Influence of Two Commercial Fibers in the Pharmacokinetics of Ethinylestradiol in Rabbits

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Accepted for publication April 8, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
Fiber formulations are used in human nutrition owing to their beneficial properties for health. It is probable that ingestion of fiber coincides with the oral administration of drugs, and therefore of its pharmacokinetics, can appear. In the present study, the compartmental and noncompartmental pharmacokinetic parameters of ethinylestradiol (EE) in rabbits after oral administration were determined. It was also studied whether the presence of two different fiber formulations [A, wheat bran (76.5%), fruit fiber (12%) and guar gum (2%) and B, Plantago ovata seeds (65%) and P. ovata seed cuticles (2.2%)] in the gastrointestinal tract modified the pharmacokinetics of EE when administered at the same time. Three groups of rabbits were used: control, fiber A and fiber B. The animals in all three groups received 1 mg/kg b. wt. EE. The estrogen was administered alone in the control group and in the presence of 4 g of fiber A and fiber B, respectively, in the other two groups. After compartmental (two-compartment open model) and noncompartmental analyses of plasma concentrations, statistical analysis revealed that the presence of fiber (both A and B) decreased between 29% and 35% the extent of EE absorbed (represented by the pharmacokinetic parameters area under the curve and the maximum plasma concentration) without affecting the rate of the absorption process (represented by the time to reach maximum concentration and the absorption rate constant).

EE is a synthetic estrogen that is widely used as a component of oral contraceptives. This compound is also used in the treatment of functional uterine bleeding and menopausal symptoms, for the inhibition of lactation and for palliative treatment of breast cancer in postmenopausal women and prostate cancer (Masterson, 1988; Godsland et al., 1992).

In order to avoid its adverse effects, EE is used at low doses; in particular, the dose of EE, when used for oral contraception, has been reduced to 30 to 50 µg per day. When other drugs and/or foods are administered at the same time, interactions may appear and EE blood concentrations may be ineffective (Goldzieher, 1994).

The bioavailability and disposition of oral medications are governed by the processes of absorption and presystemic clearance, and these may be influenced by the presence of certain diet components in the gastrointestinal tract (Melander and McLean, 1983). Dietary fiber is widely accepted as an important part of healthy human nutrition, and fiber formulations are marketed in the United States and Europe (Silk, 1989; Scheppach et al., 1990) with extensive advertising campaigns directed to the consumer. This information shows these products as highly effective in the treatment of obesity and constipation prevention, for decreasing blood cholesterol and glucose levels and even for preventing the development of gastrointestinal cancer. These aspects induce the public to consume commercial fiber preparations without medical control, in a continued and sometimes abusive way. Under these circumstances, it is highly probable that fiber ingestion coincides with oral administration of any drug, which could modify its oral absorption and therefore its pharmacokinetics. The influence of fiber on the presystemic clearance of drugs is most likely to be clinically relevant with drugs having narrow therapeutic margins (Melander and McLean, 1983), e.g., EE.

Food intake can affect the absorption and bioavailability of several drugs (Welling, 1977; Melander and McLean, 1983), but little information is available regarding contraceptive steroids. Several studies have shown that nutrition and spe-
cific dietary factors influence the metabolism of steroid compounds, which are primarily metabolized in the liver before their excretion in the urine or bile (Anderson and Kappas, 1982). An increase in the protein to carbohydrate ratio in the diet of healthy subjects can increase estrogen 2-hydroxylation (Anderson, et al. 1984), decrease androgen 5α-reduction (Kappas et al., 1983), alter the plasma levels of testosterone and cortisol in a reciprocal fashion and produce parallel changes in the binding globulin for these steroids (Anderson et al., 1987).

The purpose of the present study was to establish the compartmental and noncompartmental pharmacokinetic parameters of EE in rabbits after its oral administration and to determine whether the presence of two different fiber formulations in the gastrointestinal tract modifies the pharmacokinetics of EE when this drug and fiber formulations are administered at the same time.

Methods

Animals. Fifteen healthy, female New Zealand White rabbits weighing 2.9 to 3.5 kg were used. The animals were housed in individual metal cages, which allowed the isolation of feces in a lower container to avoid coprophagia. The environmental conditions were as follows: constant humidity (55 ± 10%), temperature (19 ± 2°C) and a 12-hr light/12-hr dark cycle. The animals were maintained on laboratory chow and water ad libitum, and they were fasted for ~24 hr before drug administration, with free access to water.

Materials. EE was obtained from Sigma Chemical Co. (St. Louis, MO), sodium pentobarbital from Barcia (Madrid, Spain), heparin from Rovi, S.A. (Madrid, Spain), fiber A from Kneipp-Werke (Würzburg, Germany) and fiber B from Madaus Cearfarma, S.A. (Barcelona, Spain). The fiber composition was as follows: for fiber A, wheat bran (76.5%), fruit fiber (12%) and guar gum (2%) (the rest of the composition appeared as excipients); for fiber B, Plantago ovata seeds (65%) and P. ovata seed cuticles (2.2%). As well, fiber B contained 18.1% succharose, and the rest of the composition appeared as excipients.

Preparation of the experimental animals. Rabbits were anesthetized with sodium pentobarbital (30 mg/kg b. wt. i.v.), and the left carotid artery was cannulated with a silicone catheter (Silastic medical-grade tubing, 1.02 mm (inner diameter) × 2.16 mm (outer diameter)). These cannulas were placed before the trial started. The end of the tubing was passed subcutaneously to emerge at the back of the neck. EE and fiber were administered to the conscious animals ~2 hr after the catheter was inserted.

Study design. Animals were randomly divided into three groups of five rabbits each: control, fiber A and fiber B and received the respective following preparations: 1 mg/kg EE p.o., 1 mg/kg EE p.o. and 4 g fiber A p.o. and 1 mg/kg EE p.o. and 4 g fiber B p.o. The five rabbits of the control group received 4 g fiber A p.o. and 1 mg/kg EE orally as a solution (1 ml) in a mixture of water and ethanol (4:1, v/v). Likewise, the 10 rabbits of groups A and B were orally treated with EE, but immediately before EE administration, they received 4 g p.o. of fiber A and B, respectively, dispersed in water. Both the EE and fiber solutions were administered by gastric intubation. A total of 50 ml water was used for fiber administration and cannula cleaning.

Blood sampling. Blood samples (3 ml) were collected through the carotid artery canula before and at 5, 10, 20, 30, 60, 90, 120, 150, 180 and 240 min after EE administration into heparinized containers. Immediately after collection, plasma was separated by centrifugation and stored at −20°C until analyzed.

Plasma concentration of EE. EE plasma concentrations were determined by high-performance liquid chromatography with electrochemical detection according to the method previously described by Fernandez et al. (1993). Intraday and interday accuracy and precision were within 10%.

Pharmacokinetic analysis. Pharmacokinetic analysis was performed on the basis of a compartmental as well a noncompartmental description of the observed data. For compartmental analysis, plasma EE concentration-time profiles were individually fitted to the following exponential equation:

\[ C_P = \sum_{i=1}^{n} C_i e^{-\lambda_i t} \]

where \( C_i \) is the \( i \)-th intercept, \( \lambda_i \) is the slope of each of \( n \) first-order rate processes, \( \varepsilon \) is the exponential function (base e) and \( t \) is time. The estimates of \( C_i \) and \( \lambda_i \) were calculated by using a computer program based on the nonlinear, iterative, least-squares regression analysis PCNONLIN 3.0 (Metzler and Weiner, 1989). The equations were fitted to the data by using a weighting factor 1/C², and the initial estimates of the parameters were determined by JANA (Dunne, 1985). The optimum number of first-order rate processes was determined by application of Akaike’s information criterion (Yamaoka et al., 1978a) and graphical analysis of weighted residuals. The other compartmental parameters were calculated by standard methods (Gibaldi and Perrier, 1982).

The model-independent pharmacokinetic parameters were calculated by using expressions based on statistical moments theory (Yamaoka et al., 1978b) and on formulae described by Gibaldi and Perrier (1982). The plasma elimination rate constant (\( \lambda \)) was calculated by least-squares regression of the logarithm of plasma concentration versus time curve over the terminal elimination phase. The area under the plasma concentration-time curve from time zero to the last determined sample time (AUC₀₋₉₅) was calculated by the trapezoidal rule, and the total area under the plasma AUC was determined by adding AUC₀₋₅ to the residual area AUC₅₋₉₅ (calculated from \( C_t \), the last experimental plasma concentration divided by the terminal slope \( \lambda \)). The total body clearance was calculated by dividing the dose by the AUC. The half-life associated with the \( \lambda \) phase (\( t_{1/2,\lambda} \)) was calculated from the quotient 0.693/A. Maximum plasma EE concentration (\( C_{\text{max}} \)) and the time to reach maximum concentration (\( t_{\text{max}} \)) were read directly from the individual plasma concentration-time curves.

Statistical evaluation. All pharmacokinetic parameters were calculated for each animal and the data presented as arithmetic means ± S.D. The data obtained from the three groups were compared for statistical significance by using the one-way analysis of variance, and Duncan’s test was used to evaluate differences between data sets when the results were significant. A \( P \leq .05 \) was taken as the level of significance for all analyses.

Results

Figure 1 shows the plot of the mean plasma concentrations of EE as a function of time after oral administration of 1 mg/kg EE for the three groups studied. This figure shows that the mean plasma concentrations of EE were higher in the control rabbits than in the fiber A and fiber B groups. The values of the pharmacokinetic parameters determined by both compartmental and noncompartmental analyses are given in tables 1 and 2, respectively.

After compartmental analysis, the plasma concentration-time curves were best resolved in all experiments into a two-compartment open model. The EE pharmacokinetic parameters determined by compartmental analysis are given in table 1. The values obtained for \( K_e \) were very similar in the control group (0.151 min⁻¹) and in both A (0.156 min⁻¹) and B (0.167 min⁻¹) groups. The values obtained for AUC were
1.4 times higher in the control group (602.47 ng·min·ml⁻¹) than in the A group (428.44 ng·min·ml⁻¹) and ~1.5 times higher than in the B group (398.19 ng·min·ml⁻¹). C₀max values were also higher in the control group (14.487 ng·ml⁻¹) than in A (10.386 ng·ml⁻¹) and B (9.501 ng·ml⁻¹) groups. With regard to the most representative parameter values of bioavailability, AUC, C₀max and t₀max (Ritchel, 1987; McGilveray et al., 1990), no significant differences were found for t₀max (9.489 min in the control group, 8.570 min in group A and 8.522 in group B), but there were significant differences for C₀max and AUC values between the control group and groups A and B.

k₀ values ranged from 0.1510 min⁻¹ (control group) to 0.1670 min⁻¹ (B group) and β-values from 0.0166 min⁻¹ (A and B groups) to 0.0171 min⁻¹ (control group). There were no significant differences when these parameters, representative of absorption and elimination rates, were compared.

The pharmacokinetic parameters derived from noncompartmental analysis are shown in table 2. In this case, AUC values were also higher in the control group (628.67 ng·min·ml⁻¹) than in A (436.60 ng·min·ml⁻¹) and B (418.60 ng·min·ml⁻¹) groups. Similar results were obtained for C₀max: 16.33 ng·ml⁻¹ (control), 11.35 ng·ml⁻¹ (A group) and 10.61 ng·ml⁻¹ (B group). The t₀max value was 10 min in all three groups. Statistical analysis revealed no significant differences for t₀max values but significant differences for AUC and C₀max when these parameters were compared between the control group and groups A and B. β-values were similar to β-values obtained after compartmental analysis.

**Discussion**

In a previous study performed in rabbits (Fernández et al., 1996), the pharmacokinetics of EE after intravenous administration was also best described by a two-compartment open model. Hümpel et al. (1979) considered EE to behave as a three-compartment open model after an intravenous dose and as a two-compartment model after oral dosing. Furthermore, Goldzieher in 1994, in a revision about this theme, indicated that in most cases the pharmacokinetics of EE after both oral and intravenous administration was best described by a two-compartment open model.

Düsterberg et al., in a study carried out in 1986 in rabbits and with the same dose of EE (1 mg/kg) in a microcrystalline suspension administered by the oral route, found a t₀max value of 15 min, which is similar to our data (10 min). However, these authors reported values for C₀max (2.53 ± 3.8 ng·ml⁻¹) and AUC (78 ± 10 ng·min·ml⁻¹) that were lower than those shown in this study (16.33 ± 3.62 ng·ml⁻¹ and 628.67 ± 136.77 ng·min·ml⁻¹, respectively). With regard to studies in women, peak concentrations of EE after a variety of doses were reached later, between 120 and 240 min after dosing (Goldzieher, 1994).

Studies of fiber-drug interactions are scarce, as indicated by Kritchevsky (1988) and Eastwood (1992) in two revisions performed on this subject. The results obtained in these studies were variable. Thus, Richter et al. (1991) showed that the consumption of a soluble fiber with pectin caused a decrease in the intestinal absorption of the hypolipidemic agent lovastatin. Retuert and Yazdani-Pedram (1992) found that fiber (especially carboxymethylcellulose) induces the decomposition of other drugs like diethylpropion hydrochloride. On the other hand, Astarola et al. (1992) found higher levels of L-dopa when administered with an insoluble fiber that contained wheat bran. However, Uusitupa et al. (1990) did not find any alteration in the absorption of glibenclamide when this drug was administered with guar gum.

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Fiber A</th>
<th>Fiber B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (ng · ml⁻¹)</td>
<td>102.046 ± 15.165</td>
<td>87.968 ± 22.671</td>
<td>81.905 ± 40.345</td>
</tr>
<tr>
<td>B (ng · ml⁻¹)</td>
<td>8.816 ± 5.455</td>
<td>5.293 ± 1.284</td>
<td>5.035 ± 2.263</td>
</tr>
<tr>
<td>α (min⁻¹)</td>
<td>0.1172 ± 0.0108</td>
<td>0.1214 ± 0.0115</td>
<td>0.1194 ± 0.0373</td>
</tr>
<tr>
<td>β (min⁻¹)</td>
<td>0.0171 ± 0.0060</td>
<td>0.0166 ± 0.0025</td>
<td>0.0166 ± 0.0028</td>
</tr>
<tr>
<td>k₀ (min⁻¹)</td>
<td>0.1510 ± 0.0080</td>
<td>0.1560 ± 0.0234</td>
<td>0.1670 ± 0.0204</td>
</tr>
<tr>
<td>k₁ (min⁻¹)</td>
<td>0.0043 ± 0.0097</td>
<td>0.0473 ± 0.0099</td>
<td>0.0457 ± 0.0190</td>
</tr>
<tr>
<td>k₁₀ (min⁻¹)</td>
<td>0.0411 ± 0.0200</td>
<td>0.0388 ± 0.0060</td>
<td>0.0431 ± 0.0155</td>
</tr>
<tr>
<td>AUC (ng · min · ml⁻¹)</td>
<td>602.466 ± 143.386</td>
<td>428.436 ± 69.6388</td>
<td>398.186 ± 110.515*</td>
</tr>
<tr>
<td>Cl (1 · kg⁻¹ · min⁻¹)</td>
<td>1.754 ± 0.501</td>
<td>2.378 ± 0.343</td>
<td>2.672 ± 0.734*</td>
</tr>
<tr>
<td>t₁/2α (min)</td>
<td>5.956 ± 0.562</td>
<td>5.753 ± 0.577</td>
<td>6.613 ± 3.280</td>
</tr>
<tr>
<td>t₁/2β (min)</td>
<td>39.242 ± 10.058</td>
<td>42.487 ± 6.540</td>
<td>42.525 ± 6.571</td>
</tr>
<tr>
<td>t₁/2α/0 (min)</td>
<td>4.513 ± 0.686</td>
<td>4.524 ± 0.686</td>
<td>4.297 ± 0.571</td>
</tr>
<tr>
<td>t₁/2α/0 (min)</td>
<td>13.201 ± 2.955</td>
<td>13.378 ± 0.546</td>
<td>34.602 ± 48.548</td>
</tr>
<tr>
<td>t₀max (min)</td>
<td>9.489 ± 1.167</td>
<td>8.570 ± 0.971</td>
<td>8.522 ± 1.331</td>
</tr>
<tr>
<td>C₀max (ng · ml⁻¹)</td>
<td>14.487 ± 3.186</td>
<td>10.368 ± 2.169*</td>
<td>9.501 ± 2.871*</td>
</tr>
</tbody>
</table>

Values are means ± S.D.
Cl, total body clearance.
* Significant difference from control at P ≤ .05.
With regard to interactions between fiber and estrogens, in recent years many studies have suggested that dietary components like fiber and fat may play a role in the regulation of the enterohepatic metabolism of these compounds, in this way influencing the estrogen levels in the body. Nevertheless, we have not found any studies about the modification of the oral absorption of EE in the presence of fiber.

Several studies suggest that fiber-rich food has a reducing effect on estrogen levels in the blood and urine. Vegetarian diets tend to contain less fat and more fiber than nonvegetarian diets, and these differences appear to influence the metabolism of endogenous estrogens (Howie and Shultz, 1985) and/or the reabsorption of biliary estrogens (Goldin et al., 1982; Adlercreutz et al., 1986). A comparison of the plasma steroid levels between omnivorous and vegetarian men has indicated that estradiol concentrations were lower in the vegetarian group (Howie and Shultz, 1985). However, Fotherby (1990) indicates that whether metabolism of contraceptive steroids differs between vegetarians and nonvegetarians is still controversial.

In many of the situations discussed above, changes occur in serum SHBG concentrations, which were increased in vegetarians. Adlercreutz et al. (1987) indicated that in the presence of lignan precursors and phytoestrogens in fiber-rich vegetables, legumes and grain, a diet rich in fiber may stimulate SHBG synthesis in the liver and may in this way reduce the levels of free estradiol and testosterone in the plasma. However, these changes will not affect EE, which does not bind to SHBG (Akpolviro et al., 1981).

A type of drug-nutrient interaction involves food as a mechanical barrier that prevents drug access to mucosal surfaces. This interaction results in decreased drug absorption and a shortened duration of action (Kirk, 1995). A meal may also influence the bioavailability by the direct binding of drugs by substances in food or by altering luminal pH, gastric emptying, intestinal transit, mucosal absorption, splanchnic-hepatic blood flow and metabolism of the drug (Anderson, 1988). In the present study we found that the presence of fiber (both P. ovata and a mixture of wheat bran, fruit fiber and guar gum) modified the extent of EE absorbed (represented by the pharmacokinetic parameters AUC and C_max) without affecting the rate of the absorption process (represented by k_a and t_max). Taking into account that C_max and AUC are the only parameters that are modified, we think that fiber acts as a mechanical barrier that prevents EE access to mucosal surfaces, resulting in decreased drug absorption and a shortened duration of action.

References

### Table 2

Pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after oral administration of 1 mg/kg EE alone (control) and in the presence of fiber A or B

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Fiber A</th>
<th>Fiber B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda (\text{min}^{-1}) )</td>
<td>0.0181 ± 0.0053</td>
<td>0.0177 ± 0.0030</td>
<td>0.0179 ± 0.0029</td>
</tr>
<tr>
<td>( \text{AUC}_{0\rightarrow\infty} (\text{ng} \cdot \text{min} \cdot \text{ml}^{-1}) )</td>
<td>7.094 ± 5.041</td>
<td>11.156 ± 4.920</td>
<td>10.519 ± 7.772</td>
</tr>
<tr>
<td>( \text{AUC}_{0\rightarrow\infty} (\text{ng} \cdot \text{min} \cdot \text{ml}^{-1}) )</td>
<td>628.668 ± 136.765</td>
<td>436.580 ± 67.702*</td>
<td>418.596 ± 110.772*</td>
</tr>
<tr>
<td>( t_{1/2z} (\text{min}) )</td>
<td>1.660 ± 0.403</td>
<td>2.337 ± 0.334</td>
<td>2.532 ± 0.686*</td>
</tr>
<tr>
<td>( C_{\text{max}} (\text{ng} \cdot \text{ml}^{-1}) )</td>
<td>40.432 ± 9.006</td>
<td>40.004 ± 6.665</td>
<td>39.502 ± 6.469</td>
</tr>
<tr>
<td>( t_{\text{max}} (\text{min}) )</td>
<td>16.326 ± 3.622</td>
<td>11.348 ± 2.201*</td>
<td>16.068 ± 3.311*</td>
</tr>
</tbody>
</table>

* Significant difference from control at P ≤ .05.


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