Pharmacokinetic/Pharmacodynamic Model of the Testosterone Effects of Triptorelin Administered in Sustained Release Formulations in Patients with Prostate Cancer

Elba Romero, Nieves Vélez de Mendizabal, Josep-Maria Cendrós, Concepción Peraire, Emma Bascompta, Rosendo Obach, and Iñaki F. Troconiz

Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, Pamplona, Spain (E.R., N.V.d.M., I.F.T.); and Drug Metabolism and Pharmacokinetics, Ipsen Pharma S.A., Barcelona, Spain (J.-M.C., C.P., E.B., R.O.)

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ABSTRACT

The objectives of the current work were to develop a predictive population pharmacokinetic (PK)/pharmacodynamic (PD) model for the testosterone (TST) effects of triptorelin (TRP) administered in sustained-release (SR) formulations to patients with prostate cancer and determine the minimal required triptorelin serum concentration (C_{TRP_min}) to keep the testosterone levels of the patients below or equal to the level of castration (TST ≤0.5 ng/ml). A total of eight healthy male volunteers and 74 patients with prostate cancer received one or two doses of triptorelin injected subcutaneously or intramuscularly. Five different triptorelin formulations were tested. Pharmacokinetic (serum concentration of triptorelin) and pharmacodynamic (TST levels in serum) data were analyzed by using the population approach with NONMEM software (http://www.iconplc.com/technology/products/nonmem/). The PK/PD model was constructed by assembling the agonist nature of triptorelin with the competitive reversible receptor binding interaction with the endogenous agonist, a process responsible for the initial and transient TST flare-up, and triggering down-regulation mechanisms described as a decrease in receptor synthesis. The typical population values of K_D, the receptor equilibrium dissociation constant of triptorelin, and C_{TRP_min} to keep 95% of the patients castrated were 0.931 and 0.0609 ng/ml, respectively. The semi-mechanistic nature of the model renders the predictions of the effect of triptorelin on TST possible regardless the type of SR formulation administered, while exploring different designs during the development of new delivery systems.

Introduction

In men, prostate cancer is the second most common cancer and the second leading cause of cancer death in the industrialized world after skin and lung cancer, respectively. Prostate cancer accounts for approximately 11% of cancer-related deaths in men, and approximately one in eight men will be diagnosed with prostate cancer during their lifetimes (Jemal et al., 2009). Surgery, radiation, and hormone therapy are currently the most common therapeutic strategies.

Prostate cancer is very sensitive to testosterone, and its pharmacological treatment is mainly based on optimal androgen blockade, which is reflected as continuous serum testosterone (TST) levels below the castration limit of 0.5 ng/ml (Mottet et al., 2011). Chemical castration can be achieved by administration of gonadotropin-releasing hormone (GnRH) analogs (agonists or antagonists). In brief, in the case of...
GnRH agonists, the pituitary gland is stimulated, leading to intense release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which stimulate gonadal production of TST. However, after continuous exposure to GnRH agonists, GnRH receptors undergo down-regulation, finally decreasing the production of TST. To avoid this refractoriness, GnRH agonists can be administered. The results obtained can be used to optimize dosing schedules and find optimal pharmacokinetic profiles matching the therapeutic needs of the patient.

**Materials and Methods**

**Study Design and Patients**

PK and PD data were obtained from four randomized, open-label clinical trials (one phase I, two phase II, and one phase III) and five SR formulations (microparticles and four different microimplants differing in the percentage of copolymers in their composition). Single doses ranging from 3 to 15 mg and two administrations of 22.5 mg of triptorelin were injected either subcutaneously or intramuscularly in the external gluteal area. Table 1 lists the main design characteristics for each clinical study.

A total of eight healthy male volunteers and 74 patients with prostate cancer were recruited. All patients, aged 18 years or more, had a histological-confirmed diagnosis of prostate cancer, locally advanced or metastatic or presenting a relapse after curative treatment, a serum testosterone level >1.5 ng/ml before the start of the treatment, and an estimated survival expectancy of at least 12 months according to the investigator’s assessment.

PK data from a phase I study (intravenous study) performed in healthy volunteers (n = 16) receiving a 0.2 mg i.v. bolus dose of triptorelin were also used to characterize the disposition of triptorelin in serum.

Written informed consent was obtained for each participant in compliance with institutional review board/independent ethics committee, informed consent regulations, the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice and local regulatory requirements.

**Blood Sampling for Triptorelin and Testosterone**

Triptorelin and testosterone were determined at the same sampling points. Several blood samples were taken during the first week at 1, 2, 4, 6, 8, 12, and 24 h and 1, 2, 3, 4, and 7 days after injection (formulations B, C, and D provided additional data at 15 and 30 min). Samples were then obtained weekly during between the first and third weeks after injection and every 10 to 14 days afterward.

Table 1 provides information about the length of the sampling periods for each of the formulations studied. In the case of the intravenous study, blood samples were taken at 2, 5, 10, 15, and 30 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, and 36 h after the start of the bolus administration.

**Determination of Triptorelin and Testosterone in Serum**

Whole-blood samples were placed in 5-ml Vacutainer tubes (BD Biosciences, San Jose, CA) (or equivalent) and left to clot for 30 min at room temperature. After complete clot reaction samples were

### TABLE 1

**Summary of data characteristics**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous Bolus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Formulation type</strong></td>
<td>Solution</td>
<td>Microtubules</td>
<td>Microtubules</td>
<td>Microparticles</td>
<td>Microtubules</td>
</tr>
<tr>
<td><strong>Dose, mg</strong></td>
<td>0.2</td>
<td>3, 6, 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td><strong>Doses, n</strong></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Injection type</strong></td>
<td>Intravenous</td>
<td>Subcutaneous</td>
<td>Subcutaneous</td>
<td>Subcutaneous</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td><strong>Subjects, n</strong></td>
<td>16</td>
<td>19</td>
<td>12</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td><strong>Healthy</strong></td>
<td>16</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>16</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td><strong>Triptorelin samples, n&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>272 (4)</td>
<td>409 (57)</td>
<td>307</td>
<td>220 (1)</td>
<td>379</td>
</tr>
<tr>
<td><strong>Testosterone samples, n&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>438 (7)</td>
<td>333 (189)</td>
<td>230 (108)</td>
<td>263 (9)</td>
<td>299 (8)</td>
</tr>
<tr>
<td><strong>Study duration, days&lt;sup&gt;d&lt;/sup&gt;</strong></td>
<td>1.5</td>
<td>84 (35–204)</td>
<td>310 (148–364)</td>
<td>148 (109–274)</td>
<td>112 (63–119)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dose of 3 mg of triptorelin was evaluated on healthy subjects only, and doses of 6 and 9 mg were evaluated on patients only.

<sup>b</sup> Second dose was administered 168 days after first administration.

<sup>c</sup> Number of samples below limit of quantification is in parentheses.

<sup>d</sup> Median (range).
centrifuged at 3000g for 15 min. After centrifugation and decantation, 1.5 ml of blood was used for the analysis. TRP and TST serum concentration levels were determined by using validated radiomunoassay methods. For TRP the limit of quantification was 0.01 ng/ml, and for TST the limit of quantification was different between studies, ranging from 0.014 to 0.1 ng/ml. The intraday and interday coefficients of variation, as well as bias, were less than 15% for both TPT and TST.

**Data Analysis**

All analyses were performed by using NONMEM version VII software (Beal and Sheiner, 2006). Part of the PK and PD data were reported as concentrations below the limit of quantification. Below the limit of quantification values were included during the analyses and treated as censored information by using the M3 method suggested by Beal (2001). The Laplacian numerical estimation method was used for parameter estimation (Beal and Sheiner, 2006).

Interindividual variability (IVV) was modeled exponentially. PK and PD data were logarithmically transformed during the analyses, and residual variability was modeled by using an additive error model on log-transformed data.

Models were sequentially developed. First, the population PK model was developed, and then the time course of testosterone levels was described, incorporating in the dataset the empirical Bayesian estimates of the individual PK model parameters.

**Pharmacokinetic Models.** Disposition of triptorelin in serum was characterized from the data obtained in healthy volunteers receiving a single 0.2 mg i.v. bolus dose, using compartmental models parameterized in apparent volumes of distribution and elimination and distribution clearances.

The time course of triptorelin concentrations obtained after administration of the SR formulations was characterized by using the PK disposition model after intravenous administration (with the parameter estimates fixed). Data from each of the formulation were characterized separately.

Different absorption models (Holford et al., 1992), including the transit compartments model (Savic et al., 2007), were used to describe drug input from the injection site. The presence of latency periods in the drug absorption processes was explored. The magnitude of the absolute bioavailability (F\textsubscript{ABS}) was also investigated by dividing the disposition pharmacokinetic parameters by F\textsubscript{ABS}. Figure 1a shows the PK model describing the time course of triptorelin in serum.

**Pharmacodynamic Model for Testosterone.** The PK/PD model developed in the current work involved the following main mechanisms:

1) Competitive interaction between endogenous agonist and TRP. At baseline the arbitrary amount of activated receptors (R\textsubscript{AC,0}) is given by eq. 1, where $R_ {T_0} \_0$ represents the total arbitrary amount of receptors, which has been set to 1, and $F_{RAC,0}$ the fraction of activated receptors (eq. 2).

\[
R_{AC,0} = F_{RAC,0} \times R_{T_0,0}
\]

\[
F_{RAC,0} = \frac{AGN}{1 + AGN}
\]

AGN represents the ratio between the endogenous agonist concentration (unknown) and its receptor equilibrium dissociation constant. After TRP administration, the amount and fraction of activated receptors ($R_{AC}$ and $F_{RAC}$, respectively) are given by eqs. 3 and 4:

\[
R_{AC} = F_{RAC} \times R_T
\]

\[
F_{RAC} = \frac{AGN + BGN}{1 + AGN + BGN}
\]

where $R_T$ reflects the amount of total receptors in the presence of triptorelin, and BGN corresponds to $C_{TRP}/K_D$, the ratio between serum concentrations of triptorelin ($C_{TRP}$) and its receptor equilibrium dissociation constant ($K_D$). Equation 4 corresponds to a reversible competitive interaction between two agonists (Ariens and Simonis, 1964).

2) Receptor down-regulation caused by prolonged exposure to TRP. Equation 5 describes the balance between receptor synthesis and receptor degradation processes occurring at baseline:

\[
\frac{dR_{T,0}}{dt} = k_{S,R} - k_{D,R} \times R_{T,0}
\]

where $k_{S,R}$ and $k_{D,R}$ are the zero- and first-order rate constants of receptor synthesis and degradation, respectively. As mentioned previously, $R_{T,0}$ was assumed equal to 1, and $k_{S,R} = k_{D,R}$. In the presence of triptorelin eq. 5 becomes eq. 6:

\[
\frac{dR_T}{dt} = k_{S,R} \times R_T - k_{D,R} \times R_T
\]

where $R_T$ reflects the down-regulation phenomena, which is described by eq. 7.

\[
D_T = \frac{D_{R_50}}{D_{R_50} + (F_{RAC} - F_{RAC,0}) \times \frac{R_T}{R_{T_0}} \times \left(2 - \frac{R_T}{R_{T_0}}\right)}
\]

$D_{R_50}$ is the value of the difference between $F_{RAC}$ and $F_{RAC,0}$ that elicits a 50% maximal reduction in $k_{S,R}$ for a given amount of $R_T$. At baseline ($F_{RAC} = F_{RAC,0}$ and $R_T = R_{T_0,0} = 1$) $D_T$ shows value of 1. An increase in $F_{RAC}$ caused by the binding of triptorelin to the GnRH receptor is associated with a decrease in $D_T$, and consequently, a reduction in the synthesis of $R_T$ (down-regulation). The term $[R_T \times (2 - R_T/R_{T_0})]$ regulates the decrease and the subsequent recovery of $R_T$, taking into account the instantaneous amount of $R_T$.

3) Stimulation of testosterone production induced by activated receptors was modeled by using eq. 8:

\[
\frac{dTST}{dt} = k_{S,T} \times R_{AC} + k_m - k_{D,T} \times TST
\]

where $k_{S,T}$ and $k_{D,T}$ correspond to the zero- and first-order constants of testosterone synthesis and degradation, respectively. The zero-order rate process, represented by $k_m$, accounts for TST production that is independent from LH and FSH (Labrie, 2010). $R_{AC}$ depends on $R_T$, and, consequently, its value is conditioned by the down-regulation process described in eqs. 6 and 7. The initial condition corresponding to eq. 8 is represented by eq. 9:

\[
k_{S,T} = (TST_0 \times R_{D,T}) - k_m
\]

$R_{AC,0}$ where TST\textsubscript{0} represents the level of testosterone at baseline. The parameters to be estimated by the model are: TST\textsubscript{0}, AGN, $K_D$, $k_m$, $k_{D,T}$, $k_{D,R}$, and $D_{R_50}$. The rest are derived from the estimated parameters by using the expressions at equilibrium.

During the development of the model, the significance of incorporating sigmoidicity parameters in eqs. 2, 4, and 7 was explored as was the contribution of the term $R_{T_0/R_{T_0}} \times (2 - R_T/R_{T_0})$ and $k_m$.  

**Model Selection.** Selection between models was based mainly on the inspection of individual versus time predicted and observed profiles and the minimal value of the objective function provided by NONMEM and approximately equal to $-2 \times$ log (likelihood). A decrease in 10.83 between nested models was considered significant at the 0.1% level. In the case of comparing the non-nested model, the Akaike Information Criteria (computed as $-2 \times$ log (likelihood) + 2 $\times$ Np, where Np is the number of the parameters in the model) was used instead (Ludden et al., 1994).

**Model Evaluation.** Parameter precision corresponding to the selected models were evaluated by computing the 2.5th, 50th, and 97.5th percentiles using the log-likelihood profiling approach (PK model) and from the analysis of 200 bootstrap datasets (PK/PD model).
The log-likelihood profiling and the bootstrap analysis were performed by using Perl-speaks-NONMEM (Lindbom et al., 2004). Model performance for the selected PK and PK/PD models was further evaluated by exploring the results from visual predictive checks, numerical predictive checks, and the individual predicted profiles corresponding to the best, median, and worst-fit subjects. The best, median, and worst-fit individuals were obtained by calculating for each subject in each formulation the root of the mean squared error, which quantifies the difference between the individual model prediction and the observed value.

<table>
<thead>
<tr>
<th>Visual Predictive Checks</th>
<th>Numerical Predictive Checks</th>
<th>Calculation of the Pharmacokinetic Descriptor $C_{\text{TRP, min}}^\text{min}$</th>
</tr>
</thead>
</table>
| A total of 1000 individual profiles per formulation were simulated, and the 5th, 50th, and 95th percentiles were calculated and represented together with the raw data. | A total of 200 datasets with the same study design characteristics as the original studies were simulated. For each dataset the median of the individual maximum concentrations of triptorelin and testosterone ($C_{\text{MAX, TRP}}$ and $C_{\text{MAX, TST}}$ respectively), area under the triptorelin serum concentration versus time curve between 0 and last time of measurement, and the castration time, defined as the period during which testosterone concentration levels remained below the castration limit (0.5 ng/ml), were calculated. Then the 5th, 50th, and 95th percentiles of the overall median distribution were computed and compared with the 50th percentiles obtained from the raw data. | The minimal required triptorelin serum concentration to maintain the...
Matlab script developed by the authors to compute differential equations systems were solved for 1000 subjects (the percentage of patients that remained under the castration level simulated every 2 days after injection of triptorelin. For each study, studies of 100 patients were simulated. Levels of testosterone were substituted by the level of castration (0.5 ng/ml).

The corresponding parameter values (estimated or derived). TST was calculated. Finally, the 2.5th, 50th, and 97.5th percentiles of the distribution of percentages were represented over time.

### Results

#### Pharmacokinetic Modeling

Disposition of Triptorelin in Serum after Intravenous Bolus Administration to Healthy Subjects. The three-compartmental model (Fig. 1a) improved significantly the description of the data compared with the one- and two-compartmental models ($p < 0.001$). IIV was included on total serum clearance (CL), and the volume of distribution of the central compartment. Parameter estimates are presented in Table 2.

Disposition of Triptorelin after SR Administration. Absorption models based on a single-depot compartment using a first- or zero-order input with or without lag time, or the transit compartments model provided a very poor description of the data. Assuming a two-depot compartments model from which the input process can follow first or zero kinetics with or without lag time, and both compartments releasing the drug in parallel or in a sequential manner, the fit was improved significantly with respect to the one-depot absorption model ($p < 0.001$); however, model predictions clearly underpredicted and overpredicted the concentration peaks and the last portion of the concentration curves, respectively. Only when a third depot absorption compartment was added was the entire pharmacokinetic profile captured properly ($p < 0.001$). For all of the SR formulations studied the release from the third compartment was best described with a zero-order model with respect to the first-order rate model ($p < 0.001$). The model for absorption represented in Fig. 1a was selected to describe the input characteristics of triptorelin after subcutaneous and intramuscular injection. The model is based on three depot compartments, two of them released the drug after first-order kinetics and one released the drug after zero-order kinetics. Absolute bioavailability was not significantly different from 1 for any of the five sustained-release formulations ($p > 0.05$). Table 3 lists the population pharmacokinetic parameters of triptorelin administered in sustained release formulations.

### Tables

#### Table 2

Population pharmacokinetic parameters of triptorelin after intravenous bolus administration to healthy volunteers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate</th>
<th>CI</th>
<th>IIV</th>
<th>Estimate</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL, L × day⁻¹</td>
<td>274.3 (260–290)</td>
<td>10 (7–15)</td>
<td></td>
<td>37 (24–58)</td>
<td></td>
</tr>
<tr>
<td>Vₐ, L</td>
<td>8.1 (6.5–10)</td>
<td></td>
<td></td>
<td>12 (11–13)</td>
<td></td>
</tr>
<tr>
<td>Vₜ₁, L</td>
<td>33.8 (3–35)</td>
<td></td>
<td></td>
<td>832.3 (720–963)</td>
<td></td>
</tr>
<tr>
<td>Residual error, log(ng/ml)</td>
<td>0.13 (0.118–0.142)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Additional Model Simulations

For each formulation, 1000 studies of 100 patients were simulated. Levels of testosterone were simulated every 2 days after injection of triptorelin. For each study the percentage of patients that remained under the castration level was calculated. Finally, the 2.5th, 50th, and 97.5th percentiles of the distribution of percentages were represented over time.

#### Table 3

Population pharmacokinetic parameters of triptorelin administered in sustained release formulations

CI is 95% confidence interval. IIV is expressed as coefficient of variation. Absolute bioavailability was nonsignificantly different from 1 for all SR formulations ($p > 0.05$)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Estimate</th>
<th>CI</th>
<th>Estimate</th>
<th>CI</th>
<th>Estimate</th>
<th>CI</th>
<th>Estimate</th>
<th>CI</th>
<th>Estimate</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>A, 3, 6, and 9 mg doses</td>
<td>B, 6 mg dose</td>
<td>C, 15 mg dose</td>
<td>D, 6 mg dose</td>
<td>E, 22.5 mg dose</td>
<td>B, 6 mg dose</td>
<td>C, 15 mg dose</td>
<td>D, 6 mg dose</td>
<td>E, 22.5 mg dose</td>
<td>B, 6 mg dose</td>
</tr>
<tr>
<td>E₁</td>
<td>1.50</td>
<td>0.7–8.6</td>
<td>0.48</td>
<td>0.3–0.8</td>
<td>4.34</td>
<td>4.2–4.4</td>
<td>1.85</td>
<td>1.5–2.2</td>
<td>0.384</td>
<td>0.3–0.5</td>
</tr>
<tr>
<td>E₂</td>
<td>0.99</td>
<td>0.6–4.2</td>
<td>0.59</td>
<td>0.4–0.7</td>
<td>0.36</td>
<td>0.2–0.29</td>
<td>0.096</td>
<td>5 × 10⁻²</td>
<td>0.6</td>
<td>0.7–1.4</td>
</tr>
<tr>
<td>F₁</td>
<td>0.25</td>
<td></td>
<td>0.49</td>
<td></td>
<td>0.18</td>
<td></td>
<td>0.34</td>
<td></td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>F₂</td>
<td>0.63</td>
<td></td>
<td>0.22</td>
<td></td>
<td>0.06</td>
<td></td>
<td>0.03</td>
<td></td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>K₁</td>
<td>0.12</td>
<td></td>
<td>0.29</td>
<td></td>
<td>0.06</td>
<td></td>
<td>0.03</td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>K₂</td>
<td>0.015</td>
<td>0.01–0.04</td>
<td>0.0257</td>
<td>0.02–0.03</td>
<td>0.047</td>
<td>0.04–0.05</td>
<td>0.032</td>
<td>0.03–0.04</td>
<td>0.019</td>
<td>0.015–0.022</td>
</tr>
<tr>
<td>K₃</td>
<td>0.099</td>
<td>0.07–0.1</td>
<td>3.5 × 10⁻³</td>
<td>2–5 × 10⁻³</td>
<td>8 × 10⁻⁴</td>
<td>8–9 × 10⁻⁴</td>
<td>0.150</td>
<td>0.1–0.2</td>
<td>0.023</td>
<td>0.015–0.036</td>
</tr>
<tr>
<td>t₁₅₀₅₀ day</td>
<td>5.49</td>
<td>4.8–6.5</td>
<td>147</td>
<td>146–148</td>
<td>69.79</td>
<td>69–70</td>
<td>111.9</td>
<td>108–115</td>
<td>0.77</td>
<td>0.7–0.9</td>
</tr>
<tr>
<td>IIW E₁, %</td>
<td>58</td>
<td>56–71</td>
<td>28</td>
<td>9–56</td>
<td>38</td>
<td>20–66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIW E₂, %</td>
<td>152</td>
<td>151–185</td>
<td>19</td>
<td>10–31</td>
<td>27</td>
<td>19–38</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IIW K₁, %</td>
<td>62</td>
<td>48–84</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual error, log(ng/ml)</td>
<td>0.491</td>
<td>0.14–0.87</td>
<td>0.355</td>
<td>0.32–0.39</td>
<td>0.62</td>
<td>0.6–0.7</td>
<td>0.269</td>
<td>0.2–0.3</td>
<td>0.60</td>
<td>0.56–0.64</td>
</tr>
</tbody>
</table>

* Dose was given twice.

** Derived parameters calculated as $F₁ = 1/1 + E₁ + E₂$, $F₂ = E₁/1 + E₁ + E₂$, and $F₅₀₅₀ = E₁/1 + E₁ + E₂$.

### Additional Model Simulations

For each formulation, 1000 studies of 100 patients were simulated. Levels of testosterone were simulated every 2 days after injection of triptorelin. For each study the percentage of patients that remained under the castration level was calculated. Finally, the 2.5th, 50th, and 97.5th percentiles of the distribution of percentages were represented over time.
model parameter estimates obtained from the five formulations where it can be observed that estimates were obtained in general with adequate precision. The duration of the zero-order process ranged between formulations from 18.5 h to almost 4 days after injection, and the fraction of the dose absorbed after zero-order kinetics varied from 3% (formulation C) to 29% (formulation B). For formulations C, D, and E, estimates of lag times associated with one absorption compartment were 147, 70, and 112 days, respectively, which allowed the capture of the delayed second concentration peaks. Some of the first-order rate constants of absorption were estimated to have very low values ($3.5 \times 10^{-3}$ and $7.9 \times 10^{-4}$ h$^{-1}$), which were compatible with slow decay in serum concentrations seen for formulations B and C. PK profiles after administration of formulation A were dose-proportional. IIV estimates were in general low to moderate (19–62%) with the exception of one absorption rate constant in formulation A (151%).

The results from the visual and numerical predictive checks are shown in Fig. 2 and Supplemental Table 1, respectively. Model evaluation indicated that the PK model provided precise parameter estimates and performed optimally in describing the entire serum profiles and important drug exposure descriptors such as $C_{\text{MAX,TTP}}$ and area under the triptorelin serum concentration versus time curve between 0 and the last time of measurement.

![Fig. 2. Visual predictive checks corresponding to the selected pharmacokinetic models. Points represent observed measurements. Solid circles represent observations below the limit of quantification. Solid lines show the median-simulated profiles. The shadowed areas correspond to the 90% prediction intervals obtained from 1000 individual simulated profiles.](image-url)
Pharmacokinetic/Pharmacodynamic Modeling

Population PK/PD parameters of the testosterone effects of triptorelin corresponding to the selected PK/PD model shown in Fig. 1b are listed in Table 4 together with the results obtained from the nonparametric bootstrap analyses. No bias is observed, and there is a good precision of the estimates. Estimates of IIV are moderate, approximately 30%. Results from visual and numerical predictive checks are presented in Fig. 3 and Supplemental Table 1. Those results indicated that the model performed optimally in describing the entire response profiles and important clinical descriptors of drug action such as the castration time and $C_{\text{MAX,TST}}$. Figure 4 shows individual model predictions for three subjects, showing the best, median, and worst fit in each of the five formulations. Deleting the term $[R_T/R_{T,0} \times (2 - R_T/R_{T,0})]$ from the model resulted in a worse fit, and the parameter $k_{in}$ was found to be significant ($p < 0.001$). Including a sigmoidicity factor in eqs. 2, 4, and 7 did not improve the fit significantly ($p > 0.05$), and the rebound mechanism represented by eqs. 10 and 11 was not supported by the data. A graphic insight in the dynamics of the main components of the selected PK/PD model is presented in Fig. 5.

Calculation of $C_{\text{TRP, min}}$

The values of $C_{\text{TRP, min}}$ to keep 5, 50, and 95% of the patients castrated were 0.0204, 0.0356, and 0.0609 ng/ml, respectively. Figure 6A shows that, assuming a constant serum concentration of 0.0356 ng/ml of triptorelin, the limit of castration was achieved after 1 year and highlights the need, with the current GnRH agonists, of achieving high triptorelin levels during the initial surge to reach a rapid castration, i.e., approximately 3 weeks. The period of time during which triptorelin levels are equal or higher than $C_{\text{TRP, min}}$ ($T_{\text{TRP, min}}$) will depend on the serum versus time profile and therefore on the type of formulation administered. $T_{\text{TRP, min}}$ to achieve 0.0609 ng/ml and calculated based on the typical PK profiles for formulations A-E was 41, 69, 47, 53, and 294 days, respectively. Figure 6B shows the effect of a second administration on the initial surge of testosterone levels, where it can be observed that during the prevalence of the down-regulation progress the flare-up was negligible.

Supplemental Fig. 1 represents the time profiles of the percentages of castrated patients over time obtained from 1000 studies with 100 subjects each and simulated for each formulation. Because all formulations shared the same model for TST effects, the observed differences were caused by differences in the absorption/release profiles among formulations.

Discussion

In the current work a population PK/PD model has been developed for five different SR formulations of triptorelin administered subcutaneously or intramuscularly. The PK model used to characterize drug disposition after intravenous administration provided a good description of the data. The estimates of the model parameters were similar or in the range of those calculated by using noncompartmental analysis after intravenous bolus administration of 0.5 mg of triptorelin to healthy volunteers (Müller et al., 1997): $CL$ ($L \times h^{-1}$), 11.6 (current) versus 12.6, and $V_{SS}$, apparent volume at steady state (L), 53 (current) versus 31.2. During the modeling of pharmacokinetic data obtained from the sustained formulations, it has been assumed that triptorelin disposition is not altered by the disease, which represents a limitation in our analysis. Absorption from SR formulations was very slow, resulting in triptorelin concentrations maintained for long periods of time and indicating the presence of flip-flop kinetics. Despite the differences seen in the estimates of the parameters between formulations, a common absorption model was selected, which was similar to the one reported previously after SR administration of triptorelin (Tornøe et al., 2007). Differences in parameter estimates can be attributed to differences in the formulation and administration route.

Population PK/PD models have been developed in the past for different antagonists (Fattinger et al., 1996; Pechstein et al., 2000; Tornøe et al., 2007) and different agonists (Gries et al., 1999; Tornøe et al., 2007) of the pituitary receptor. In the case of triptorelin a model developed for LH and testosterone after SR administration of triptorelin at the dose of 3.75 mg has been proposed (Tornøe et al., 2007). There are substantial differences in the structure between the model selected in the current study and the one published previously (Tornøe et al., 2007). We have combined indirect response-based models resembling synthesis and degradation processes with receptor binding and reversible competitive interaction. The current model showed very good performance, describing very sharp response profiles as shown for some individuals in Fig. 4 without requiring high sigmoidicity in the relationships represented by eqs. 2, 4, and 7, decreasing nonlinearity and providing stability to the model. One additional characteristic of the current model is the fact

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Median, 2.5–97.5th</th>
<th>IIV</th>
<th>Median, 2.5–97.5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{TST}}$, ng/ml</td>
<td>3.98</td>
<td>3.98 (3.43–4.55)</td>
<td>35.07</td>
<td>35.2 (31.2–41.4)</td>
</tr>
<tr>
<td>$K_T$, ng/ml</td>
<td>0.93</td>
<td>0.93 (0.81–1.09)</td>
<td>31.36</td>
<td>31.9 (30.8–41.1)</td>
</tr>
<tr>
<td>$D_{\text{TST}, 50}$, unitless</td>
<td>0.024</td>
<td>0.025 (0.022–0.027)</td>
<td>32.09</td>
<td>32.6 (31.8–39.7)</td>
</tr>
<tr>
<td>$h_{in, T}$, day$^{-1}$</td>
<td>0.185</td>
<td>0.21 (0.19–0.24)</td>
<td>35.77</td>
<td>36.3 (34.4–47.1)</td>
</tr>
<tr>
<td>$h_{out, T}$, day$^{-1}$</td>
<td>0.041</td>
<td>0.036 (0.031–0.042)</td>
<td>0.55</td>
<td>0.59 (0.52–0.72)</td>
</tr>
<tr>
<td>AGN, unitless</td>
<td>0.31</td>
<td>0.34 (0.31–0.39)</td>
<td>0.52</td>
<td>0.77 (1.63–1.88)</td>
</tr>
</tbody>
</table>

$T_{\text{TST}}$ serum levels of TST at baseline.

* Additive error in log-scale.
that the down-regulation process, with a time profile represented in Fig. 5, middle, was described with just one parameter ($D_{R,50}$). The additional term involving $R_T$ in eq. 7 avoided an increase in model complexity. The selected model, on the other hand, does not account for rebound phenomena. The degree of perturbation (number of different dose levels, study duration, routes of administration, etc.) is essential to make the system react and provide data reflecting the complexities inherent to the system studied. We speculate that the reason for the discrepancies seen between the previous model and the one presented here is that we used data from studies in which testosterone levels were measured up to 1 year and dose levels ranged from 3 to 22.5 mg.

Because of differences in the structure of the models, it is difficult to perform comparisons in parameter estimates across different models. Yet, the estimated parameters reported in the current analysis for $k_{D,T}$ was 0.023 h$^{-1}$, a value similar to those published previously: 0.01 h$^{-1}$ (Gries et al., 1999) and 0.09 h$^{-1}$ (Tornøe et al., 2007). Synthesis of testosterone was split in two zero-order rate processes ($k_{S,T}$/$k_{in}$), one depending on the activated pituitary receptors and showing a value at baseline of 9.51 ng × day$^{-1}$ (a derived parameter obtained from eqs. 1, 2, and 9), and the second represented by $k_{in}$, which is insensitive to the presence of the agonist and associated with a much lower estimate (0.041 ng × day$^{-1}$). The latter might be representing the synthesis of testosterone from dehydroepiandrosterone in the general circulation (Labrie, 2010).

In the current model, triptorelin exerted its action while increasing the fraction of activated receptors, therefore the pharmacodynamic parameter $K_D$ should not be interpreted as the concentration of triptorelin eliciting half of maximal testosterone reduction. In a complex system such as the one studied in the present work, and if a link between triptorelin concentrations and testosterone levels is to be done, a system analysis such as the one performed to calculate $C_{TRP,min}$ is required. It is noteworthy that the value of $C_{TRP,min}$ needed to keep 50% of the patients castrated (0.0356 ng/ml) was very similar to the $EC_{50}$ estimate of 0.047 ng/ml for triptorelin reported by Tornøe et al. (2007).

Serum concentrations of triptorelin of 8 ng/ml already were associated with a fraction of activated receptors of 0.9; therefore, higher levels of drug concentrations will not elicit a further increase in the initial flare-up seen in testosterone levels shortly after injection.

Supplemental Fig. 1 shows one of the applications of every PK/PD evaluation, especially performed on a small population of patients. Once a PK/PD model has been developed, it can be used to generate for each condition (tested formulation in the current study) the distribution of a response indicator (e.g., the predicted percentage of patients castrated over time).

In conclusion, a PD population model was developed that described the effects of triptorelin on testosterone in healthy volunteers and patients with prostate cancer independent of the dose administered, route of administration, and type of formulation, once a specific PK model was already available. System analyses were undertaken to compute the PK descriptor $C_{TRP,min}$, with important implications on establishing optimal dosing schedules. Results have been provided (selected model and parameter estimates) that can be used to select an optimal plasma concentration versus time profile through multiobjective optimal control methods. It can be considered multiobjective be-
Fig. 4. Individual observed (points) and model-predicted (lines) serum testosterone levels versus time profiles for the best (top), median (middle), and worst (bottom) individual fit for each of the sustained release formulations studied. RMSE, root mean squared error. Solid circles represent observations below the limit of quantification.
cause ideally the PK profile should produce minimal flare-up in the testosterone levels, rapid castration (within 3 weeks after injection), and long-term castration. In addition, investigations aiming to establish a model relating the PK/PD characteristics between animals and humans are currently ongoing and will allow for earlier formulation selection.

**Fig. 5.** Graphical insight into the dynamics of the main components of the selected PK/PD model, using the typical parameter estimates and the population PK profile obtained from formulation D. The fraction of activated receptors (top right) follows the kinetics of triptorelin in serum (top left); however, the total amount of activated receptors (bottom left) remains at a very low level because of the down-regulation process that reduces receptor synthesis. Middle images show how the term \( R_T / R_{T,0} \) regulates the effect of the term \( \frac{F_{RAC} - F_{RAC,0}}{2 - R_T / R_{T,0}} \) as a function of the amount of the total receptors. Typical profile of testosterone after injection of formulation D (bottom right).

**Fig. 6.** A, typical testosterone profile (solid line) in the presence of constant serum concentrations of triptorelin equal to \( C_{TRP_{min}} \) (0.0356 ng/ml). B, typical testosterone profiles after one or two injections of formulation D. The second injection occurred at 53, 100, or 125 days after the start of the treatment.

**Authorship Contributions**

Participated in research design: Romero, Vélez de Mendizabal, Cendrós, Peraire, Bascompta, Obach, and Troconiz.

Conducted experiments: Cendrós, Peraire, and Bascompta.

Performed data analysis: Romero, Vélez de Mendizabal, Cendrós, Peraire, and Troconiz.
Wrote or contributed to the writing of the manuscript: Romero, Vélez de Mendizabal, Cendrós, Peraire, Obach, and Troconiz.

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Ariuens EJ and Simonis AM (1964) A molecular basis for drug action. The interaction of one or more drugs with different receptors. J Pharm Pharmacol 16:289–312.


Address correspondence to: Dr. Inaki F. Troconiz, Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, Pamplona 31080, Spain. E-mail: itroconiz@unav.es

Supplementary material

Table 1. Results from the numerical predictive check

<table>
<thead>
<tr>
<th>Formulation</th>
<th>( C_{\text{MAX,TRP}} ) (ng/mL)</th>
<th>( C_{\text{MAX,TST}} ) (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed Median</td>
<td>Simulated(^a) Median (2.5-97.5(^{th}))</td>
</tr>
<tr>
<td>IV solution</td>
<td>21.13</td>
<td>19.93 (16.6-24.4)</td>
</tr>
<tr>
<td>A</td>
<td>2.024</td>
<td>3.4 (2.4 – 4.5)</td>
</tr>
<tr>
<td>B</td>
<td>1.971</td>
<td>2.0 (1.4 – 2.8)</td>
</tr>
<tr>
<td>C</td>
<td>10.98</td>
<td>13.6 (9.6 – 19.5)</td>
</tr>
<tr>
<td>D</td>
<td>3.261</td>
<td>2.9 (2.4 – 3.6)</td>
</tr>
<tr>
<td>E</td>
<td>47.146</td>
<td>70.3 (41.3 – 117.7)</td>
</tr>
</tbody>
</table>

\(^a\) results obtained from 200 simulated datasets; \( C_{\text{MAX,TRP,TST}} \), maximum serum concentrations of TRP and TST, respectively; \( AUC_{\text{last}} \), area under the TRP serum concentration vs time curve between time 0 and last time of measurement.
Figure 1. Percentage of castrated patients over time. Dashed lines represent the median profiles over 1000 simulated studies. The shadowed areas cover the 2.5 and 97.5th percentiles.