Relevance of Arteriovenous Concentration Differences in Pharmacokinetic-Pharmacodynamic Modeling of Midazolam

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ABSTRACT
In the present investigation, the extent of arteriovenous concentration differences of midazolam in rats was quantified, and the consequences of these differences on the pharmacodynamic estimates were determined. The arterial concentration-effect relationships were analyzed by a traditional-effect compartment model that characterizes the delay between blood and the effect site with the rate constant $k_{eo}$. Venous concentration-effect relationships where analyzed according to the traditional model and an extended-effect compartment model that, by incorporating an additional rate constant $k_{vo}$, can characterize the delay between the arterial and venous sampling site. Significant hysteresis was observed in the arterial but not the venous concentration-effect relationships. Rate constants for $k_{eo}$, $k_{vo}$, and terminal half-life were (mean ± S.E.M.) 0.32 ± 0.062, 0.093 ± 0.013 and 0.0217 ± 0.0008 min⁻¹, respectively, indicating the existence of significant arteriovenous concentration differences. Pharmacodynamic estimates as determined on basis of the arterial concentrations and the traditional-effect compartment model were $EC_{50} = 104$ ± 1 ng/ml, $E_{max} = 151$ ± 4 μV/sec and $\gamma = 0.83 ± 0.06$. Analysis of the venous concentration-effect relationships on basis of the traditional- or extended-effect compartment model led to similar pharmacodynamic estimates, indicating that the observed arteriovenous concentration differences did not result in biased pharmacodynamic estimates. This is due to the fact that the effect relevant elimination rate constant of midazolam is relatively small compared with its $k_{vo}$. The observed results are consistent with earlier reports based on computer simulations.

The observed hysteresis in non-steady-state pharmacodynamic investigations can often be explained by a distributional disequilibrium between the site at which the drug concentration is measured and the site at which the drug exerts its action. Traditional-effect compartment models have been proposed that characterize the equilibration between the arterial concentrations and the concentrations at the effect site. This is achieved by postulating a first-order rate constant ($k_{eo}$) between the central plasma compartment and the compartment at which the drug exerts its effect (Fuseau and Sheiner, 1989; Segre, 1968; Sheiner et al., 1979; Veng-Pedersen et al., 1991; Verotta and Sheiner, 1988).

The existence of profound arteriovenous concentration differences has been documented for a large number of drugs. In many pharmacodynamic investigations, drug concentrations are determined in venous blood. The pharmacological effect, however, is primarily determined by the concentration in arterial blood; therefore in pharmacokinetic-pharmacodynamic investigations, the delay from the arterial circulation to the venous sampling site should be taken into account (Chiou, 1989; Chiou et al., 1981). Hence, if venous concentrations do not reflect the arterial concentrations, postulation of a simple rate constant between the central and the effect site compartment may not be sufficient to characterize correctly the subsequent delays from the venous sampling site to the arterial site and from the arterial site to the effect site (fig. 1). To account for this, extended effect-compartment link models have been proposed in which an equilibration delay between arterial and venous concentrations has been incorporated (Gumbleton et al., 1994; Sheiner, 1989; Verotta et al., 1989). Typically, different compartments for concentrations of the drug in arterial and venous blood and at the effect site are postulated. The distribution of drug between these compartments is characterized on basis of first-order rate constants: $k_{vo}$ for the distribution between arterial and venous blood and $k_{eo}$ for the distribution between arterial blood and the effect site (fig. 1) (Gumbleton et al., 1994; Verotta et al., 1989). The analytical solution to the equations characterizing the distribution among arterial blood, venous blood and the effect site has been resolved (Tuk et al., 1997), allowing the

ABBREVIATIONS: $A$, intercept of the plasmaconcentration vs. time profile; $\alpha$, first-order rate constant of the plasmaconcentration vs. time profile; $k_{eo}$, first-order rate constant for the distribution between arterial blood and the hypothetical effect compartment; $k_{vo}$, first-order rate constant for the distribution between arterial and venous blood; $E_{o}$, base-line effect value; $E_{max}$, maximal effect; $EC_{50}$, concentration at half-maximal effect; $\gamma$, constant expressing the sigmoidicity of the concentration-effect relationship; EEG, electroencephography; $\sigma$, variance of parameter estimate.
incorporation of the routine in nonlinear regression programs.

It was demonstrated recently by means of computer simulations that the use of the traditional-effect compartment model in the presence of arteriovenous concentration differences can lead to significantly biased pharmacodynamic estimates. Depending on the ratio of the rate constants determining the delay to the effect site, delay to the venous sampling site and half-life of the drug, bias up to 90% in EC₅₀ was observed (Tuk et al., 1997). The extended-effect compartment model in each situation yielded unbiased although imprecise results.

In the present investigation, the extent of arteriovenous concentration differences of midazolam in rats was quantified and the consequences of these differences on the pharmacodynamic estimates were determined using quantitative EEG analysis as a measure of effect intensity. Pharmacokinetic-pharmacodynamic relationships were investigated using either arterial or venous concentrations. The “true” value of kₑo was determined on the basis of the observed hysteresis loop in the arterial concentration-effect relationship and the true value for kᵛo was determined by simultaneously fitting the arterial and venous concentrations. In addition, venous concentration-effect relationships were analyzed by the traditional- and extended-effect compartment models. These estimates were compared with their independently derived true estimates to evaluate the performance of both models.

The purpose of the present study was to (1) determine the extent of arteriovenous concentration differences for midazolam in rats, (2) determine the impact of these differences on the pharmacodynamic estimates of midazolam when using the traditional-effect compartment model to analyze venous concentration-effect relationships and (3) apply the extended-effect compartment model on experimental pharmacokinetic-pharmacodynamic data.

Materials and Methods

Study design. The study was designed according to a parallel-group design. Rats were assigned randomly to one of two treatment groups in which blood sampling in rats of the arterial group was performed via the femoral artery and of the venous group via the tail vein.

Animals. Two groups of seven male Wistar rats (Sylvius Laboratory Breeding Facility, Leiden, The Netherlands) weighing (mean ± S.E.M.) 245 ± 3 g were used in the study. The animals were housed individually in plastic cages with a normal 12-hr light/dark cycle and fed a commercially available diet (Standard Laboratory Rat, Mouse and Hamster Diets, RMG-TM; Hope Farms, Woerden, The Netherlands) and water ad libitum. From the night preceding the experiment, the animals were deprived of food but had free access to water.

Surgical procedures. For the measurement of EEG signals, chronic cortical EEG electrodes were implanted into the skull of the animals 1 week before the kinetic-dynamic experiments as described previously (Mandema et al., 1991). One day before the experiment, indwelling canulae were implanted in all rats in the right jugular vein (for drug administration) and right femoral artery (for blood collection in rats of the arterial sampling group). One hour before the experiment, a single small incision was made in the tail vein of all rats (for blood collection in rats of the venous group). This procedure was used because peripheral rather than central venous blood samples are required to examine the role of (distributional) arteriovenous concentration differences (Gumbleton et al., 1994).

Drug dosage and blood sampling. Both groups of rats received 10 mg/kg midazolam intravenously during a 15-min infusion. Midazolam was dissolved in 0.9% saline with the aid of an equimolar quantity of hydrochloric acid. To determine the pharmacokinetics of midazolam, blood samples of 100 or 200 µl near the end of the experiment, were collected at fixed-time intervals after drug administration over a period of 280 min. Both groups of rats were stimulated in their tails for purpose of sampling, whereas only in the venous group were actual samples taken. Rats in the arterial group had blood taken from the femoral artery, whereas for the venous group, the sampling procedure was mimicked without an actual sample being taken. Heparinized blood samples were centrifuged and plasma was separated and stored at −35°C until analysis. One hour after the experiment, the animals were killed. In all rats, a final blood sample was obtained by aortic puncture to be used for protein binding measurements.

EEG measurements. The output form bipolar EEG leads was continuously recorded using a Nihon Kohden EEG system consisting of a bioelectric input box (model JB-682G), bioelectric amplifier (model AB-621G) and bioelectric input panel (model PB-680G). The low-pass filter was set at 100 Hz, and the time constant was 0.3 sec. During the course of the experiment, the animals were forced to walk in a slowly rotating drum to prevent spontaneous fluctuations in the level of vigilance (Mandema et al., 1991). EEG recordings were commenced 15 min before the administration of midazolam for baseline determination. Two EEG leads, the frontocentral and central occipital lead on the left hemisphere, were quantified online by an aerodynamic analysis (Gregory and Pettus, 1986) as described previously (Mandema et al., 1991). The amplitudes (µV/sec) in the 11.5- to 30-Hz frequency band of the frontocentral lead were calculated and used as a measure of drug effect intensity.

Drug analysis and plasma protein binding. The plasma concentrations of midazolam were determined by a gas chromatographic assay using electron-capture detection as described previously (Mandema et al., 1992). The extent of plasma protein binding of midazolam was determined for each individual animal by ultrafiltration at 37°C using the Amicon Micropartition System (Amicon Division, Danvers, MA). This procedure has been described in detail previously (Mandema et al., 1991).

Data analysis. Pharmacokinetics of midazolam after arterial and venous sampling were fitted simultaneously using a pooled fit, according to a monoexponential venous link:

\[
C_v = R_v \ast f(t)
\]

\[
C_v = R_v \ast g(t) \ast f(t)
\]

where R(t) is the infusion regimen normalized for body weight, f(t) is the unit impulse disposition function and g(t) is the arteriovenous link model. Under the assumption that no metabolism across the
arteriovenous bed occurs, \( f(t) \) and \( g(t) \) are described by:

\[
\begin{align*}
  f(t) &= \int_{0}^{t} A_i \cdot e^{-\alpha_i t} \\
  g(t) &= k_{vo} \cdot e^{-k_{vo} t}
\end{align*}
\]

In this equation, \( A_i \) and \( \alpha_i \) are the coefficients and exponents of the equation, respectively, and \( k_{vo} \) is the rate constant characterizing the delay from the arterial to the venous compartment (fig. 1).

**Pharmacodynamics.** Concentrations at the effect site are assumed to be monoexponentially linked to the arterial concentrations:

\[
C_e = R_i \cdot h(t) \cdot f(t)
\]

where:

\[
h(t) = k_{wo} \cdot e^{-k_{wo} t}
\]

where \( k_{wo} \) is the rate constant characterizing the delay from the arterial to the effect site. Concentration-effect relationships were characterized using the sigmoid \( E_{max} \) pharmacodynamic model:

\[
E = E_0 + E_{max} \left( \frac{C_e^\gamma}{C_e^\gamma + EC_{50}^\gamma} \right)
\]

where \( E \) is the observed effect at effect site concentration \( C_e \), \( E_0 \) is the baseline effect value, \( E_{max} \) is the maximal effect, \( EC_{50} \) is the concentration at half-maximal effect and \( \gamma \) is a constant expressing the sigmoidicity of the concentration-effect relationship. Data from the arterial group were fitted on basis of the traditional-effect compartment model, and data from the venous group were analyzed both on basis of the traditional- and extended-effect compartment model.

**Results**

Figure 2 shows the time course of the plasma concentrations of midazolam for the arterially and venously sampled rats. The solid line represents the best fit to the combined pharmacokinetic analysis of the arterial and venous concentrations. The curve through the terminal phase slightly underestimates the actual data points. In analysis of the data, we examined the possibility to further improve the fit by using different weighting factors. Based on the log likelihood criterion as a measure of the goodness of fit, no further improvement of the fitting could be obtained. Significant arteriovenous differences were observed. During the infusion, arterial concentrations were higher than the venous concentrations. On cessation of the infusion, the arterial concentrations dropped sharply, with the result that the venous concentrations were higher during the remainder of the experiment (fig. 2C).

Simultaneous fitting of the pharmacokinetic profiles of both groups allowed the determination of the pharmacokinetic parameters of midazolam and quantification of the equilibration delay between the arterial and venous sampling site; the so-derived (mean ± S.E.M.) "true" estimate for \( k_{vo} \) was 0.093 ± 0.013 min\(^{-1}\), and it characterized the equilibration delay between the arterial and venous (sampling) sites. The terminal rate constant of midazolam was estimated at 0.0217 ± 0.001 min\(^{-1}\). No differences in protein binding were observed between the arterial (\( f_u = 4.2 ± 1.0% \)) and venous (\( f_v = 4.5 ± 0.7% \)) groups. The administration of midazolam led to an increase of effect intensity that, when concentrations declined after stopping the infusion, gradually returned to preinfusion values. No differences in the time-effect profiles were observed between the arterial and venous sampled groups.

A counterclockwise hysteresis loop was observed in the concentration-EEG relationship (fig. 3) when the EEG was linked to the arterial concentrations. Analysis according to a traditional-effect compartment model yielded a \( k_{vo} \) estimate of 0.32 ± 0.06 min\(^{-1}\). Neither hysteresis nor prototresis was observed in the venous concentration-effect relationship (fig. 3), as confirmed by the \( k_{vo} \) estimate of 313 ± 110 min\(^{-1}\) (table

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**Fig. 2.** Time course of midazolam plasma concentrations after arterial (A) and venous (B) sampling and mean fitted concentration profiles in arterial (—) and venous (— — —) blood (C) after a 15-min infusion of 10 mg/kg midazolam.
and no significant improvement in fit was observed compared with the model without delay between concentration and effect.

Fittings based on arterial and venous concentrations according to the traditional- and extended-effect compartment model are shown in figure 4. Pharmacodynamic estimates as determined on basis of the arterial concentrations and the traditional-effect compartment model were $EC_{50} = 104 \pm 11$ ng/ml, $E_{\text{max}} = 151 \pm 4 \mu V/sec$ and $\gamma = 0.83 \pm 0.06$. Analysis of the venous concentration-effect relationships on basis of the traditional- or extended-effect compartment model led to similar pharmacodynamic estimates (table 2). Both the traditional- and extended-effect compartment models failed to retrieve true estimates for $k_{\text{eo}}$ and $k_{\text{vo}}$ on the basis of the venous concentration-effect relationship. Analysis of the venous concentration-effect relationships according to the extended-effect compartment model did not result in a significant improvement of the log-likelihood estimates over those observed for the traditional-effect compartment model (table 2).

**Discussion**

The existence of arteriovenous concentration differences has been documented for a large number of drugs. The pharmacokinetic implications of these differences have been widely studied, and they are acknowledged to have a profound impact on the pharmacokinetic profile of many drugs (Chiou, 1989). The impact of these differences on pharmacodynamic estimates only recently have been subject of investigation (Gumbleton et al., 1995; Tuk et al., 1997). By means of computer simulations, the existence of arteriovenous concentration differences has been shown to lead to significant

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pharmacokinetic parameter estimates of midazolam as determined by simultaneously fitting the pharmacokinetic profiles of both groups of rats according to equations 1 through 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (ng/ml)</td>
<td>7.0 ± 2.3</td>
</tr>
<tr>
<td>A2 (ng/ml)</td>
<td>0.294 ± 0.025</td>
</tr>
<tr>
<td>$\alpha_1$ (min$^{-1}$)</td>
<td>1.22 ± 0.45</td>
</tr>
<tr>
<td>$\alpha_2$ (min$^{-1}$)</td>
<td>0.093 ± 0.013</td>
</tr>
<tr>
<td>$k_{\text{eo}}$ (min$^{-1}$)</td>
<td>0.076 ± 0.012</td>
</tr>
<tr>
<td>$\sigma^2_a$</td>
<td>0.192 ± 0.028</td>
</tr>
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Values are mean ± S.E.M.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Pharmacodynamic parameter estimates of midazolam according to the sigmoid $E_{\text{max}}$ model (equation 5)</th>
</tr>
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<tbody>
<tr>
<td>Arterial or venous concentrations were used to drive the pharmacokinetic-pharmacodynamic fit using the traditional or extended effect compartment model. No significant differences were observed in pharmacodynamic estimates $P &lt; .05$ by analysis of variance.</td>
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<thead>
<tr>
<th></th>
<th>Arterial traditional</th>
<th>Venous traditional</th>
<th>Venous extended</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0$ (μV/sec)</td>
<td>78 ± 4</td>
<td>90 ± 5</td>
<td>91 ± 5</td>
</tr>
<tr>
<td>$EC_{50}$ (ng/ml)</td>
<td>104 ± 11</td>
<td>86 ± 11</td>
<td>89 ± 11</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (μV/sec)</td>
<td>151 ± 4</td>
<td>167 ± 6</td>
<td>166 ± 5</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>0.83 ± 0.06</td>
<td>0.78 ± 0.07</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>$k_{\text{eo}}$ (min$^{-1}$)</td>
<td>0.32 ± 0.062</td>
<td>313 ± 110</td>
<td>2.5 ± 2.0</td>
</tr>
<tr>
<td>$k_{\text{vo}}$ (min$^{-1}$)</td>
<td>1.4 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>720 ± 45</td>
<td>1060 ± 50</td>
<td>1050 ± 50</td>
</tr>
<tr>
<td>$-2LL$</td>
<td>3175</td>
<td>3913</td>
<td>3908</td>
</tr>
</tbody>
</table>

**Fig. 3.** Concentration-effect relationships for two typical rats on arterial (A) or venous (B) sampling. Anticlockwise hysteresis was observed on arterial but no on venous sampling.

**Fig. 4.** A, Concentration-EEG effect relationship after arterial sampling. The line represents the best fit according to the traditional-effect compartment model. B, Concentration-EEG effect relationship after venous sampling. The (overlapping) lines represent the best fittings according to the traditional- and extended-effect compartment model.
bias in pharmacodynamic estimates. Bias up to 90% in estimates of EC\textsubscript{50} were reported, depending on the ratio of k\textsubscript{eo}, k\textsubscript{vo} and the half-life of a drug (Tuk et al., 1997). In the present investigation, the extent of arteriovenous concentration differences of midazolam in rats was quantified. In addition, the consequences of these differences on the pharmacodynamic estimates of midazolam were determined by analyzing the venous concentration-effect relationships according to the traditional-effect compartment model. The sensitivity of this model to arteriovenous concentration differences was investigated by comparing parameters derived from the venous concentrations with the parameters derived from analyzing the arterial concentration-effect relationships. The venous concentrations were also analyzed according to an extended-effect compartment model, which takes into account the equilibration delay between the arterial and venous sampling site. Within the context of pharmacokinetic-pharmacodynamic modeling especially, distributional arteriovenous concentration differences are important (Gumbleton et al., 1994). Such differences are observed in particular when peripheral venous blood samples are obtained from poorly perfused tissues. For this reason, the tail was chosen as the sampling site for peripheral venous blood.

The time course of concentrations of the arterial and venous groups after the administration of 10 mg/kg midazolam is shown in figure 2. The marked distribution phase for the arterial concentrations was not seen for venous samples, indicating the existence of arteriovenous concentration differences. The extent of arteriovenous concentration differences was quantified by simultaneously analyzing the concentrations of the arterial and venous group (see equations 1–4). The rate constant characterizing the delay from the arterial to the venous sampling site k\textsubscript{vo} was 0.093 ± 0.013 min\textsuperscript{-1} (mean ± S.E.M.) (table 1), indicating the existence of a significant equilibration delay between the arterial and venous sampling site, with a half-life of 7 minutes.

The identical time-effect profiles observed after arterial and venous sampling indicate that the effect measurements were not influenced by differences in sampling sites and that there were no differences in pharmacodynamics between the two groups of rats. Arterial and venous concentrations were used to characterize the pharmacokinetic-pharmacodynamic relationship of midazolam. Figure 3 shows the arterial concentration-effect profile, which clearly displays hysteresis. Using the traditional-effect compartment model to solve the hysteresis, the k\textsubscript{eo} was estimated to be 0.32 ± 0.062 min\textsuperscript{-1} (see table 2). Because this k\textsubscript{eo} value is determined on the basis of arterial concentrations, it can be considered to be the “true” value for delay to the effect site.

Interestingly, minimal hysteresis was observed in the venous concentration-effect relationship of midazolam (fig. 3). Solving this small loop in the venous concentration-effect relationship resulted in an estimate of the apparent delay to the effect site k\textsubscript{vo} which is too large to be of any relevance. Although at first this may seem inconsistent with the estimated delay to the effect site as estimated on the basis of the arterial concentrations, this can be attributed to the coinciding occurrence of arteriovenous concentration differences. Apparently, for midazolam, the delay from the arterial to the effect site is masked by the delay from the arterial to the venous sampling site. The fact that the true k\textsubscript{vo} (as estimated from the arterial concentration-effect relationship) is in the same range as the true k\textsubscript{vo} (as estimated from the simultaneous fit of the arterial and venous concentrations), in combination with the variability due to EEG measurement error, accounts for this observation. This does not mean that no delay to the effect site is present in the venously sampled rats. It is there, but it is masked by a delay to the venous site of a similar magnitude. Based on venous concentrations, the net result will be an unique concentration-effect relationship without hysteresis. This behavior is consistent with earlier observations for thiopental in humans, in whom hysteresis was observed on arterial but not venous sampling (Stanski et al., 1984). This shows that the existence of arteriovenous concentration differences may also be a relevant issue in integrated pharmacokinetic-pharmacodynamic investigations in humans. This is particularly the case for drugs with a very short terminal half-life and in the situation in which a profound decline in the pharmacological response intensity occurs during the rapid distribution phase, as was recently demonstrated on the basis of computer simulations (Tuk et al., 1997).

Concentration-effect relationships were simultaneously analyzed by estimating the k\textsubscript{vo} and the pharmacodynamic estimates by the traditional- or extended-effect compartment model. Despite the marked differences in arterial and venous time-concentration profiles, no differences were detected in pharmacodynamic estimates of midazolam (table 2), nor did the extended-effect compartment model significantly improve the log-likelihood estimate of the fit of the venous concentration-effect relationship. This indicates that estimating the extra parameter k\textsubscript{eo} did not improve the quality of the fit (see also fig. 4) or the accuracy of the pharmacodynamic estimates. This is consistent with the earlier reported computer simulations, in which bias was reported only for certain combinations of k\textsubscript{vo}, k\textsubscript{vo} and the rate constant of the half-life of the drug in the time period in which the decline in pharmacological effect is most pronounced is <5 times k\textsubscript{vo} and k\textsubscript{vo} is smaller than k\textsubscript{vo} (as is the case for midazolam here), there is no need to model the underlying arteriovenous equilibration delay. Under these conditions, a traditional first-order link between venous and effect-site concentrations yields accurate and reliable estimates of pharmacodynamic parameters such as E\textsubscript{max}, EC\textsubscript{50} and \gamma. Because in the current study these criteria are all met for midazolam, no bias in pharmacodynamic estimates should be expected, and indeed none are observed. This conclusion, however, cannot be generalized to all situations in which venous pharmacokinetic-pharmacodynamic relationships of midazolam are investigated. An interesting question is whether a significant bias in the pharmacodynamic parameter estimates would have been observed when most of the decline of the pharmacological response would have occurred during the rapid distribution phase. Earlier computer simulations show that bias in the pharmacodynamic parameter estimates is particularly prominent when the ratio between the values of t\textsubscript{1/2} and the half-life of the rapid distribution rate phase is <5 (Tuk et al., 1997). In the present study, this ratio is ~4. This indicates that (some) bias in the pharmacodynamic parameters EC\textsubscript{50} and \gamma factor may be expected in this situation.

In summary, significant arteriovenous concentration differences exist for midazolam in rats as reflected in the half-
life for equilibration between the arterial and venous sampling site of ~7.5 min. Because the rate constant characterizing the phase during which most of the effect occurs is <5 times \( k_{\text{eo}} \) and \( k_{\text{eo}} \) is smaller than \( k_{\text{vo}} \), no bias in pharmacodynamics estimates was observed. Because the disappearance of midazolam effect was most pronounced in the second pharmacokinetic phase, there was no need to model the underlying arteriovenous equilibration delay. These results are consistent with earlier reported computer simulations, in which for these circumstances, minimal bias in pharmacodynamic estimates was predicted.

References