Altered Disposition and Effect of Lerisetron in Rats with Elevated Alpha1-Acid Glycoprotein Levels

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Purpose. To examine the effect of changes in plasma α1-acid glycoprotein (AAG) levels on the pharmacokinetics (PK) and pharmacodynamics (PD) of lerisetron, a novel serotonin 5-HT_3 receptor antagonist, in the rat.

Methods. After subcutaneous administration of turpentine oil, AAG was significantly elevated compared with controls. The PK of unchanged lerisetron (UL; high-performance liquid chromatography with radioactivity monitoring) and total lerisetron (TL; unchanged + changed, scintillation counting) was characterized post intravenous (i.v.) ^{14}C lerisetron (50 μg/kg) in control and turpentine oil pretreated rats. The PK (0 – 180 min) was described by a two-compartmental model. Protein binding of lerisetron *in vitro* was measured using an ultrafiltration technique. The effect of lerisetron (5 μg/kg, i.v.) over 180 min was measured in anesthetized rats (control and pretreated) with the Bezold–Jarisch reflex (inhibition of bradycardia after 16 μg/kg serotonin i.v.) as the endpoint. PD parameters were estimated by sigmoid E_{max} models.

Results. The unbound fraction was significantly diminished in pretreated rats (mean ± SEM) (6.60 ± 1.23% vs. control 14.4 ± 1.40%, $P < 0.05$). Volume of distribution (V) and clearance for UL and TL were significantly decreased when compared to the controls ($P < 0.0001$ for UL and $P < 0.05$ for TL). Plasma clearance based on unbound concentration for UL did not differ between groups but the unbound V and steady-state unbound V remained decreased ($P < 0.05$ and $P < 0.0001$). Pretreated rats showed a significantly diminished drug effect: the area under the E-t curve over 180 min was (mean ± SEM) 5189 ± 657.7 in control animals vs. 3486 ± 464.4 in the pretreated group ($P < 0.05$). The EC_{50} (concentration at half maximum effect) for UL and TL were increased in pretreated rats and were not compensated when the unbound concentration was used.

Conclusions. An increase in AAG causes alterations in the PK and PD of lerisetron, and because this is not compensated with the unbound concentration, we suggest that mechanisms not linked to protein binding may be involved.

KEY WORDS: lerisetron; serotonin; α1-acid glycoprotein; unbound pharmacokinetics; effect alterations; Bezold-Jarisch reflex.

INTRODUCTION

Lerisetron is a novel 5-HT_3 antagonist, a piperazinylbenzimidazole derivative, with high selectivity that is effective in the treatment of nausea and vomiting induced by emetogenic cytotoxic drugs. This compound, like other agents such as tropisetron, ondansetron, and granisetron, possesses high-affinity binding for the 5-HT_3 receptors (Pki = 9.4), as well as a potent ability to inhibit the Von Bezold–Jarisch reflex (1). This reflex is used extensively to investigate the capacity of 5-HT_3 receptor antagonists to inhibit vomiting in the rat (1,2). The relation between doses (1 to 10 μg/kg) and effect (B–J reflex) of lerisetron was also studied in Wistar rats demonstrating the existence of a linear relationship between dose-effect with an ED_{50} value of 2 μg/kg intravenous (i.v.) (1).

Lerisetron is highly bound to serum proteins (both in humans and rats) and exhibits a high volume of distribution (V) and systemic clearance (CL), which is mainly due to metabolism (3). Lerisetron can bind to serum albumin and α1-acid glycoprotein (AAG), but the latter appears to be the major binding protein (4). Serum AAG levels are increased in a number of disease states, including depression, rheumatoid arthritis, severe burns, cancer, and acute inflammation (5).

Several studies have demonstrated that this change in AAG levels leads to a significant increase in drug binding and altered pharmacokinetics (PK) of drugs, e.g., prazosin (6), propranolol (7), lidocaine (8), disopyramide (9), penbutolol (10), mianserine (11,12), methadone (13), and imipramine (14). In most cases, the pharmacologic action is decreased and correlates with the unbound concentration. These results are in accordance with the free drug hypothesis that only the unbound drug is available for transport and interacts with the pharmacological receptors. However, this appears not to be always true because other authors have shown that the drug bound to proteins can also cross some membranes in the body, a behavior known as the "free intermediate hypothesis" (15). This seems to occur when liposoluble drugs and AAG, or lipoproteins, are implicated in the binding process (16). Thus, at present it is unclear whether drug effect, after an increase in drug binding, correlates better with the unbound or the total drug concentration.

On the other hand due to a lack of combined PK/PD studies the distinction between alterations in disposition and drug-receptor interactions has not been made. The consequences in the relation between concentration and effect (PD), have been studied in scarce cases, such as β-adrenoceptor antagonists (7), methadone (13), and prazosin (6). The matter is of interest because it has been reported that AAG may activate or interact with various receptors and lymphocytes in the body (17) and it is therefore apparent that AAG possesses abilities to alter pharmacological response independently of the modifications in binding. So, for example, the α1-adrenergic blocking activity of prazosin *in vitro* was lower in the presence of AAG although the unbound concentration remained unchanged (6,18).

To date, it is unknown whether the PK/PD of lerisetron is altered by elevated AAG serum levels. This protein is substantially elevated in cancer patients where lerisetron is being evaluated for its antiemetic activity.

In the present study, we performed an integrated PK/PD evaluation to characterize the consequences of increased AAG plasma levels in the dosing-to-effect pathway of this drug. Taking into account that AAG also appears to be re-

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sponsible for changes in metabolism, we studied both unchanged lerisetron (UL) and total lerisetron (changed + unchanged, TL).

MATERIALS AND METHODS

Chemicals and Reagents

Lerisetron hydrochloride is a 2-piperazinylbenzimidazole, 7-methoxy derivative synthesized by FAES SA laboratories (Bilbao, Spain). ^{14}C lerisetron hydrochloride (specific activity 28.07 mCi/mM) was obtained from Huntington (UK). Radiochemical purity was over 98%. Chemicals for high-performance liquid chromatography (HPLC) analyses were of at least HPLC grade. Turpentine oil ('purissimum' grade) was obtained from Panreac. All other reagents and solvents were obtained from commercial sources.

Animals

Female Sprague–Dawley rats (n = 53, 180–285 g) were used in the experiments. Animals were maintained under laboratory standard conditions on a 12-h light/dark cycle with light from 8:00 am to 8:00 pm, in a temperature (21–22°C)- and humidity (70%)-controlled room and were acclimated a minimum of 4 days before experiments were performed. Food (standard laboratory rat, mouse and hamster diets, Panlab, Barcelona, Spain) and water were available *ad libitum.* The protocol of the study was approved by the Committee on Animal Experimentation of the University of the Basque. The day before the experiment, animals were fasted overnight. During the experiments, the animals' body temperature was monitored using a rectal thermometer probe and maintained at 37°C by placing the animal on a temperature-regulated heating pad.

Two distinct sets of rats were used for concentration and effect measurements since the doses for each study had to be different. Kinetic studies were performed at a dose of 50 μg/kg because it is among the range of doses that is being used in human studies. This dose cannot be used for effect measurement because the Bezold–Jarisch surrogate effect is obtained at much lower doses, so a dose of 5 μg/kg had to be used, which produces nonquantifiable concentrations. Animals were divided in two groups: control and turpentine-oil (pretreated). The former received 0.5 ml of saline and the latter an injection of 0.5 ml of turpentine oil subcutaneously (s.c.) and 48 h before the experiments.

Protein Levels

Albumin and AAG levels were measured in control and pretreated rats. One-hundred microliters of plasma were used to evaluate AAG levels based on a fluorimetric determination method of AAG in plasma with quinaldine red (19). Albumin reagent BCG (bromcresol green) was used for the quantitative, colorimetric determination of albumin in 10 μl of plasma at 628 nm. This procedure is a modification of the method of Doumas.

Pharmacokinetic Studies

To characterize the PK disposition of lerisetron in control and pretreated rats (n = 24), a 50 μg/kg dose of ^{14}C-Lerisetron was used, which was related to therapeutic dose in human volunteers (data from FAES)

The day of the study (48 h after turpentine oil administration) and before drug administration, the animals were anesthetized with urethane (1.25 g/kg i.p.). Jugular vein catheters (polyethylene catheters 0.3 mm i.d., 10 cm length, Vygon, France) for drug administration and intra-arterial carotid catheters (0.5 mm i.d., 10 cm length) for blood sample withdrawal were implanted, respectively. After a surgical incision in the throat, a polyethylene tube was introduced and fixed into the trachea to help ventilation in the spontaneously breathing animal. Then two different groups of rats, control (n = 14: 5 for UL measurement and 9 for TL) and pretreated (n = 10, 4 for UL measurement and 6 for TL) animals received a single 50 μg/kg bolus i.v. dose of ^{14}C-lerisetron via the jugular vein cannula. The drug was administered at the same time of the day to eliminate circadian variations in the PK parameters. Blood samples were collected from the arterial cannula immediately predose and at selected intervals: 1, 3, 5, 15, 30, 45, 60, 90, 120, 150, and 180 min postdose and placed into heparinized tubes. Different volumes of blood were drawn (210 μl or 100 μl) depending on if UL or TL was going to be measured, respectively. Blood was replaced with an equal volume of saline. The maximum total amount of blood withdrawn was 2 ml per rat. Plasma was separated by centrifugation and all samples were stored at –20°C until analysis for UL and TL.

Drug Assay

Analysis of UL

To 0.1 ml of heparinized plasma, obtained from five control rats and four pretreated rats, 10 μl of internal standard (Naftol, 9 mg/ml) were added. After stirring for 10 s, 150 μl of acetonitrile was added to precipitate proteins. The samples were mechanically shaken for 2 min and then centrifuged for 20 min at 2000 g at 4°C. After centrifugation, a 200-μl aliquot was analyzed for lerisetron content by HPLC, using a Kontron chromatograph on a Lichrosper 60 RP Select B column (Merck), 25 × 4 mm and with a 5-μm particle size. The mobile phase was a mixture of solution A (triethylamine 0.01 M; pH adjusted to 2.5 with phosphoric acid) and solution B (acetonitrile) using a proportion, which varied throughout the chromatogram in a manner so that, at the beginning, the mobile phase consisted of 20% of solution B and 80% solution A. At minute 13, the column was 100% solution B and ended at minute 20 with the initial proportions. The flux was of 1 ml/min.

Two different detectors were used and disposed serially. One was a radioactivity monitor to measure lerisetron and the other was a fluorimetric device to measure the internal standard. The retention time was 6 min for lerisetron and 12 min for Naftol. This analytical procedure (for a 100-μl sample) was sensitive to 6.5 ng/ml for UL and linear over the range from 6.5 ng/ml to 250 ng/ml. The mean intra and interday coefficients of variation of the assay were 13.4% (at 25 ng/ml), 3.8% (at 100 ng/ml), 5.9% (at 250 ng/ml), and 4.6% (at 25 ng/ml), 6.68% (at 100 ng/ml), 1.9% (at 250 ng/ml), respectively (n = 3 per concentration).

Analysis of TL

Aliquots of plasma (40 μl) from rats (n = 6) in the pretreated group and rats (n = 9) in the control group were placed in vials to which scintillation counting liquid (10 ml) of (Cocktail Biogreen 1) was added. The radioactivity was counted by a Packard Tricarb model 2200 CA. The limit of detection of the assay was 0.5 ng/ml for 40 μl and the within and between-day coefficients of variation were less than 10%.

Protein Binding *In Vitro* Studies

^{14}C-lerisetron (^{14}C-L) (10 μL) was added to 0.99 ml of plasma from control rats (n = 5) and pretreated rats (n = 5) to obtain a final concentration of 100 ng/ml (C_T), which was within the range (5–200 ng/ml) of lerisetron in rats after 50 μg/kg i.v. Samples were mixed and incubated at 37°C for 10 min. Aliquots of all samples (1 ml) were then transferred to Amicon Micropartition Units (MPS-1). The devices contain a membrane filter of controlled porosity with a cut-off molecular weight of 10,000 Daltons that retains plasma protein and allows free drug in solution to pass through. The MPS-1 were centrifuged at 3000 r.p.m. for 8 min at 37°C. This procedure is considered as a reliable and relatively easy system to use for separating protein-free from protein-bound ligand for several drug families (14). The free concentration obtained as ultrafiltrate (C_u) was measured by scintillation spectrophotometry (Packard Tricarb). Samples (100 ml) were mixed with 10 ml of scintillation liquid (Cocktail Biogreen 1) and radioactivity (d.p.m.) was counted in the spectrometer for 5 min. With each series of samples, vials containing aliquots of the ultrafiltrate and known quantities of the labeled compound in the range 0.5 to 100 ng/ml, were used as standards. The limit of detection of the assay was 0.5 ng/ml (for a 40-μl sample). No binding of lerisetron to the membrane was observed.

The per cent fraction of unbound lerisetron (fu) was determined as

$$\%\,\text{Unbound} = \frac{C_u}{C_T} \times 100$$

This concerns UL only because binding determination is done *in vitro* where no metabolite is present.

Effect measurement: The von Bezold-Jarisch reflex

We determined the mode of action of lerisetron by evaluating the ability to block serotonin-induced bradycardia in anesthetized rats during 180 min. This method has been broadly used to characterize pharmacologic profiles *in vivo* of drugs in this family (2,3).

A different set of female Sprague rats (n = 29) from our facilities were used according to the requirements of the European Convention for the protection of vertebrate animals used for research and other scientific purposes. Animals were allocated randomly to two groups (control, n = 15 and pretreated, n = 14) and fasted overnight before the experiment. At the time of the experiment (48 h after turpentine oil or saline subcutaneous administration), rats were anesthetized with urethane (1 g/kg i.p.) (Aldrich Chemie, Steinheim, Germany). After a surgical incision in the throat, a polyethylene tube was introduced and fixed into the trachea to help ventilation in the spontaneously breathing animal. A polyethylene catheter (PE50), filled with heparinized saline (50 I.U. heparin/ml physiological saline solution) (Rovi, Barcelona, Spain), was placed into the right carotid artery and connected to a pressure transducer (Abbot Critical Care, Ireland) and coupled to a MacLab/4e system. Arterial blood pressure and heart rate were monitored using a computer (Macintosh Performa 5200) and the complete experiment recorded in diskette for further analysis and evaluation. Another polyethylene catheter (PE10) was placed and fixed into the right jugular vein to perform drug administration. A heated pad was used to maintain the animal rectal temperature at 37°C throughout the experiment.

In preliminary experiments, we studied the dose–response relationship for serotonin-induced bradycardia (as part of the von Bezold–Jarisch reflex) over the range 4–128 μg/kg i.v. administered at 10- to 15-min intervals. A dose of 16 μg/kg i.v. (n = 4) of 5-HT resulted in a submaximal reproducible response over a 4-h period when administered at the above intervals so this dose of 5-HT was used throughout the present study for induction of the von Bezold–Jarisch reflex.

After completion of the catheter placement, animals were allowed to stabilize during a 30-min period. This was followed by a challenge with 5-HT, which was repeated three times at 10-min intervals to establish the control bradycardic response as the index (basal heart rate – 5-HT rate)/(basal heart rate). Then, lerisetron was administered via i.v. bolus injection (5 μg/kg) and the degree of inhibition of the bradycardic response to 5-HT (expressed as % with respect to the control response) was checked at 5, 15, 30, 45, 60, 90, 120, 150, and 180 min afterwards.

Data Analysis

Plasma lerisetron (UL and TL) concentration vs. time profiles for each rat were analyzed using a nonlinear regression package (WINNONLN 1.5, Pharsight, Palo Alto, CA, USA). Model selection was based on the Akaike Information Criterion (AIC) and analysis of the weighted residuals, as well as the correlation parameters (r and r^2) between predicted and observed concentrations. The following two-compartmental model optimally fitted individual concentration vs time data from each group,

$$Cp(t) = A\exp(-\alpha t) + B\exp(-\beta t) \qquad (1)$$

where $C_p(t)$ is the plasma concentration at time t, α is the distribution rate constant, β is the terminal elimination rate constant, and A, B are intercept parameters. The intercompartmental mass transfer rates k_{12}, k_{21}, elimination rate k_{10}, as well as CL, volume of distribution at steady state (Vss), and elimination half life ($t_{1/2}\beta$) are also estimated. Area under the plasma concentration-time curve from time zero to the last sample time point and then to infinity ($AUC_{0-\infty}$) was calculated with the trapezoidal rule and then by mono compartmental extrapolation to infinite time.

The parameters obtained by fitting the model to data were then used to simulate the concentrations at the times and doses of effects after a 5 μg/kg i.v. dose of lerisetron (which produces nonquantifiable concentrations) in a different group of rats. The linearity had been previously verified (3).

Unbound concentration profiles were obtained by multiplying plasma UL concentrations by the unbound fraction (fu), in both the pretreated and control groups. PK param-

eters of unbound lerisetron were estimated from fits to these concentrations using the model of Eq. 1. Previous binding studies (4) have shown that the per cent unbound lerisetron was independent of lerisetron concentration ranging from 50 ng/ml to 2 μg/ml.

The effect, bradycardia reduction, was related to simulated plasma UL and TL concentrations by the sigmoid E_{max} relation

$$E^* = E_{max} \cdot \frac{Cp^{s\gamma}}{EC_{50}{}^{\gamma} + Cp^{s\gamma}} \quad (2)$$

where E is the model effect, E_{max} the maximum effect (fixed to 100%), Cp^5 is the simulated concentration at the time of the response observation, EC_{50} is the concentration in plasma at half the maximum effect, and γ (the Hill exponent) is related to the number of binding sites per receptor molecule for the drug and results in the sigmoidicity of the curve. The same model fitting package was used to obtain the PD parameters EC_{50} and γ for UL and TL. Assessment of goodness-of-fit was based on AIC, weighted residual plots, and the standard error of the parameter estimates.

Statistical Analysis

The compartmental PK and PD parameter estimates of UL and TL were compared statistically by the unpaired student's *t*-test. All averaged data are reported as mean ± standard error of the mean (SEM) in the tables and plotted as mean ± SEM. PD parameters are reported as mean with per cent coefficient of variation (%CV) obtained as 100 × SEM/Mean Estimate.

RESULTS

All animals in the pretreated group showed an inflammatory reaction in the leg produced by s.c. injection of turpentine oil. The AAG levels in this group were significantly increased when compared to the control (mean ± SEM) (1.75 ± 0.09 g/l vs. 0.60 ± 0.04 g/l, $P < 0.05$). No changes in albumin levels were observed between groups. The unbound fu of lerisetron *in vitro* was significantly decreased in the preteated group compared to controls (6.6 ± 1.23 % vs. 14.4 ± 1.4%, respectively, $P < 0.05$).

Pharmacokinetics

Plasma concentrations of UL and TL vs. time were best described, in both control and pretreated animals, by a two-compartment model. The model fits for UL concentrations in both groups, control and turpentine, are shown in Fig. 1A and estimated parameters are in Table I. UL plasma concentrations were significantly higher in pretreated rats compared to the controls up to 60 min, and globally in the $AUC_{0-\infty}$ ($P < 0.05$). V, Vss, and CL were significantly decreased in pretreated animals ($P < 0.0001$, $P < 0.05$, $P < 0.0001$, respectively). Mean $t_{1/2}\beta$ was also reduced, but not significantly, in the pretreated group (mean ± SEM) (30.2 ± 3.7 min and 19.2 ± 3 min in control and pretreated rats, respectively). The unbound parameters for UL (Table II) show that CL did not differ between control and pretreated groups. However, V unbound and Vss unbound remained significantly decreased in the pretreated group ($P < 0.0001$, $P < 0.05$).

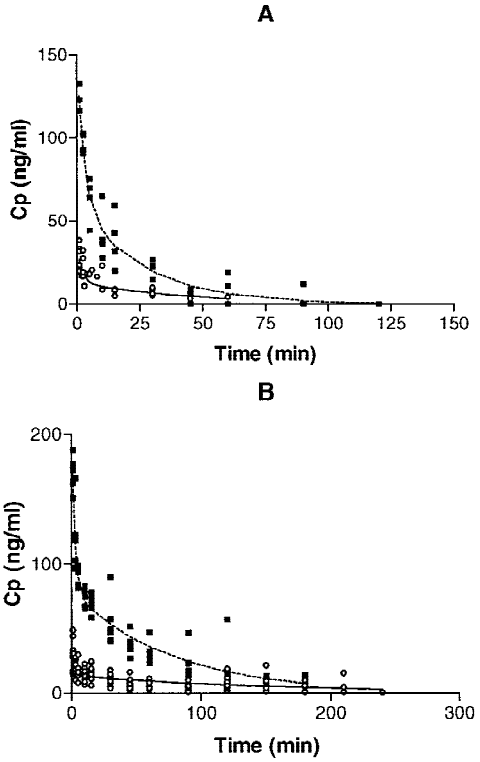

Fig. 1. Observed plasma concentrations (Cp) vs. time of unchanged lerisetron in control rats, (open circles, n = 5), and turpentine-pretreated rats (filled squares, n = 4) (A) and total lerisetron in control rats (n = 6) and turpentine-pretreated rats (n = 9) (B), obtained after i.v. bolus of ^{14}C-Lerisetron (50 μg/kg. Also, bi-compartmental model predictions for the control group (solid line) and for the pretreated group (dashed line).

Figure 1B also shows the mean PK model fit for TL after 50 μg/kg ^{14}C lerisetron i.v. in the control and pretreated rats with levels of the latter visibly above the controls. The mean PK parameter estimates and errors are listed in Table I. A significant increase in the $AUC_{0-\infty}$ ($P < 0.05$) was observed in the pretreated group with a significant reduction in V, Vss and CL ($P < 0.05$, $P < 0.05$, $P < 0.05$). No significant difference was observed in $t_{1/2}\beta$. (mean ± SEM) (123.0 ± 30.0 min and 52.6 ± 5.10 min for control and pretreated, respectively.)

Although the PK parameters for TL were different from those for UL, the

$$\frac{\text{AUC} - \text{control}}{\text{AUC} - \text{pretreatd}}$$

ratios for TL and UL were not significantly different, (mean ± SEM) 0.41 ± 0.06 and 0.39 ± 0.03, respectively ($P > 0.05$).

In Vivo Drug Effect

The temporal evolution of effect observations after 5 μg/kg lerisetron i.v. to control and pretreated rats are shown in Fig. 2. The figure depicts the average bradycardia reduction per cent (with SEM) for all animals. Five minutes after lerisetron i.v., reduction of bradycardia in controls was 83.5% (64–100%). However, pretreated animals showed only a 60.8% reduction of bradycardia being the range from 32.0% to 95.4%. The differences were statistically significant at 5 min ($P < 0.05$), 15 min ($P < 0.0001$), 30 min ($P < 0.05$), 45 min

Table I. Pharmacokinetic Parameters and Standard Error of the Estimate (mean ± SEE) in Control and Turpentine-Pretreated Rats of Unchanged Lerisetron and Total Lerisetron

	Unchanged lerisetron		Total lerisetron	
Pharmacokinetic parameters	Control (n = 5)	Pretreated (n = 4)	Control (n = 9)	Pretreated (n = 6)
V (l)	0.30 ± 0.02	0.080 ± 0.002[b]	0.33 ± 0.06	0.060 ± 0.003[a]
k_{10} (min^{-1})	0.060 ± 0.005	0.089 ± 0.008[a]	0.020 ± 0.006	0.040 ± 0.005[a]
k_{12} (min^{-1})	0.20 ± 0.08	0.180 ± 0.008	0.42 ± 0.12	0.24 ± 0.06
k_{21} (min^{-1})	0.15 ± 0.07	0.22 ± 0.05	0.22 ± 0.06	0.150 ± 0.033
$AUC_{0-\infty}$ (μg.min/l)	642 ± 55.0	1926 ± 299.0[a]	2414 ± 580.3	5756 ± 506.0[a]
CL (1/min)	0.017 ± 0.001	0.007 ± 0.001[b]	0.008 ± 0.002	0.0021 ± 0.0002[a]
Vss (1)	0.70 ± 0.08	0.150 ± 0.009[a]	0.97 ± 0.17	0.144 ± 0.003[a]

[a] Significantly different from control ($P < 0.05$)
[b] Significantly different from control ($P < 0.0001$)

($P < 0.05$), and 60 min ($P < 0.05$). After this time, the reduction of bradycardia was parallel in both groups except for the 180-min point, where a significant difference between groups (mean ± SEM) (11.6 ± 3.6% in C and 2.0 ± 0.9% in the pretreated group) was also observed ($P < 0.05$). We had verified previously that turpentine pretreatment does not affect the response to 5-HT. Mean per cent bradycardia after serotonin in control rats was 55.2 ± 14.4% (n = 14) and for pretreated rats 56.5 ± 11.3% (n = 15).

Considering the total evolution of the effect, there is a significant difference ($P < 0.05$) in the AUE (0–180 min) curve between control and pretreated rats (mean ± SEM) (5189.3 ± 657.7 and 3486 ± 464.4, respectively).

Pharmacodynamics

The PK parameters of TL and UL after i.v. 50 μg/kg of ^{14}C-lerisetron were used to simulate plasma concentrations at the time points and dose of the effect measured (5 μg/kg). Plasma concentrations of both forms of the drug were directly related to the effect and the relationship between UL or TL and effect was successfully described by the sigmoid E_{max} model. The estimated PD parameters are summarized in Table III.

Figure 3A shows the best fit for the model when plasma UL concentrations vs effect were used. The curve for pretreated rats is shifted to the right indicating that a higher concentration is needed for this group to reach the same effect as the control. The PD curve for TL (Fig. 3B) with pretreated rats is also shifted to the right, implying that a higher concentration is necessary in this group also in order to produce the same effect as in the control.

The relationship between effect and unbound concentration of UL in the two groups is shown in Fig. 4. The unbound concentrations do not relate as expected with the drug effect, so EC_{50} unbound remains different between the control and pretreated groups (Table III).

Table II. Unbound Pharmacokinetic Parameters and Standard Error of the Estimate (mean ± SEE) of Unchanged Lerisetron in Control and Turpentine-Pretreated Rats

Unbound pharmacokinetic parameters	Control (n = 5)	Pretreated (n = 4)
V_u (l)	2.14 ± 0.006	1.25 ± 0.2[a]
CL_u(l/min)	0.12 ± 0.007	0.11 ± 0.007
Vss_u (l)	4.99 ± 0.07	2.33 ± 0.29[b]

[a] Significantly different from controls ($P < 0.05$).
[b] Significantly different from controls ($P < 0.0001$).

DISCUSSION

In this study, explored the changes in plasma disposition kinetics and the effect of lerisetron in rats with elevated AAG levels and protein binding. An integrated PK/PD study was performed for two forms of drug in plasma: total concentration (TL = changed + unchanged) and UL that had been shown to display different PK characteristics (3). The unbound concentrations were also obtained by multiplying total concentration of UL by the fu.

The method used here to induce the increase in AAG has been employed in previous research (20–22). The idea is to create an experimental model where presumably the only change is the increase in AAG levels and where the importance of the changes in binding of basic drugs can be studied. Nevertheless, there is controversy regarding whether the inflammation or the raised. AAG levels can produce changes in the PK and the effect of drugs by mechanisms not related to protein binding.

For example, the presence of an inflammatory reaction was seen to decrease the total CL of tolbutamide (23) and Kobusch *et al.* (24) found that the acetylation of sulphametazine was not altered in turpentine treated rabbits. Holla-

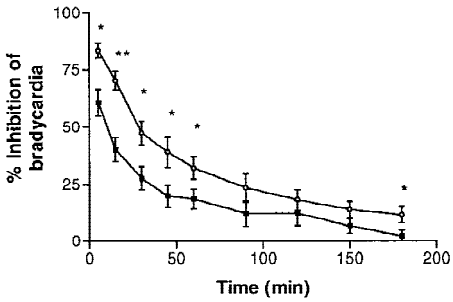

Fig. 2. Mean (± SEM) observed lerisetron effect (% inhibition of serotonin induced bradycardia) vs. time in control (open circles, n = 15), and turpentine-pretreated rats (filled squares, n = 14) after 5 μg/kg lerisetron i.v. Points are simply connected. *Significantly different from controls ($P < 0.05$). **Significantly different from controls ($P < 0.0001$).

Table III. Pharmacodynamic Parameters Estimates and Percent Coefficient of Variation (%CV = 100 × SEE/Mean) of Unchanged Lerisetron and Total Lerisetron in Control and Turpentine-Pretreated Rats[a]

	EC_{50} (ng/ml)	γ	EC_{50u} (ng/ml)
Unchanged lerisetron			
Control (n = 14)	0.44 (5.9%)	2 (12.2%)	0.064 (15.6%)
Pretreated (n = 15)	4.2 (9.5%)	1.1 (14.5%)	0.28 (10.7%)
Total lerisetron			
Control (n = 14)	0.9 (4.4%)	2.3 (8.7%)	—
Pretreated (n = 15)	7.4 (5.4%)	2.1 (12.4%)	—

[a] Unbound EC_{50} is also shown for UL.

day *et al.* (25) investigating the effects of elevated serum AAG levels (in transgenic mice) on the PK disposition of fluoxetine and its antidepressant activity observed changes in the PK and a decrease in the effect without changes in the unbound drug fu. These conflicting results may complicate the interpretation of observations for other drugs, especially when an integrated PK/PD is not performed and only specific changes in pharmacologic response are evaluated.

In this work we found elevated levels of both TL and UL in the pretreated group (Fig. 1) and saw that the presence of a high concentration of AAG in pretreated rats produced a significant decrease in the activity of lerisetron after i.v. administration (Fig. 2). Thus, the pharmacologic effect doesn't correlate with the total plasma concentration and if we represent plasma concentration vs effect of all rats (control and pretreated), a hysteresis is observed (figure not shown). So, we analyzed the PK/PD in both groups separately and afterwards we compared the parameter estimates. The unbound parameters were also calculated and compared between groups.

Pharmacokinetics

Both CL and distribution volume were decreased, but there was a higher decrease in the latter, and this fact may be responsible for the (non significant) reduction in $t_{1/2}\beta$. As a result of the PK changes, we have seen high levels of total lerisetron (bound + unbound) in plasma, both for TL and UL.

The reduction found in volume of distribution (V and V_{ss}) agrees with the literature on other drugs with similar PK characteristics (13,26). Lerisetron is an extensively distributed drug (V = 0.3 L), which allows *a priori* prediction of a proportional change in volume due to the change in fu in plasma (27). Indeed, we have found that V and Vss are significantly decreased in the pretreated rats for the two forms UL and TL. However, when correcting with the fu, the unbound Vss and V remain decreased, which suggests that the presence of AAG could alter the intrinsic drug distribution (i.e., tissue binding) of lerisetron regardless of binding to plasma protein. This has also been observed for some other drugs (10) and could be linked to the liposolubility properties of the agent (15,16).

Nevertheless, it is not clear what are the consequences for the half-life and CL, of changes in protein binding. Generally, drugs with a low or intermediate CL, would have this parameter correlate positively with the free fraction (13,27). The half-life increases or remains unchanged depending on what occurs to the volume. High extraction ratio drugs, like

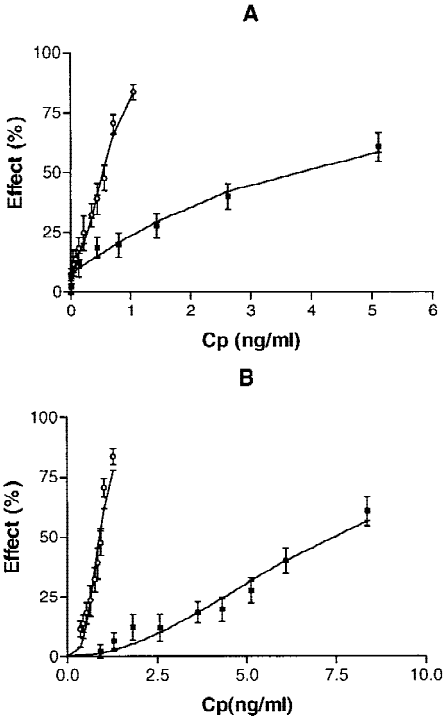

Fig. 3. Observed drug effect (mean ± SEM per cent inhibition of serotonin-induced bradycardia) vs. simulated plasma concentrations (Cp) for unchanged lerisetron (A) in control (open circles, n = 15) and pretreated rats (filled squares, n = 14), and for total lerisetron (B), after 5 μg/kg lerisetron i.v. The lines represent the best model fits.

imipramine (14), lidocaine (8), or disopyramide (9) show no alteration in CL with changes in the bound fraction. Curiously however, the systemic CL of some other highly extracted drugs like propranolol (7), has been reported to be significantly reduced.

Changes in fu should not have affected the total plasma CL of lerisetron because it is considered a high extraction drug (high CL). But, as in the case of propranolol, we have observed a decrease in CL for both forms of the drug (UL and TL). A possible explanation for this is that the elimination of drugs can be affected by plasma proteins themselves. In this sense, results from several studies have suggested a decrease

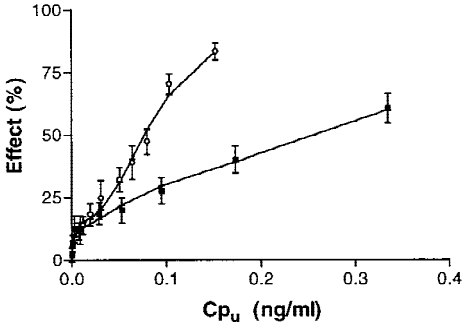

Fig. 4. Observed drug effect (mean ± SEM per cent inhibition of serotonin induced bradycardia) vs. simulated plasma unchanged lerisetron unbound (free) concentrations (Cp_u) in control (open circles, n = 15) and pretreated rats (filled squares, n = 14) after i.v. administration of lerisetron (5 μg/kg). The model fits are drawn as solid lines.

in the intrinsic CL of drugs in situations in which the AAG levels are increased (28), even though there are no changes in the fu, as it happens with fluoxetine (25). On the other hand, AAG is known to decrease the CL of unbound prazosin without altering its half-life (29) and this is attributed to accumulation or alteration of the active metabolites, in the presence of AAG. However, in our study the CL was corrected when transformed into unbound CL, (unbound CL was the same for both control and pretreated groups). Therefore, the change in total plasma CL is related to changes in the fu (unbound fraction) suggesting that this drug has a PK behavior similar to intermediate-sized CL agents. It should be noted that lerisetron binds to erythrocytes in the rat *in vivo* (fu = 45.7 ± 5.86%, experimental data not shown) and therefore blood CL (smaller than plasma CL) would be smaller than the liver blood flow in the rat (60 ml/min/kg). Hence lerisetron could be considered an intermediate extraction drug. The binding to erythrocytes was decreased in the treated group (fu = 82.8 ± 5.4%; $P < 0.05$) due to higher protein binding in plasma. This could explain the lower plasma CL value observed in pretreated rats (decreased fu and CL vs the control group). The half-life decreased, because CL decreased less than V.

Another interesting outcome in this study is that there are parallel results for UL and TL, and the ratio

$$\frac{\text{AUC} - \text{control}}{\text{AUC} - \text{pretreatd}}$$

is the same for both UL and TL. Thus, although PK parameters of UL are different from those of TL, indicating the existence of at least one metabolite (hydroxylated OH according to preliminary data), there is strong evidence that changes in fu do not affect the metabolic behavior of the drug.

Pharmacodynamics

On the basis of our results regarding the decrease in the extent of drug distribution, we could suggest that the observed, reduced, effect could be caused by kinetic modifications. Nevertheless, the lack of relation between total, and unbound, plasma concentrations and effect required an analysis of eventual alterations in the PD process, hence PD modeling.

A sigmoid E_{max} model optimally fit the values in both control and pretreated groups. In this latter group the value of EC_{50} was significantly higher than in controls (both for UL and TL), suggesting that in the pretreated group a higher concentration of total lerisetron (bound + unbound) is needed to produce the same effect. Although preliminary data show that the OH metabolite has a similar pharmacologic activity as the parent drug, we could discard its influence on the observed PD changes because the metabolite concentrations are an order of magnitude less than those of the parent drug. Additionally, the unbound EC_{50} remained increased in the pretreated group.

The consequences in PD of an increase in AAG have been studied in very few cases i.e., propranolol (30) penbutolol (10), disopyramide (9), and methadone (13). In these studies, the pharmacologic response is better correlated with the unbound concentration in plasma than with the total, so in most cases a change in EC_{50} with no change in $EC_{u,50}$ has been observed. However, AAG can apparently alter pharmacological activity beyond processes related to drug-protein binding, via changes in the drug-receptor interaction, as in the case of prazosin (6) or changes in distribution to the effect site, e.g., fluoxetine (26). These two agents showed lack of correlation between plasma unbound drug concentration and effect.

Considering that the pharmacological effect is related to unbound rather than total drug concentration one could expect a significant shift in the effect-total concentration relationship but not in the effect-unbound concentration relationship. In general, no change in E_{max} should be observed. However, in the case of prazosin and imipramine (8,16) at equal unbound concentration, the PD parameters were different in the presence of high AAG levels in plasma, as in our case, and the implicated mechanism has not been completely explained. For example, for prazosin (8) it was suggested that there may be an interaction of AAG with the receptors, which would prevent the drug from blocking the effect of α-1 adrenergic agonists and thus explain the alteration in its intrinsic antagonistic activity. In the present study we could suggest an interaction with the 5-HT_3 receptor site.

Overall in this study, elevated serum AAG levels, and subsequently increased protein binding, resulted in a significant reduction in the pharmacological activity of lerisetron as well as in the PK parameters (CL and V) of the drug. The activity did not correlate with the total or unbound plasma concentrations, suggesting that AAG possess properties that may modify the pharmacological response by mechanisms that go beyond protein binding changes.

Further studies should be carried out in search of these mechanisms, but meanwhile and according to the results of this work, a decreased effect of lerisetron should be expected in patients showing high AAG levels, e.g., cancer patients.

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