Pharmacokinetic Study of the Digoxin-acenocoumarol Interaction in Rabbits

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A study was carried out to evaluate the potential pharmacokinetic interaction between digoxin and acenocoumarol. The binding of digoxin to rabbit cardiac tissue homogenates was assessed in vitro, using the equilibrium dialysis technique. An increase in the first-order constant (p < 0.05) and a reduction in the partition coefficient in the equilibrium situation (p < 0.001) of digoxin were observed when the cardiac homogenates were previously treated with acenocoumarol. In the in vivo study, the kinetics of digoxin administered in single and multiple dosage regimens were compared in control rabbits and in rabbits treated simultaneously with acenocoumarol. Kinetic analysis of the results was performed using Non-linear Mixed Effects Modeling (NONMEM). In the presence of acenocoumarol, the population distribution volume (Vd) of digoxin was increased by 40—60%, no differences being found as regards the elimination clearance. Also, joint administration of both drugs led to a reduction in digoxin concentrations in the heart (p < 0.01) at the end of the dosage regimen. Both sets of results point to the hypothesis of a hitherto unreported possible pharmacokinetic interaction between the two drugs affecting the distribution process. This interaction could lead to lower plasma digoxin levels, in view of the increased Vd, and a possible reduction in the therapeutic effect, owing to the decrease in affinity and in concentration in heart tissue.

Key words digoxin-acenocoumarol interaction; equilibrium dialysis; population pharmacokinetic; distribution volume; Non-linear Mixed Effects Modeling (NONMEM)

Digoxin remains among the most frequently administered cardiac drugs. Its narrow therapeutic range, together with the existence of many factors that modify the pharmacological response to digoxin, make routine monitoring of its plasma levels crucial. Among such factors, one of the most important is the large number of interactions shown by the drug, most of them with a pharmacokinetic basis.1,2

Aenidoconmarol is the oral anticoagulant most widely used in continental Europe. Despite its widespread use, the literature contains few references to this drug, particularly in comparison with the large body of information on warfarin, the most widely used anticoagulant in other areas of the world. The important pharmacokinetic differences shown by acenocoumarol mean that the results obtained with other anticoagulants cannot be extrapolated to this drug.3—5

Treatments with digoxin and acenocoumarol are usually chronic and often simultaneous. At two hospitals in our region it was observed that patients receiving treatment with acenocoumarol required higher doses of digoxin to control their conditions and for digoxin levels within the therapeutic range to be obtained.6,7 The literature contains no references to this possible interaction. Indeed, in a review paper on the interactions of anticoagulants Harder and Thürmann7 specifically cite the absence of references to interactions between cardiotonic glucosides and coumarin derivatives, and they highlight the need for controlled studies of these drugs. Accordingly, the aim of the present work was to evaluate the potential pharmacokinetic interaction between digoxin and acenocoumarol in rabbits both in vitro and in vivo.

MATERIALS AND METHODS

The interaction between digoxin and acenocoumarol was studied in in vitro and in vivo experiments. In the in vitro studies, the binding of digoxin to cardiac tissue homogenates was assessed in the presence and absence of acenocoumarol, using the equilibrium dialysis technique. In the in vivo experiments, the kinetics of digoxin administered in single- and multiple-dose regimens were compared in a group of control rabbits and in another group treated with both drugs simultaneously. The levels of digoxin in heart tissue at the end of the dosage regimen were also studied.

In Vitro Study The in vitro studies of the binding of digoxin to heart homogenates were carried out using the equilibrium dialysis technique.8,9

Heart Tissue Homogenates: Homogenates were prepared from rabbit hearts at a concentration of 50 mg/ml in phosphate buffer (1/15 M, pH 7.4) to study the binding of digoxin to heart tissue. Untreated homogenates and homogenates previously treated with a solution of acenocoumarol (1 mg/ml) over 4 h before the dialysis experiments were compared.

Prior to the study, experiments had been conducted that showed that digoxin is not adsorbed onto the dialysis membrane. Neither was any significant shift across the membrane observed. The process was carried out at physiological temperature (37°C). Using this technique, two types of experiments were performed.

Study of Digoxin Binding to Heart Homogenates in the Equilibrium Situation: Study of digoxin binding to heart homogenates in the equilibrium situation was carried out at initial concentrations of 10, 25, 50, 75, 100, 150, 200, 250 and 300 ng/ml in phosphate buffer. Experiments were repeated three times for each concentration. The time of dialysis required for distribution equilibrium to be reached was 4 h.

Study of Digoxin Binding to Heart Homogenates as a Function of Time: One milliliter of homogenate was placed in one compartment of the dialysis cell against 1 ml of a solution of digoxin of 100 ng/ml prepared in phosphate buffer.
Unbound concentrations were determined at 0.17, 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4 and 5 h. The total number of experiments conducted for each experimental situation was 8.

In Vivo Study This part of the study was carried out on four groups of adult male New Zealand rabbits (n=40) with an age range between 63 and 83 d and weight between 2.00 kg and 2.60 kg (mean 2.33±0.17 kg). All experiments were performed in compliance with the “Principles of Laboratory Animal Care” and the Spanish laws for animal welfare.

Single-Dose Study: Group I: Digoxin i.v. (Digoxin Boehringer®, Boehringer Mannheim) dose=200 μg/kg. Group II: Digoxin (same treatment)+acenocoumarol (1.5 mg/kg/d s.c.,10) starting 4 d before treatment with digoxin.

Blood samples were obtained 8, 12, 22 and 24 h after digoxin administration.

Multiple-Dose Study: All animals received digoxin treatment over 7 d. Group III: Digoxin i.v. loading dose=150 μg/kg and 12 maintenance doses=75 μg/kg every 12 h. Group IV: Digoxin (same treatment)+acenocoumarol (1.5 mg/kg/d s.c., starting 4 d before treatment with digoxin. From the fifth day of treatment the dose of acenocoumarol was reduced to half because of the high prothrombin times observed.

Blood samples were obtained at the 1st (8, 10, 12 h), 9th (12 h), 12th (12 h) and 13th (8, 10, 12 h) doses, according to the pre-established protocol.

To control the anticoagulant effect of acenocoumarol, prothrombin times were evaluated in plasma from the rabbits of group IV prior to starting acenocoumarol treatment and on the 5th, 7th and 11th days of treatment with acenocoumarol.

Administration of the digoxin doses and blood withdrawal were performed through the marginal ear vein of the rabbits when conscious and immobilised. Blood samples were collected in Eppendorf tubes containing heparin and were centrifuged immediately to separate the plasma.

After the multiple dose experiments, the rabbits were sacrificed by an overdose of sodium pentobarbital and the hearts were removed. Plasma and removed hearts were stored at −30° pending analysis.

Analytical Technique Digoxin concentrations in samples of plasma and heart tissue from the in vivo study were determined using a fluorescence polarization immunoassay (TDx Digoxin II assay, Abbot Laboratories, Ltd.). The intra- and inter-assay errors had a variation coefficient of <10%.

Samples of heart tissue were conditioned prior to determinations.11) A sample of 1 ml of heart tissue homogenate (100 mg/ml) was subjected to double extraction, obtaining a dry residue. For digoxin determination, the dry residue was dissolved in 1 ml of plasma.

Since the TDx technique is a commercial method for the determination of drugs in biological samples, for the determination of unbound digoxin in buffered medium in the in vitro assays a reverse phase HPLC technique with fluorimetric detection and pre-column derivatization was used. The samples required extraction with dichloromethane before pre-column derivatization. Derivatization was accomplished using the technique of Embree and McErlane.12) Analysis was performed on a 5 μm LiChrospher® RP-18 column 125 mm in length and 4 mm i.d. The mobile phase was acetonitrile/phosphate buffer (38/62 v/v), pH=5, at a flow rate of 2 ml/min at room temperature. The detection limit of the technique was lower than 2 ng/ml (considering as the detection limit the concentration whose chromatographic peak was greater than twice the noise signal) and the limit of quantification was lower than 5 ng/ml.

With both analytical techniques used, it was observed that acenocoumarol did not interfere in digoxin assays. For this, both blanks and solutions of digoxin of known concentrations were determined in the absence and presence of acenocoumarol.

Kinetic Analysis In Vitro Study: For the in vitro studies on digoxin binding to heart homogenates, saturable and non-saturable binding models were tested, using non-linear regression and statistical criteria of goodness of fit. A single class of non-saturable binding sites was selected. This model was described by the following equation:

\[ C_u = nC_sK_a - C_u \]

where \( C_s \) is the bound concentration, \( n \) the number of protein-binding sites per class of binding site, \( C_p \) the protein concentration, \( K_a \) the equilibrium association constant and \( C_u \) the unbound concentration in the dialysis cell.

Equation 1 can be expressed as:

\[ C_u = P \cdot C_u \]

where \( P \) represents a linear binding partition coefficient.

The binding kinetics of digoxin as a function of time was characterised as a first-order kinetic process by the equation:

\[ C_u = A_0 + B_0 \cdot e^{-Kt} \]

where \( A_0 \) is the free concentration in the equilibrium situation, \( B_0 \) is the coefficient of the exponential equation, and \( K \) is the first-order constant related to the permeability of drug through semi-permeable membrane and the binding to heart homogenates.

The \( P \) parameter in the experiments performed in the equilibrium situation and \( A_0, B_0 \) and \( K \) in the experiments conducted at different times were optimised using the PCNONLIN 4.2 nonlinear regression program.13)

In Vivo Study: Kinetic analysis of digoxin and its interaction with acenocoumarol in vivo was carried out using population pharmacokinetics.

A one-compartment open population model with first-order elimination was used. Although digoxin kinetics is multicompartamental, this is the model most widely used in clinical practice.13) The corresponding population pharmacokinetic parameters of plasma clearance (\( CL_p \)) and distribution volume (\( V_d \)) were modelled as a function of the acenocoumarol (ACN) covariate:

\[ CL_p = \theta_1 (1 + \theta_2 \cdot ACN) \quad \text{and} \quad V_d = \theta_3 (1 + \theta_4 \cdot ACN) \]

The categorical covariate has an ACN value of 1 when there is concomitant treatment with acenocoumarol and an ACN of zero in the opposite case. \( \theta_1, \theta_2, \theta_3 \) and \( \theta_4 \) are the fixed-effect parameters of the regression. Thus, \( \theta_2, \theta_3 \) and \( \theta_4 \) directly quantify the influence of acenocoumarol in the pharmacokinetic parameter.

The statistical model selected to describe the intersubject variabilities in the clearance and distribution volume of digoxin was proportional:
where $V_d$ and $Cl_p$ are the individual estimates, $\bar{Cl}_p$ and $\bar{V}_d$ represent the typical values of the kinetic parameters, and $\eta$ are the individual variations from this mean value. These were assumed to be normally distributed, with a mean of 0 and variance $\omega^2$.

To describe the residual error in the concentration, an additive model was selected:

$$c_{ij} = \hat{c}_{ij} + e_{ij}$$

where $c_{ij}$ are the observed and $\hat{c}_{ij}$ the predicted concentrations of the $i$th individual on the $j$th sampling occasion. $e_{ij}$ are the residual errors that describe the differences between the measured and predicted concentrations. These were assumed to be normally distributed, with a mean of 0 and a variance of $\sigma^2$.

The population pharmacokinetic parameters of digoxin were estimated using Non-linear Mixed Effects Modelling (NONMEM), as implemented in the NONMEM software package (version IV, level 1). Statistical comparison of population models was based on a $\chi^2$ test of the difference in the objective function. Other criteria used in evaluating alternative population models were: weighted residuals plots, minimisation in the magnitude of residual variability and Akaike’s Information Criterion. Statistical comparison of pharmaco-kinetic parameters was performed using parametric statistics, after confirming distribution normality using the Shapiro-Wilks test.16)

RESULTS

Figure 1 shows the linear correlation established between unbound and bound digoxin concentrations in heart homogenates in the equilibrium situation in the presence and absence of acenocoumarol. The partition coefficient of digoxin had a mean value of $23.5 \pm 6.8$ in the control group, which decreased to $15.0 \pm 8.17$ in the presence of acenocoumarol, the difference being statistically significant ($p<0.001$).

Figure 2 shows the mean concentration of unbound digoxin as a function of time in the study of binding to heart homogenates. Table 1 shows the mean in vitro binding parameters of digoxin estimated by non-linear regression and the

<table>
<thead>
<tr>
<th></th>
<th>Digoxin</th>
<th>Digoxin + Acenocoumarol</th>
<th>Statistical significance</th>
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</thead>
<tbody>
<tr>
<td>Mean (ng/ml)</td>
<td>51.22</td>
<td>36.20</td>
<td>$p&lt;0.01$</td>
</tr>
<tr>
<td>CV %</td>
<td>24.26</td>
<td>19.30</td>
<td></td>
</tr>
<tr>
<td>$A_0$ (ng/ml)$^a$</td>
<td>49.18</td>
<td>42.40</td>
<td>$p&lt;0.05$</td>
</tr>
<tr>
<td>$B_0$ (ng/ml)$^a$</td>
<td>11.98</td>
<td>16.20</td>
<td></td>
</tr>
<tr>
<td>$K$ (h$^{-1}$)$^b$</td>
<td>2.75</td>
<td>5.65</td>
<td>$p&lt;0.05$</td>
</tr>
<tr>
<td>$P$</td>
<td>23.50</td>
<td>15.01</td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td>CV %</td>
<td>28.94</td>
<td>54.43</td>
<td></td>
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</table>

a) Study as a function of time ($C_{\text{unbound}} = A_0 + B_0 \cdot e^{-kt}$). b) Study in the equilibrium situation ($P$: partition coefficient).
statistical comparison with the data found in the presence of acenocoumarol. These results show that the process of the binding of digoxin to heart homogenates \textit{in vitro} is a first-order kinetic process. Under the experimental conditions of the \textit{in vitro} study, the uptake of digoxin by heart tissue homogenates was non-specific and concentration-independent. A statistically significant ($p<0.05$) increase was seen in the constant ($K$) in the presence of acenocoumarol.

Figure 3 shows the digoxin concentrations in the hearts of the rabbits at the end of the dosage regimen in the \textit{in vivo} study. Joint administration of digoxin and acenocoumarol led to a statistically significant reduction ($p<0.01$) in the accumulation of digoxin in heart tissues.

Table 2 show the value of the objective function obtained for the basic and final population model chosen. Introduction of acenocoumarol administration, as a categorical variable, into the equation of the apparent distribution volume elicited a statistically significant reduction in the objective function. Table 3 shows the fixed-effect and random population parameters of digoxin for the two models considered.

Figure 4 shows the plasma digoxin levels for the multiple-dose rabbit groups and the population prediction.

### DISCUSSION

The results obtained from the \textit{in vitro} study by equilibrium dialysis point to the existence of differences in the process of digoxin uptake by heart homogenates in the presence of acenocoumarol. In the equilibrium situation, digoxin displayed a high affinity for heart homogenates with high partition coefficients. A reduction was seen in the partition coefficient of digoxin to heart homogenates in the presence of acenocoumarol (Fig. 1). These differences were also reflected in the increase in the first-order constant (Table 1, Fig. 2) when the binding study was carried out in the non-equilibrium situation.

The differences in digoxin levels in heart tissue ($p<0.01$) obtained in the \textit{in vivo} study (Fig. 3) confirm those obtained in the \textit{in vitro} study since they point to a possible reduction in the affinity of digoxin for heart tissue in the presence of acenocoumarol. These observations suggest that modifications could occur in the pharmacokinetic parameters of digoxin distribution when the drug is administered concomi-

### Table 2. Population Pharmacokinetic Models Selected with NONMEM

<table>
<thead>
<tr>
<th>Study</th>
<th>Objective function</th>
<th>Akaike's criterion</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic model: $Cl_p = \theta_1$; $V_d = \theta_3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single dose</td>
<td>$-133.7$</td>
<td>$-123.7$</td>
<td>—</td>
</tr>
<tr>
<td>Multiple dose</td>
<td>$-306.5$</td>
<td>$-296.5$</td>
<td>—</td>
</tr>
<tr>
<td>Final model: $Cl_p = \theta_1$; $V_d = \theta_3(1+\theta_4 \cdot ACN)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single dose</td>
<td>$-142.4$</td>
<td>$-130.4$</td>
<td>$p&lt;0.005$</td>
</tr>
<tr>
<td>Multiple dose</td>
<td>$-311.5$</td>
<td>$-299.5$</td>
<td>$p&lt;0.05$</td>
</tr>
</tbody>
</table>

ACN: Association with acenocoumarol, yes = 1 and no = 0.

### Table 3. Values of the Population Parameters of Digoxin Obtained Using the Models Proposed in Table 2

<table>
<thead>
<tr>
<th>Study</th>
<th>$Cl_p$ (ml/min/kg)</th>
<th>$V_d$ (l/kg)</th>
<th>$\eta_{Cl_p}$ (c.v.%), $\eta_{V_d}$ (c.v.%), $\epsilon$ (±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic model: $Cl_p = \theta_1$; $V_d = \theta_3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>123.87 (9%)</td>
<td>—</td>
<td>66.67 (12%) 32% (33%) 37% (29%) ±0.13 (13%)</td>
</tr>
<tr>
<td>Multiple</td>
<td>113.46 (6%)</td>
<td>—</td>
<td>69.31 (12%) 25% (42%) 20% (145%) ±0.21 (21%)</td>
</tr>
<tr>
<td>Final model: $Cl_p = \theta_1$; $V_d = \theta_3(1+\theta_4 \cdot ACN)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>125.37 (10%)</td>
<td>—</td>
<td>51.35 (10%) 0.60 (34%) 35% (39%) 31% (74%) ±0.12 (12%)</td>
</tr>
<tr>
<td>Multiple</td>
<td>114.13 (6%)</td>
<td>—</td>
<td>60.54 (13%) 0.40 (59%) 25% (44%) 8% (—) ±0.21 (21%)</td>
</tr>
</tbody>
</table>

Values in brackets represent the standard error of the estimate expressed as coefficient of variation (%). The coefficient of variation for residual variability was calculated for the approximate mean value of the observed concentration (1 ng/ml; range: 0.2—2 ng/ml).
tantly with acenocoumarol, supporting the hypothesis of the existence of a possible interaction at pharmacokinetic level between the two drugs. Such findings would account for the need to increase digoxin doses in patients treated with acenocoumarol in order to obtain a similar therapeutic response.

As seen in Table 2, the population analysis of plasma digoxin concentrations reveals that the Vd of digoxin increases in the presence of acenocoumarol when the drug is administered both as a single dose ($p<0.005$) and on a multiple dose regimen ($p<0.05$). This increase in Vd would explain the lower digoxin plasma levels seen in patients treated simultaneously with acenocoumarol. No improvements in the goodness of fit are observed when the ACN covariate is introduced in the prediction of Cl. The greater degree of significance of the interaction in the single-dose study could be attributed to the fact that in the multiple-dose study it was necessary to reduce the dose of acenocoumarol to avoid hemorrhages. The low statistical power could also be attributed to the large interindividual variability, which is common in studies on drug interactions, and to the limited number of animals that can be included in a prospective study of the type performed here.

The values of the population pharmacokinetic parameters shown in Table 3 reflect the influence of acenocoumarol in the distribution kinetics of digoxin. In the final model, the value of Vd increases in the presence of acenocoumarol, this increase being higher in the single-dose study (60%) than in the multiple-dose one (40%). The interindividual and residual variabilities observed for the final model have acceptable values, within the usual range for this type of study. The difficulty in estimating intersubject variability in the Vd with precision as encountered in the present study has already been reported in the literature for drugs with a long half-life.17,18

Although the modifications observed in the partition coefficient of digoxin in heart both in vitro and in vivo suggest a decrease- and not an increase- in the digoxin Vd, this is not necessarily in conflict with the results obtained in the population kinetic analysis. However, such a decrease would only occur if the decrease in the partition coefficient were to occur in all tissues and not only in the heart, the site of action of the drug.

The possible mechanism involved in this interaction could be related to competitiveness phenomena between the two drugs for binding sites in certain organs and tissues; specifically in the heart. This would explain the modifications in the partition coefficient of digoxin observed in vitro and in the accumulation of digoxin in heart tissues when administered in vivo concomitantly with acenocoumarol. These modifications in the distribution processes would give rise to alterations in the apparent distribution volume and in the plasma concentrations of digoxin.

CONCLUSION

A pharmacokinetic interaction between digoxin and acenocoumarol was observed in both in vitro and in vivo studies. The results obtained point to the notion that distribution was the pharmacokinetic process most affected. In view of the narrow therapeutic range of digoxin and the frequent joint use of these two drugs in humans, further complementary studies are necessary to confirm the possible interaction and its clinical implications.

REFERENCES