The Pharmacokinetics and Absorption of Recombinant Human Relaxin in Nonpregnant Rabbits and Rhesus Monkeys After Intravenous and Intravaginal Administration

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Recombinant human relaxin (rhRlx) is being developed as a potential cervical ripening agent to be applied intravaginally or intracervically prior to parturition. The pharmacokinetics and absorption of rhRlx were determined in nonpregnant female rabbits and rhesus monkeys after intravenous bolus (iv) and intravaginal administration of 0.1 mg/kg; additionally, rabbits were dosed with 0.5 mg/kg intravaginally. In rabbits (n = 6), mean (±SD) peak concentrations following iv bolus administration were 1554 ± 296 ng/mL. The weight-normalized clearance (CL/W) was 5.9 ± 0.4 mL/min/kg, initial volume of distribution (V_1/W) was 57 ± 9 mL/kg, and volume of distribution at steady state (V_{ss}/W), assuming central compartment elimination, was 240 ± 20 mL/kg. V_{ss}/W could be as large as 2000 ± 400 mL/kg without this assumption. The estimated amounts of rhRlx absorbed in rabbits following intravaginal administration of 0.1 and 0.5 mg/kg (n = 5/dose) were 3.1 ± 1.4 and 0.7 ± 0.3%, respectively; peak concentrations were 600 ± 297 and 1066 ± 584 pg/mL, respectively. In rhesus monkeys (n = 5) after iv administration, peak concentrations were 971 ± 277 ng/mL; CL/W was 4.1 ± 0.6 mL/min/kg, V_1/W was 78 ± 25 mL/kg, and V_{ss}/W, assuming central compartment elimination, was 690 ± 220 mL/kg. The upper limit for V_{ss}/W was 1600 ± 200 mL/kg when no assumptions were made regarding site (compartment) of elimination. After intravaginal administration (n = 6), two monkeys had undetectable rhRlx concentrations throughout the 48-hr sampling interval; one monkey had only one sample containing measurable rhRlx (51 pg/mL) at 24 hr; and three monkeys absorbed <2% of the 0.1 mg/kg dose. Peak concentrations in these three animals ranged from 64 to 1475 pg/mL. The absorption of rhRlx was low and variable in both species, and similar results have been observed in women.

KEY WORDS: relaxin; pharmacokinetics; absorption; intravenous administration; intravaginal administration.

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INTRODUCTION

Ripening the cervix before induction of labor at term has been shown to increase the success rate of vaginal delivery and to shorten the induction-delivery interval (1). Intravaginal estradiol (1), prostaglandin $F_{2\alpha}$ (2), and purified porcine relaxin (3) have been used individually and in combinations (4) to ripen the cervix in humans. In several animal efficacy models, porcine relaxin directly inhibited spontaneous uterine contractions and, in conjunction with estrogen, caused the breakdown of the collagen matrix within the uterus, cervix, and breast, allowing these tissues to be remodeled during pregnancy and parturition. These properties of porcine relaxin are described in a comprehensive review (5).

The success of these studies led to interest in developing recombinant human relaxin (rhRlx) as a potential therapeutic agent to be applied intravaginally or intracervically in a topical gel to facilitate childbirth. Since the vagina is permeable to a wide variety of both organic and inorganic compounds (6), we designed a series of preclinical studies to determine the extent of rhRlx intravaginal absorption before the initiation of clinical trials. Two species (rabbits and rhesus monkeys) that have different vaginal lining characteristics were studied: rabbits have a simple cuboidal or columnar epithelium, and monkeys have a stratified squamous epithelium that is similar to the lining of the human vagina (T. Terrell, personal communication). A similar group of rabbits and rhesus monkeys received rhRlx by iv administration to characterize the serum pharmacokinetics and to determine the fraction absorbed after intravaginal administration.

MATERIALS AND METHODS

Materials

The relaxin that was used in these studies was composed of an A chain containing 24 amino acids and a B chain containing 29 amino acids held together by two disulfide bonds. The separate A and B chains were produced in *Escherichia coli*, purified, and combined to form recombinant human relaxin in a manner similar to that described previously for a chemically synthesized human relaxin analogue (7). The iv formulation was a solution of rhRlx in a sterile, isotonic buffer that was administered at a concentration of 0.5 mg/mL (rabbits) or 0.1 mg/mL (rhesus monkeys). For intravaginal administration, rhRlx was formulated in a 3% methylcellulose gel. *In vitro* testing demonstrated that rhRlx was immediately and completely released from the gel into any suitable buffer upon mixing. The 0.1 and 0.5 mg/kg intravaginal doses in rabbits were achieved by administering a uniform volume of 0.27 mL/kg at concentrations of 0.38 and 2.00 mg/mL, respectively. The 0.1 mg/kg intravaginal dose in rhesus monkeys was achieved by administering 0.1 mL/kg at a concentration of 1.00 mg/mL.

Experimental Design

Rabbits. Sixteen female, nonpregnant, New Zealand White rabbits (2.9 to 3.3 kg) (Elkhorn, Modesto, CA) were quarantined for 5 days prior to study. For iv administration (n = 6), a dosing catheter was placed in the left ear vein and

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a blood collection catheter placed in the right ear artery. After obtaining a predose blood sample, the rabbits received 0.1 mg/kg rhRlx as a rapid bolus into the venous catheter. Twenty-two blood samples (1.0 mL each) were collected from the arterial catheter over the next 12 hr. After each blood sample was withdrawn, the catheter was flushed with normal saline containing 1 U/mL heparin to clear the catheter and replace blood volume. The saline was removed before a sample was collected from the catheter. For intravaginal administration, blood collection catheters were placed in the right ear artery. After the animals were anesthetized with a mixture of ketamine and xylazine, a predose blood sample was collected. Rabbits were placed in a prone position and dosed with either 0.1 or 0.5 mg/kg rhRlx intravaginally (n = 5/dose) via a 5-mm-diameter glass rod that was fully inserted into the vagina. The material was deposited in the proximal vagina adjacent to the cervix based on the length of the glass rod (5 in.). The animals remained prone until they fully recovered from the anesthesia (approximately 1 to 2 hr), then they were returned to individual cages. Twenty-five blood samples (1.0 mL each) were removed from the arterial catheter over a 48-hr period after dosing.

Rhesus Monkeys. Eleven female nonpregnant rhesus monkeys were selected for the study. For iv administration, five animals (3.9 to 6.5 kg) were anesthetized with ketamine, a catheter was placed in the saphenous vein for blood collection, and a second catheter was placed in the cephalic vein for dosing. The animals were chair restrained. When the animals were determined to be alert (i.e., head and eyes followed staff and reaction to visual stimuli), a predose blood sample was collected, and 0.1 mg/kg rhRlx was administered as an iv bolus over 5 sec. Thirty-four blood samples (1.0 ml each) were collected over a 14-hr period following dosing. The animals were removed from their chairs 5 hr after dosing. Blood samples were collected from the saphenous vein up to the 5-hr sample. Blood samples were collected via venipuncture from a leg or a vein in the nondosing arm following the 5-hr sample or when the blood sampling catheter was not patent. For intravaginal administration, six rhesus monkeys (4.4 to 7.1 kg) were anesthetized with ketamine, and a blood collection catheter was placed in the saphenous vein. A predose blood sample was collected, then the perineal area was cleaned using a betadine solution. While the animals were anesthetized and lying in a prone position, the dose (0.1 mg/kg rhRlx) was administered intravaginally via an 18-G catheter that was inserted approximately 3 in. into the proximal vagina. The animals were chair restrained shortly after dosing and allowed to recover from the anesthesia. Twenty-eight blood samples (1.0 mL each) were collected over a 48-hr period after dosing. Blood samples were collected from these animals as described for the iv dose group.

Sample Processing and Assay

The blood samples were allowed to clot at room temperature for a minimum of 30 min. After centrifugation, the serum was separated and stored frozen at −60 to −80°C until analysis. The relaxin immunoreactive protein concentrations were determined in serum using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) specific for rhRlx (8). An affinity-purified goat anti-human relaxin polyclonal antibody was used for coating, and a horseradish peroxidase-conjugated affinity-purified rabbit anti-human relaxin antibody was used for detection. This assay uses rhRlx as a standard calibrator, and not an analogue of relaxin as reported previously (9). Goat IgG (in quantities proportional to the concentration of the goat anti-human relaxin capture antibody) was added to each standard, control, and sample to prevent the nonspecific interactions that are common for assays of this type (10). The interassay precision (%CV) was less than 15% for control preparations at 500, 150, and 45 pg/mL; the within-run precision (%CV) ranged from 5 to 12% for these high, mid, and low controls. The assay range was 20 to 1250 pg/mL.

Pharmacokinetic Analyses

Bi- and triexponential equations were fitted to the immunoreactive serum protein concentration–time data [weight = 1/predicted concentration)^2] from individual animals after iv dosing using nonlinear least-squares regression analysis (NONLIN84, Version 1987, Statistical Consultants, Lexington, KY). The optimal number of exponentials was established using the Akaike criterion (11,12). The coefficients and exponents of the exponential equations were used to calculate the area under the serum concentration versus time curve (AUC), clearance (CL), half-lives ($t_{1/2}$), and initial volume of distribution (V_1) (13), as well as the volume of distribution at steady state (V_{ss}), permanence time in the serum (T_1), mean exit time from the serum (Ω_1), and mean exit time from the body (Ω_{body}) (14), assuming that the system was linear and state-determined. The V_{ss} and Ω_{body} were calculated as minimum (min) and maximum (max) values, because only serum concentration versus time data were available and because no assumptions were made regarding site (compartment) of elimination (14). The ratio $\Omega_{body/max}/\Omega_{body,min}$ is exactly equal to the ratio $V_{ss,max}/V_{ss,min}$; in addition, $\Omega_{body,min}$ is equal to Ω_1, when assuming elimination only from the central compartment.

The area under the serum concentration versus time curve from 0 to 48 hr (AUC_{0-48}) after intravaginal administration was computed using the linear trapezoidal method (15). The fraction of rhRlx absorbed was calculated for each animal as the dose-corrected ratio of AUC_{0-48}/AUC_{iv}, using the AUC_{iv}, where both AUCs were computed over the same interval of time. The formula for computing the 48-hr AUC_{iv} is

$$AUC_{iv} = \sum_{i=1}^{n} \frac{a_i}{\lambda_i}(1 - e^{\lambda_i t})$$

where n is the optimal number of exponentials, a_i are the coefficients and λ_i are the exponents of the mean exponential equation, and time (t) is equal to 48 hr. The serum concentration in a blood sample taken 5 to 30 min prior to treatment, maximum concentration (C_{max}), and time of C_{max} (T_{max}) were the observed values.

RESULTS

The rhRlx serum concentration versus time data for the

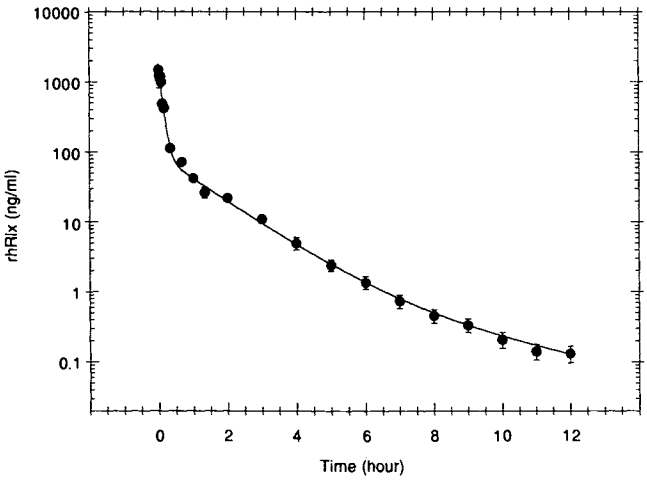

Fig. 1. Mean serum concentration versus time data for rhRlx in nonpregnant rabbits after iv bolus administration of 0.1 mg/kg. Filled circles are the mean ± SD (n = 6). The mean fitted equation is superimposed on the data [$C(t) = 1719e^{-0.17t} + 86e^{-0.013t} + 2.2e^{-0.0040t}$, where C is in ng/mL and t is in min].

Table I. Pharmacokinetic Parameters for rhRlx in Nonpregnant Female Animals After Intravenous Bolus Administration of 0.1 mg/kg (Mean ± SD Unless Noted Otherwise)

Parameter	Rabbits (n = 6)	Rhesus monkeys (n = 5)
Weight (kg)	3.1 ± 0.1	5.2 ± 1.0
V_1/W (mL/kg)	57 ± 9	78 ± 25
$V_{ss,min}/W$ (mL/kg)	240 ± 20	690 ± 220
$V_{ss,max}/W$ (mL/kg)	2000 ± 400	1600 ± 200
CL/W (mL/min/kg)	5.9 ± 0.4	4.1 ± 0.6
$t_{1/2\lambda 1}$ (min)	4.0 ± 0.4 (58% AUC)	2.0 ± 0.5 (13% AUC)
$t_{1/2\lambda 2}$ (min)	54 ± 4 (39% AUC)	24 ± 7 (44% AUC)
$t_{1/2\lambda 3}$ (min)	180 ± 50 (3% AUC)	250 ± 50 (43% AUC)
T_1 (min)	9.6 ± 1.2	19 ± 7
Ω_1 (min)[a]	42 ± 3	180 ± 80
$\Omega_{body,max}$ (min)	350 ± 70	410 ± 70

[a] $\Omega_{body,min}$ is equal to Ω_1, when assuming elimination only from the central compartment.

six rabbits and five rhesus monkeys after iv administration of 0.1 mg/kg were best described by triexponential equations using the Akaike criterion (11,12). The individual coefficients and exponents of the triexponential equations were averaged, and the resulting mean curves were superimposed on the mean data for each species (Figs. 1 and 2). Table I summarizes the mean calculated pharmacokinetic parameters. The CL, V_1, $V_{ss,min}$, and $V_{ss,max}$ were normalized for animal weight (W) and are reported as CL/W, V_1/W, $V_{ss,min}/W$, and $V_{ss,max}/W$, respectively. The measured peak concentrations of rhRlx after iv bolus doses of 0.1 mg/kg in rabbits and rhesus monkeys were 1554 ± 296 and 971 ± 277 ng/mL, respectively.

The serum concentration data following intravaginal administration of rhRlx to rabbits and to rhesus monkeys are shown in Figs. 3 and 4, respectively. Table II summarizes the C_{max}, T_{max}, and fraction absorbed that were observed in

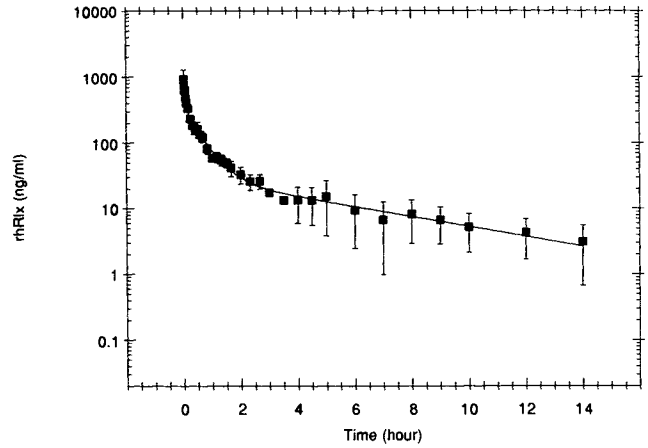

Fig. 2. Mean serum concentration versus time data for rhRlx in nonpregnant rhesus monkeys after iv bolus administration of 0.1 mg/kg. Filled squares are the mean ± SD (n = 5). The mean fitted equation is superimposed on the data [$C(t) = 1106e^{-0.37t} + 330e^{-0.031t} + 30.3e^{-0.0029t}$, where C is in ng/mL and t is in min].

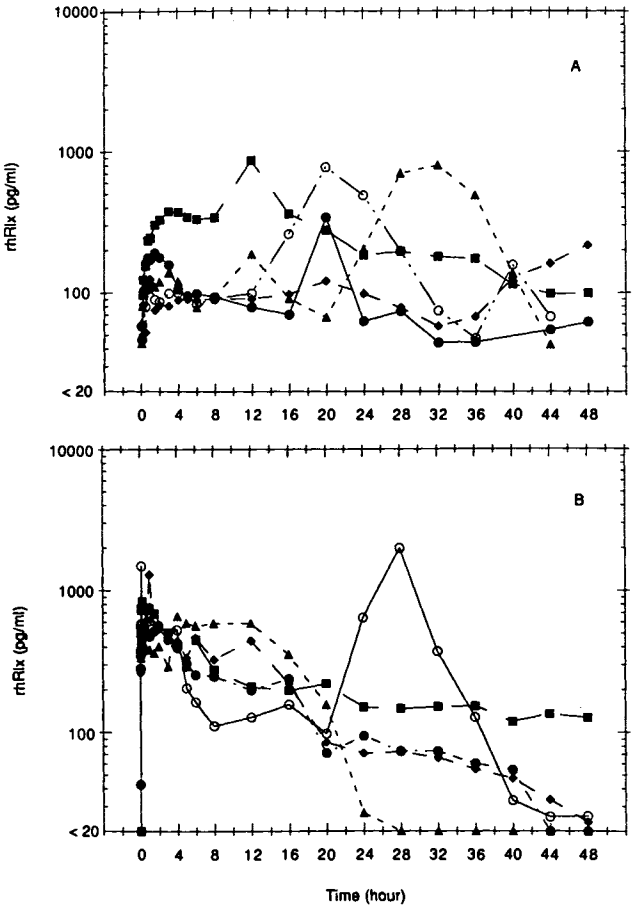

Fig. 3. Individual serum concentration versus time data for rhRlx in nonpregnant rabbits after intravaginal administration. Symbols are the observed data. (A) The rabbits were dosed with 0.1 mg/kg; (B) the rabbits were dosed with 0.5 mg/kg.

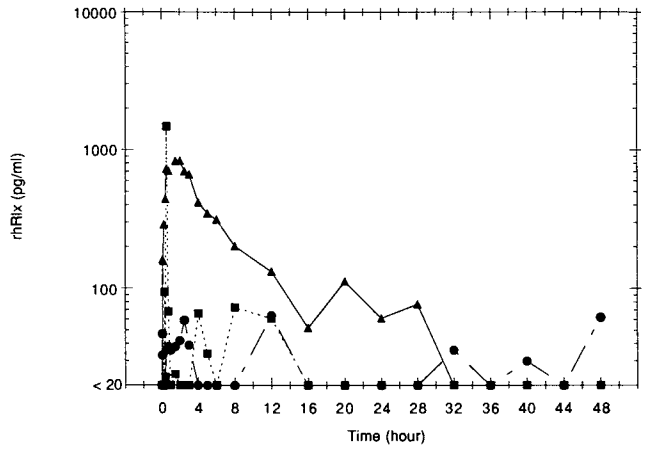

Fig. 4. Individual serum concentration versus time data for rhRlx in nonpregnant rhesus monkeys after intravaginal administration of 0.1 mg/kg. Symbols are the observed data from the three monkeys that had more than one sample with measurable rhRlx serum concentrations.

each species. The absorption in rabbits following administration of 0.1 and 0.5 mg/kg was 3.1 ± 1.4 and 0.7 ± 0.3%, respectively. Two rhesus monkeys had undetectable rhRlx concentrations (<20 pg/mL) throughout the 48-hr sampling interval; one monkey had only one sample containing measurable rhRlx (51 pg/mL) at 24 hr; and the three remaining monkeys absorbed 0.2, 0.5, and 1.4% of the dose.

DISCUSSION

After iv administration, V_1/W was approximately equivalent to the weight-normalized plasma volume (~60 mL/kg) (16) in rabbits and rhesus monkeys; and $V_{ss/min}/W$ was approximately equivalent to the weight-normalized extracellular volume (~200 mL/kg) (16) in rabbits but was larger in rhesus monkeys. Since only the serum was sampled and since no assumptions about the site (compartment) of elimination were made, the ratio $(V_{ss,max}/W)/(V_{ss,min}/W)$ is an indicator of the uncertainty of the amount of drug in the body following chronic administration (14). This uncertainty factor is 8.3 in rabbits and 2.4 in primates. The uncertainty factor in humans (4.8) is within this range (Chen, unpublished data), which is not unexpected given the similarities in distribution volumes across species for biomacromolecules, in general, and for relaxin, in particular (17). Current analytical methodologies for proteins do not allow further resolution of this uncertainty; however, it is expected to be clinically inconsequential, given the acute nature of the therapy and the minimal absorption following intravaginal application of rhRlx.

The permanance time in the serum (T_1) is 9.6 and 19 min in rabbits and rhesus monkeys, respectively. The difference between the mean exit time from the body (Ω_{body}) and T_1 is the time relaxin spends in the tissues before being eliminated; this value ranges from 32 to 340 and from 160 to 410 min in rabbits and rhesus monkeys, respectively.

In a previously reported study, the disposition of rhRlx made by solid phase synthesis was determined in nonpregnant and pregnant rhesus monkeys after iv administration of 0.088 mg/kg (8). All reported pharmacokinetic parameters for the synthetic process material were similar to the pharmacokinetic parameters for the recombinant process material, except $t_{1/2\gamma}$ (139 min) and $V_{ss,min}/W$ (319 ± 34 mL/kg). $V_{ss,max}/W$ was not reported. The differences between the pharmacokinetic parameters of rhRlx in rhesus monkeys in the two studies may reflect normal variation in the estimation of these parameters or subtle differences in the proteins due to the manufacturing techniques (synthetic versus recombinant). Importantly, that study showed that the disposition of synthetic rhRlx was similar in pregnant and nonpregnant animals.

The serum concentration data following intravaginal administration of rhRlx to rabbits and to rhesus monkeys revealed low and variable absorption. In rabbits, the 0.5 mg/kg dose produced higher C_{max} values that seemed to occur earlier after dosing, but resulted in relatively lower estimates for the fraction absorbed than those obtained after the 0.1 mg/kg dose. It should be noted, however, that these conclusions are based on trends in parameters that are highly variable.

Table II. Pharmacokinetic Parameters for rhRlx in Nonpregnant Female Animals After Intravaginal Administration (Mean ± SD Except Where Noted)

Species	Dose (mg/kg)		Weight (kg)	T_{max} (hr)	C_{max} (pg/mL)	Fraction absorbed (%)
Rabbit	0.1	Range	3.0–3.2	6–48	217–862	1.6–4.7
(n = 5)		Mean	3.1	20[a]	600	3.1
		SD	0.1		297	1.4
Rabbit	0.5	Range	2.9–3.3	0.25–28	560–1981	0.5–1.2
(n = 5)		Mean	3.1	1.0[a]	1066	0.7
		SD	0.2		584	0.3
Rhesus monkey						
(n = 6)	0.1	Range	4.4–7.1	2.0–24[b]	51–1475[b]	0.2–1.4[c]

[a] Median.

[b] The range of data from four animals. All the samples collected from two additional animals had concentrations of rhRlx that were below the minimum detectable concentration (20 pg/mL).

[c] The range of data from three animals. All the samples collected from two additional animals had concentrations of rhRlx that were below the minimum detectable concentration (20 pg/mL), and another animal had only one sample collected with a concentration of rhRlx that was within the range of the assay.

The low systemic exposure to rhRlx after intravaginal administration contributed to this variability, and the apparent differences in absorption between doses probably will not result in discernably different systemic effects. Although only the lining of the monkey vagina is histologically similar to that of a woman (T. Terrell, personal communication), the serum concentrations and absorption estimates from both species were similar to the results observed in healthy nonpregnant women follow intravaginal administration of a similar rhRlx gel formulation (A. Perlman, unpublished data).

In conclusion, this study has determined that there is limited permeability of rhRlx through the vagina of nonpregnant rabbits and rhesus monkeys after local administration. Furthermore, both preclinical studies were predictive of the results observed in nonpregnant women.

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