concentration-time profiles of rapamycin-28-*N,N*-dimethylglycinate in mice treated by 1 min i.v. injection with doses of **A** 10, **B** 25, **C** 50 and **D** 100 mg/kg. Experimental *points* (•) are the geometric mean of observed plasma levels in at least three mice per time point. The *solid line* represents the best-fit curve of the experimental data determined by nonlinear regression

Table 1. Rapamycin-28-*N,N*-dimethylglycinate pharmacokinetic and derived parameters

Parameter[a]	Prodrug dose (mg/kg)				Units
	10.0	25.1	50.1	100.3	
Phases	2	2	2	3	
$C(\tau)$	14.6	21.6	24.7	61.7	μM
C_1	11.5	17.7	21.5	42.2	μM
C_2				16.6	μM
C_z	3.1	3.9	3.2	2.8	μM
$t_{1/2,1}$	10.6	7.6	12.4	5.6	min
$t_{1/2,2}$				35.5	min
$t_{1/2,z}$	2.06	2.29	2.78	4.81	h
MRT	2.32	2.67	2.80	3.78	h
CL	12.5	23.8	39.3	38.6	$ml \cdot min^{-1} \cdot kg^{-1}$
V_1	0.63	1.06	1.85	1.48	$l \cdot kg^{-1}$
V_{ss}	1.73	3.80	6.57	8.75	$l \cdot kg^{-1}$
V_Z	2.22	4.71	9.45	16.0	$l \cdot kg^{-1}$
AUC	12.2	16.1	19.4	39.6	$\mu M \cdot h^{-1}$
AUC_1	24.1	20.5	32.9	14.3	%
AUC_2				35.9	%
AUC_Z	75.9	79.5	67.1	49.8	%

[a] Abbreviations: Phases, number of disposition phases observed in plasma concentration-time curve; $C(\tau)$, plasma concentration of drug at the end of infusion; C_i, *y*-intercept of the *i*-th disposition phase; λ_i, elimination rate constant of *i*-th disposition phase; $t_{1/2,i}$, half-life of the *i*-th disposition phase; AUC_i, contribution of the *i*-th disposition phase to AUC

Table 2. Parameters characterizing the time course of rapamycin in plasma following i.v. administration of rapamycin-28-*N,N*-dimethylglycinate

Parameter[a]	Prodrug dose (mg/kg)				Units
	10.0	25.1	50.1	100.3	
C_{max}	4.95	6.61	7.43	8.12	μM
t_{max}	1.90	3.06	2.33	0.0[b]	h
$t_{1/2,z}$	12.8	10.4	6.10	6.03	h
AUC	53.2	90.1	136.6	120.7	$\mu M \cdot h^{-1}$
AUMC	0.60	1.02	1.75	1.44	$mM \cdot h^{-2}$
MRT	11.3	11.3	12.8	11.9	h
CL/f	2.86	4.24	5.58	12.6	$ml \cdot min^{-1} \cdot kg^{-1}$
V_{ss}/f	1.95	2.88	4.28	9.03	$l \cdot kg^{-1}$

[a] Abbreviations: C_{max}, peak plasma concentration; t_{max}, time of the peak plasma concentration; AUMC, area under the first moment curve from time zero to infinity; CL/f, ratio of total plasma clearance to the bioavailable fraction of drug; V_{ss}/f, ratio of volume of ditribution at steady state to the bioavailable fraction of drug

[b] The maximum plasma concentration of rapamycin was observed at the first time point

The magnitude of the terminal rate constants decreased linearly as the RG dose was increased from 10 to 100 mg/kg ($r = -0.999$). Despite a prolongation of $t_{1/2,z}$ from 2.1 to 4.8 h, the apparent CL increased from 12.5 to 39.3 ml · min^{-1} · kg^{-1} as the RG dose was escalated from 10 to 50 mg/kg. Values of CL calculated for the 50 and 100 mg/kg doses were similar. These observations suggested that the dose-dependent pharmacokinetic behavior of RG was not associated with saturable elimination, typically characterized by an inverse relationship between CL and dose. Consideration of the apparent volumes of distribution suggested that

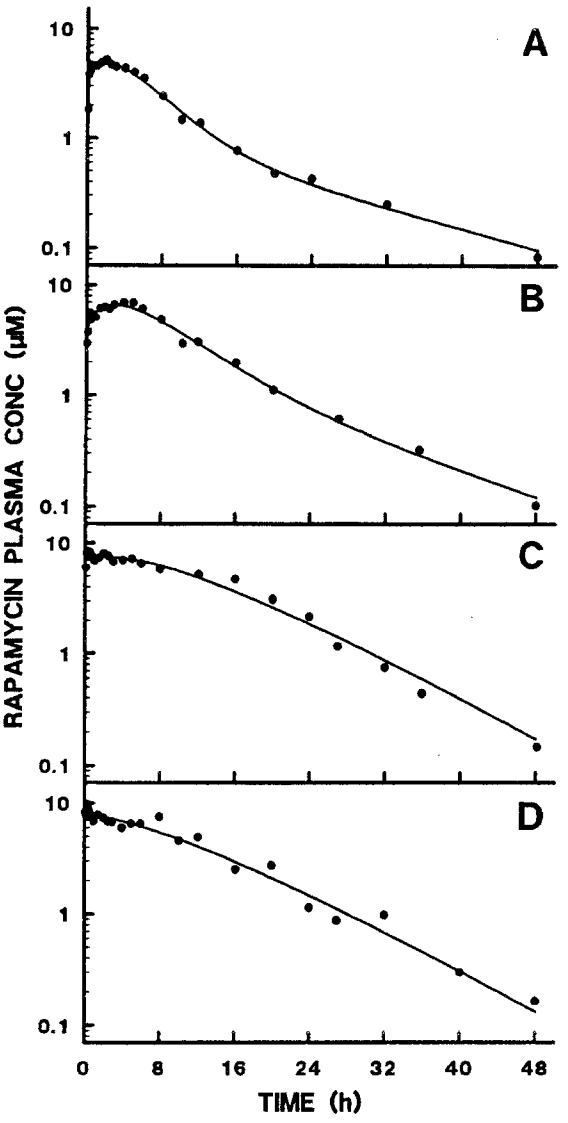


Fig. 3A–D. Plasma concentration-time profiles of rapamycin generated in vivo following the administration of **A** 10, **B** 25, **C** 50 and **D** 100 mg/kg of rapamycin-28-*N,N*-dimethylglycinate to mice by 1 min i.v. injection. Experimental *points* (•) are the geometric means of observed plasma levels in at least three mice per time point. The *solid line* represents the best fit curve of the experimental data determined by nonlinear regresion

this behavior may arise from enhanced tissue distribution at the higher doses. Values of V_1 increased by a factor of 2–3 in the dose range evaluated, while V_{ss} and V_z increased from 1.7 to 8.8 l/kg and 2.2 to 16.0 l/kg, respectively.

Prodrug conversion to RA

Time courses of the observed RA geometric mean plasma concentration and best-fit curves in mice treated with RG are shown in Fig. 3. Parameters characterizing the RA plasma profiles, determined by nonlinear regression analysis of the experimental values, appear in Table 2. For each of the RG doses, ranging from 10 to 100 mg/kg, measurable plasma levels of RA were rapidly achieved and exceeded

the prodrug concentration within 30 min after treatment. Thus, the clearance of RA from plasma was considerably slower than RG. Plasma concentrations of RA were sustained near peak levels, which ranged from 4.95 to 8.12 μM, for approximately 8 h. The plasma profiles of generated RA also exhibited marked dose-dependency. This was characterized by a reduction in the number of observed decay phases with a concurrent decrease of the apparent $t_{1/2,z}$, from 12.8 to 6.0 h, and less than proportionate increase in AUC as the prodrug dosage was escalated. However, the MRT of the drug in the body, 11.8 ± 0.7 h, expressed as the harmonic mean $\pm$SD of the values observed at each dose [23], remained relatively constant.

Discussion

RA may be categorized among the continually increasing number of promising chemotherapeutic agents for which the identification of a suitable delivery system has become a primary impediment toward their development. The macrocylic structure and absence of ionizable functional groups impart a high degree of hydrophobicity upon the molecule which, consequently, exhibits very limited solubility in water, in the order of 20 μg/ml [19]. Oral administration did not present an acceptable alternative due to inefficient absorption from the gastrointestinal tract. This was indicated by diminished antifungal [16] and antitumor [13] potency and lower acute toxicity [1] in mice treated with the compound p.o. as compared to the i.p route. In addition, a microbiological assay for RA revealed that serum levels of the drug in mice treated p.o. were approximately 50% lower than s.c. administration [16].

A clinically acceptable dosage form was therefore required for the continued evaluation of RA [11]. The mixed solvent approach was unsuccessful because solubility in aqueous media was not appreciably enhanced with less than 50–60% of a miscible cosolvent, and precipitation occurred upon dilution (unpublished results). Attempts to develop an injectable dosage form employing surfactants to effect solubilization were not favorably evaluated due to vehicle toxicity [17, 18]. This prompted the National Cancer Institute to initiate development of a water-soluble prodrug to facilitate parenteral dosing. The approach was considered feasible owing to the presence of secondary hydroxyl groups at positions C-28 and C-43 of the molecule, representing sites for structural modifications that are potentially bioreversible. Selective esterification of RA with amino acids at the 28-hydroxyl group was achieved through appropriate control of the reaction conditions [19]. Salts of these monosubstituted derivatives exhibited water solubilities exceeding 50 mg/ml.

Among the reported derivatives of RA, the methanesulfonate salt of the 28-*N,N*-dimethylglycinyl ester (RG) was selected for preclinical evaluation based primarily on in vivo activity against i.c. brain tumor models (unpublished results). However, RG exhibited a relatively high degree of stability in aqueous solution and biological fluids at physiological temperature, with half-lives of 45 h in a pH 7.4 buffer, 5 h in human plasma, 1.8 h in rat plasma and 4.5 h in a 20% rat liver homogenate preparation [19]. Thus, the potential for systemically circulating prodrug necessitated the quantitation of both RA and RG in specimens obtained during pharmacokinetic studies with RG. An assay was desired with greater inherent specificity than the microbiological method previously used to monitor RA in the plasma of rodents and dogs [16]. These objectives were achieved by developing an analytical method based upon reversed-phase HPLC with UV detection. Conditions were found to separate RA and RG from their minor *cis-trans* isomers about the amide bond in the macrocylcic ring [3], drug-related degradation products, metabolites and endogenous components. Plasma specimens were prepared for analysis by rapid deproteinization with methanol to facilitate the concurrent isolation of the two compounds. Extensive validation of the assay unequivocally demonstrated that both RA and RG were quantitatively recovered with negligible prodrug conversion or degradative loss of either analyte during sample workup and chromatographic analysis.

The assay was sufficiently sensitive to permit RG to be monitored in the plasma of mice for 5 h after treatment with the lowest dose of 10 mg/kg and longer than 12 h at the 100 mg/kg dose level. The persistence of systemic RG, further exemplified by an MRT that ranged from 2.3 to 3.8 h, would usually be considered unacceptable for a prodrug designed exclusively to facilitate parenteral injection [24]. Ideally, once the delivery function is achieved, the prodrug should rapidly and quantitatively convert to the parent drug in the blood stream. However, although not an intended effect, RG appeared to mimic a slow-release drug delivery system, serving as a depot to prolong the duration of RA. The plasma concentration of RA exceeded RG within 30–90 min after i.v. administration of 10 to100 mg/kg doses and decreased less than 10-fold from peak levels, which ranged from 4.95 to 8.12 μM, during the subsequent 24 h. The MRT of RA in the body, 11.8 ± 0.7 h, was considerably longer than that of its precursor (2.3–3.8 h) and independent of the prodrug dose. These observations suggest that it may be possible to maintain therapeutic plasma levels of the drug with a daily i.v. bolus dosing regimen of RG. Avoiding administration by an extended continuous infusion schedule would not only enhance patient convenience, but also conserve limited clinical resources.

Although the appearance of individual RG plasma profiles at each dose level was typical of apparent first-order elimination, disposition of the prodrug was nevertheless highly dose-dependent. A significant prolongation of $t_{1/2,z}$ with a concurrent increase in the magnitude of apparent CL values occurred as the prodrug dosage was escalated. This behavior differs from the more frequently observed situation characterized by disproportionately large increments in the AUC with increasing doses of a drug subject to elimination by saturable processes. As reviewed by Gibaldi and Perrier (see p. 301 in [20]), several different mechanisms have been proposed for compounds exhibiting similar dose-dependent effects. A hypothesis regarding the nature of the nonlinear pharmacokinetic behavior of RG was formulated by considering the influence of dose on the apparent volumes of distribution. The magnitudes of V_1 and V_{ss} at the lowest dose, 10 mg/kg, were both relatively small, with respective values of 0.63 and 1.73 l/kg. However, at the

100 mg/kg dose level, the value of V_{ss} was more than 5 times greater, while V_1 only exhibited a 2.3-fold increase in magnitude. These observations are consistent with nonlinear binding of the prodrug to plasma proteins while tissue binding remains linear. Thus, it appears that the fraction of RG bound to plasma protein decreases with escalating dose due to saturation of binding sites, with a corresponding increase in the amount of prodrug distributed into tissue regions, from which it is slowly released back into the systemic circulation or eliminated by mechanisms that may include conversion to drug. Although the values of λ_z and V_z both exhibited a functional dependence on prodrug dose, V_z increased to a greater extent than λ_z decreased, accounting for the seemingly anomalous trends in $t_{1/2,z}$ and CL with increasing dose. The high affinity of RA for binding to plasma proteins has been qualitatively demonstrated [16]. Attempts to experimentally verify the existence of saturable plasma protein binding at the concentrations of RA and RG achieved in vivo were encumbered by their propensity for adsorption onto membrane surfaces, instability in plasma [19] and limitations of assay sensitivity.

In summary, the present studies were conducted to pharmacokinetically evaluate the water-soluble 28-*N,N*-dimethylglycinyl ester of RA as a potential produg to enable parenteral administration of this promising, although highly insoluble, antitumor agent. Detectable plasma concentrations of RG persisted for 5–12 h in mice treated i.v. with doses ranging from 10 to 100 mg/kg. The disposition of RG exhibited an atypical dose-dependency that appeared to originate from saturable binding of the compound to plasma proteins while binding to tissue remains linear. Although systemically circulating prodrug may be subject to elimination by a variety of pathways, RG effectively served as a slow-release delivery system for the parent compound. Single doses of 10 to 100 mg/kg RG given by bolus i.v. injection provided plasma concentrations of RA that were sustained at near-peak levels for approximately 8 h and remained above 0.1 μM for 48 h. These observations imply the possibility of maintaining therapeutic plasma levels of the drug on a more convenient dosing regimen than a continuous infusion schedule. The present findings, coupled with the demonstrated activity of RG against in vivo brain tumor models, warrant continued development of the rapamycins as much-needed chemotherapeutic agents for the treatment of brain neoplasms.

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