

POPULATION PHARMACOKINETIC STUDY OF [D-TRP⁶]-LHRH AGONIST ANALOG AFTER SINGLE INTRAVENOUS AND INTRAMUSCULAR INJECTIONS IN RATS.

H. Colom¹, C. Peraire¹, A. Casanovas¹, Ll. Campmany², D. Martinez², JM. Cendros², R. Obach², J. Domenech¹

¹ Biopharmaceutics and pharmacokinetics Department, Faculty of Pharmacy, University of Barcelona. Avda Joan XXIII s/n. 08028 Barcelona. Spain

² Ipsen Pharma S.A. Ctra. Laureà Miró. 396, 08980 Sant Feliu de Llobregat Barcelona. Spain.

Abstract

A population pharmacokinetic analysis was performed after single i.v. (25 µg/kg) and i.m. (10, 50 and 100 µg/kg) injections in rats of triptorelin, a LHRH agonist analogue with increased affinity for the pituitary receptor with respect to the natural hormone. A sparse sampling strategy was applied. Blood samples were obtained up to 720 min post-i.v. administration and up to 1440 min post-i.m. administration. Triptorelin plasma levels were assayed using a validated radioimmunoassay method (RIA). The non linear mixed effects modeling using the NONMEM ver. V program was used for the data analysis. The two open compartment first order input (ADVAN4) was the best pharmacokinetic structural model. Intraindividual and measurement/analytical residual error was best described by a model with both additive and proportional components and the interindividual variability for K_a was best described by the exponential error model. The plasma clearance (CL), apparent central (V_c) and peripheral compartment (V_{p1}) distribution volumes, intercompartmental clearance (CL_{p1}) and absorption rate (K_a) values were 261 ml/h, 38.8 ml, 122.0 ml, 276.0 ml/h, 2.79 h⁻¹, respectively. The intramuscular bioavailability was complete. The interindividual variability for K_a was of 40.80% (expressed as coefficient of variation %), the residual variability resulted in 36.0% (expressed as coefficient of variation %) and 0.0318 ng/ml for the proportional and additive components, respectively.

Introduction

Triptorelin, is a LHRH agonist analogue with increased resistance to enzymatic degradation and affinity for the pituitary receptor. It causes a down-regulation of GnRH receptor number and a post-receptor desensitization of the gonadotropic cell resulting in reversible biochemical castration. Experimental animal and clinical studies have proved its efficacy in hormone-dependent disorders as prostate cancer, endometriosis, among others. No pharmacokinetic data have been previously reported after single doses in rats. The objective of this study was to find a population PK model after i.v. and i.m. administration of triptorelin in aqueous solution in rats.

Material and Methods

Compound

Triptorelin acetate was purchased from Ipsen-Pharma and administered as an aqueous solution both intravenously at the dose of 25 µg/kg and intramuscularly at the doses of 10, 50 and 100 µg/kg (expressed as pure peptide).

Animals

Male Sprague-Dawley rats weighing 275-300 g were used. The animals were acclimatized at a temperature of 20-22°C and a relative humidity of 50% under natural light/dark conditions for a minimum of one week before the beginning of the study.

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Design

A sparse sampling design was applied. Triptorelin was administered to groups of eight rats/route/dose.

Blood samples were obtained by cardiac puncture and collected in tubes containing EDTA and aprotinin to prevent any enzymatic degradation, then centrifuged and stored at -20°C until the analysis day. The sampling times were before and at 1, 3, 5, 15, 30, 60, 90, 120, 150, 180, 240, 360, 480, 600 and 720 min post-i.v. injection, and at 3, 5, 15, 30, 60, 90, 120, 150, 180, 240, 360, 480, 600, 720 and 1440 min post-i.m. injection.

Analytical determination

Triptorelin plasma levels were determined by a specific and previously validated radioimmunoassay method. Polyclonal Triptorelin antiserum from rabbit was incubated with rat plasma and RIA buffer during 24 hours and then the tracer ^{125}I -triptorelin added. After 24 hours of incubation, the separation of free and antibody bound triptorelin was achieved after precipitation with 1-propanol. Each sample was analysed by triplicate (curve standards) or duplicate (Quality control samples and rat samples). The lower limit of quantitation was 15.625 pg/ml. The acceptance criteria for each RIA batch was that 4 of 6 QC standards (70, 45 and 25 pg/ml) had to show an accuracy $<15\%$ (CV%) and the 2 outliers should not be of the same concentration. Validation for measurement in rat plasma resulted in accuracy (relative error) and precision (CV%) $<15\%$.

Population pharmacokinetic analysis

Non linear mixed effects modeling using the NONMEM ver. V program was used for the data analysis. The first order conditional estimation method was used in all the analyses. Model selection was based on the objective function value from the NONMEM output and graphical analysis using the Xpose ver 2.0 program. A drop of the objective function value of 3.84 between two nested models ($p < 0.05$) was considered as statistically significant.

Open models of one, two and three compartments with first order absorption were fitted to the data (ADVAN2, ADVAN4 and ADVAN12). The population values of the main pharmacokinetic parameters (clearance, distribution volumen and absorption rate) were estimated. The models with and without bioavailability as fixed parameter were assayed in order to know if a significative drop in the objective function occurred after inclusion of this parameter in the model. The additive and exponential models were assayed to describe the interindividual variability, the additive, proportional and additive-proportional models were assayed to explain the intraindividual and measurement/residual variability.

Results and discussion

Triptorelin plasma levels found after intravenous (25 $\mu\text{g}/\text{kg}$) and intramuscular injections (10, 50 and 100 $\mu\text{g}/\text{kg}$) in rats are shown in Figure 1.

A pharmacokinetic analysis identified the two open compartment first order input (ADVAN4) as the best pharmacokinetic structural model. Intraindividual and measurement/analytical residual error was best described by a model with both additive and proportional components and the interindividual variability for K_a was best described by the exponential error model.

The final model population fixed effects and random effects parameter estimates (with the relative standard error in parenthesis) are summarized in Table 1. The apparent clearance (CL), central (V_c) and peripheral compartment (V_{p1}) distribution volumes, intercompartmental clearance (CL_{p1}) and absorption rate (K_a) values were 261 ml/h, 38.8 ml, 122.0 ml, 276.0 ml/h and 2.79 h^{-1} , respectively. Although the inclusion of bioavailability in the model showed a value of 100%, the model without this parameter was considered due to the fact that no significant drop in the objective function value was found after the inclusion of it. The intramuscular bioavailability was complete. The interindividual variability for K_a was of 40.80% (expressed as coefficient of variation %), the residual variability resulted in 36.0% (expressed as coefficient of variation %) and 0.0318 ng/ml for the proportional and additive components, respectively. The

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pharmacokinetic linear behaviour within the dose range tested was proved after being assayed the dose as covariable in the model.

Table 1. Population values of the fixed effects parameters (main pharmacokinetic parameters) estimated

Parameter	Units	Estimate	IIV%
CL	ml/h	261.0 (0.74)	-
Vc	ml	38.8 (0.32)	-
Clp1	ml/h	276.0 (0.24)	-
Vp1	ml	122.0 (0.18)	-
Ka	h ⁻¹	2.79 (0.13)	40.80 (0.62)
Proportional error	%	36.0 (0.18)	-
Additive error	Ng/ml	0.0318 (0.33)	-

Estimates are shown with the relative standard error in parenthesis. IIV: inter-subject variability expressed as CV.

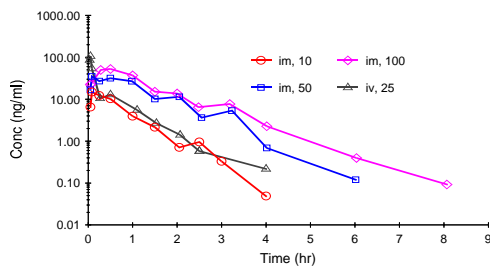


Figure 1. Triptorelin plasma levels after i.v. (25 µg/kg) and i.m. (10, 50 and 100 µg/kg) single injections to rats.

References

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2. Beal SL, Sheiner LB. NONMEM Users Guides. San Francisco (CA): NONMEM Project Group, University of California at San Francisco, 1992.

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Contact Address:

Helena Colom Codina

helena.colom@ub.edu

Faculty of Pharmacy, University of Barcelona

Avda. Joan XXIII s/n

08028 Barcelona

Tel.:93-4024560

Fax:93-4024563