Original research article

Effect of glucomannan and the dosage form on ethinylestradiol oral absorption in rabbits

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Abstract

To the beneficial properties of dietary fiber in human health, several disadvantages can be added as the possible modification of the bioavailability of other drugs when administered by the oral route. In this study, the influence of glucomannan in the oral bioavailability of ethinyl estradiol (EE), when administered to female rabbits in two different dosage forms (enteric capsules and dispersed in water), was established. To carry out the study, three groups of six animals each were used. All animals received 1 mg kg^{-1} oral EE, and rabbits in groups 2 and 3 received 1.5 g glucomannan dispersed in water or in enteric capsules, respectively, immediately before EE. When comparing the results obtained after the administration of EE/glucomannan dispersed in water with those obtained after the administration of this estrogen without fiber, we can see that C_{max} is 1.4 times lower, AUC 1.9 times lower and that t_{max} is identical (10 min). However, after the administration of fiber in enteric capsules, AUC and C_{max} are higher (4.1 and 7.8 times, respectively) than when the estrogen was administered alone, and also, there is a delay in t_{max} (20 min). After the administration of glucomannan in the enteric capsule, the fiber forms, as in the stomach, a highly viscous solution in the gut that would limit EE access to the mucosal surface delaying its absorption. However, this effect could be compensated by a reduction of EE metabolism in the intestinal wall, leading to a higher absorption of the estrogen.
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1. Introduction

Dietary fiber plays an important role in human health: preventing or treating hyperlipidemia [1], cardiovascular diseases [2], obesity [3], colorectal cancer [4], gastrointestinal disorders [5] and diabetes [6].

Glucomannan is a polysaccharide obtained from the tubers of the *Amorphophallus konjac* plant, a member of the family Araceae found in east Asia [7,8]. The chemical structure of this dietary fiber consists, mainly, of mannose and glucose in the ratio 8:5 linked by $\beta(1{\rightarrow}4)$ glycosidic bonds. This soluble fiber has an extraordinarily high water-holding capacity, forming highly viscous solutions when dissolved in water [9]. It has the highest molecular weight and viscosity of any known dietary fiber [8].

It has been demonstrated that this product is highly effective in the treatment of obesity due to the satiety

sensation that it produces [10,11]; as a remedy for constipation, because it increases the feces volume [12]; as hypocholesterolemic agent, interfering in the transport of cholesterol and of bile acids [10,13]; and as hypoglycemic and hypoinsulinemic agent probably by delaying gastric emptying and slowing glucose delivery to the intestinal mucosa [14].

To the beneficial properties of fiber, several disadvantages can be added, such as the possible modification of the bioavailability of other drugs when administered by the oral route at the same time. There are not many studies regarding this aspect, and the results obtained are controversial: several studies show that fiber can increase the bioavailability of other drugs, other authors find a decrease, or no modification is observed [15–29]. The main mechanism of interaction may be that the fiber retains by adsorption part of the administered dose, which would be higher with a very viscous fiber.

Ethinyl estradiol (EE) is a synthetic estrogen widely used for oral contraception, in the treatment of functional uterine bleeding and menopausal symptoms [30,31]. This estrogen

is usually administered at very low doses (20–35 µg in oral contraception) in order to avoid its adverse effects [32]. Due to the low doses employed, the presence of other drugs and/ or foods in the gastrointestinal tract can modify the bioavailability of this compound, increasing or decreasing its plasma concentrations; this may lead, respectively, to adverse reactions or to therapeutic failures.

In two previous studies performed in female rabbits, it was shown that when EE was administered with two commercial fibers containing a high proportion of insoluble components [(A) wheat bran (76.5%), fiber fruit (12%) and guar gum (2%); (B) *Plantago ovata* seeds (65%) and *P. ovata* seed cuticles (psyllium) (2.2%)] [15] or with guar gum [16], the extent of EE absorbed decreased without modifying the rate of absorption. However, when animals received EE with psyllium, the extent absorbed did not change, but there was a delay in the absorption of the estrogen [16].

This drug is mainly absorbed in the stomach [33]. When EE reaches the intestine, it is widely metabolized, being that the gut wall metabolism is an important factor in the low systemic bioavailability of this estrogen [34,35]. Considering this fact, as well as the aspects reported before regarding how fiber can modify EE absorption, we have designed this study taking into account the following considerations:

- As glucomannan is a very viscous fiber, it can retain part of the oral administered dose of the estrogen when administered at the same time.
- If fiber is administered as an enteric formulation, it would not modify EE bioavailability, which would be the same or very similar to that obtained administering the estrogen alone.

Therefore, the purpose of this study was to establish the influence of glucomannan on the bioavailability of EE, when administered to female rabbits by the oral route in two different dosage forms: enteric capsules and dispersed in water. If glucomannan retains by adsorption part of the EE administered, when the fiber is administered in enteric capsules, the bioavailability of the estrogen would not be modified because EE is mainly absorbed in the stomach.

2. Methods

2.1. Animals

Eighteen healthy female New Zealand White rabbits with a body weight ranging from 2.640 to 3.400 kg were employed. The animals were housed in individual metal cages, which allowed the isolation of feces in a lower container thus avoiding coprophagia, under controlled conditions of temperature ($19\pm2^{\circ}C$), humidity ($55\pm10\%$) and light–dark cycle (12:12 h). The rabbits were maintained under these conditions for at least 1 week before the assay, with free access to water and standard laboratory chow, and they were fasted for 24 h before drug administration, with water ad libitum. Protocols and procedures were approved by the Institutional Animal Care and Use Committee of the University of León.

2.2. Materials

Ethinyl estradiol was obtained from Sigma (St. Louis, MO, USA), sodium pentobarbital from Barcia (Madrid, Spain), heparin from Rovi (Madrid, Spain), Eudragit L from Drogfesa (Mollet del Vallés, Barcelona, Spain) and fiber from Daker-Farmasimes (San Just Desvern, Barcelona, Spain).

2.3. Preparation of the experimental animals

Rabbits were anesthetized with sodium pentobarbital (30 mg kg^{-1} iv body weight) and the left carotid artery 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. Ethinyl estradiol and fiber were administered to the conscious animals approximately 2 h after the catheter was inserted.

2.4. Study design

Rabbits were randomly divided into three groups of six animals each. All animals of the first group received 1 mg kg^{-1} oral EE dissolved in a mixture of water and ethanol (4:1 v/v). Rabbits in the other two groups received 1.5 g glucomannan immediately before drug administration dispersed in water and in enteric capsules, respectively. Enteric capsules were gelatin-coated capsules with Eudragit L. Both the EE and the fiber were administered by gastric intubation. A total of 50-ml water was given to all animals after drug administration.

2.5. Blood sampling

Blood samples (3 mL) were obtained from the left carotid artery through the cannula into heparinized containers, before and at 5, 10, 20, 30, 60, 90, 120, 150, 180, 210 and 240 min after EE administration. Immediately after collection, plasma was separated by centrifugation and stored at $-20^{\circ}C$ until analyzed.

2.6. Plasma concentration of EE

Ethinyl estradiol concentrations in plasma were measured by high-performance liquid chromatography with electrochemical detection following the method previously described [36]. Intraday and interday accuracy and precision were determined by performing the injection of several standard solutions (0.2–100 ng mL^{-1}) each day and the values obtained ranged from 2.26% to 9.85%. The limit of detection of the assay was 50 pg mL^{-1}. Neither heparin nor pentobarbital had any effect on the assay.

2.7. Pharmacokinetic analysis

Pharmacokinetic analysis was performed on the basis of a noncompartmental description of the observed data.
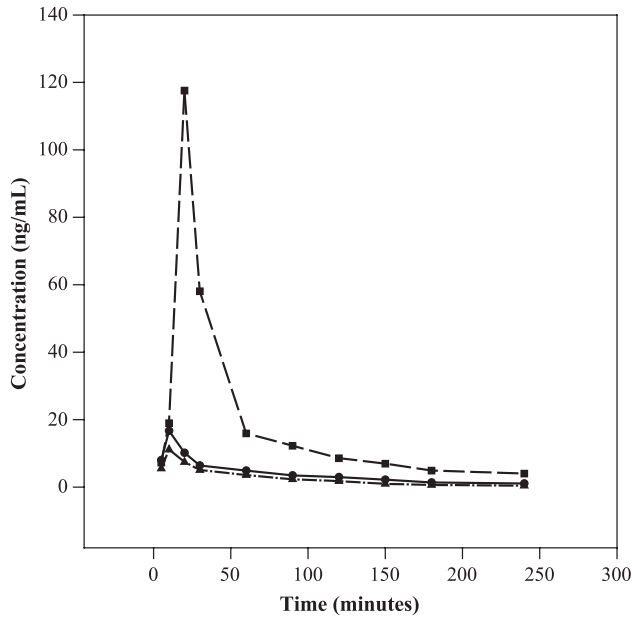
Fig. 1. Mean plasma concentrations of EE in rabbits after oral administration of 1 mg kg^{-1} body weight alone (●) and in the presence of glucomannan dispersed in water (▲) or in enteric capsules (■).

Expressions based on statistical moments theory [37] and on formulae described by Gibaldi and Perrier [38] were used to calculate the model-independent pharmacokinetic parameters. The plasma elimination rate constant λ was determined 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 (AUC$_{0-t}$) was calculated by the linear trapezoidal rule, and the total area under the plasma concentration–time curve (AUC$_{0-\alpha}$) was determined by adding AUC$_{0-t}$ to the residual area AUC$_{t-\alpha}$ (calculated from C_t, the last experimental plasma concentration divided by the terminal slope λ).

The half-life associated with the λ phase ($t_{1/2\lambda}$) was determined from the quotient $0.693/\lambda$. The total body clearance (Cl) was calculated by dividing the dose by the

AUC and the apparent volume of distribution (V_a) as Cl/λ. The maximum plasma EE concentration (C_{max}) and the time to reach maximum concentration (t_{max}) were read directly from the individual plasma concentration–time curves.

2.8. Statistical analysis

All pharmacokinetic parameters were calculated for each animal, and the data are reported as arithmetic mean±standard deviation. The data obtained for the three groups were compared for statistical significance by Kruskal–Wallis test, at $p<.05$, and when the results were significant, Wilcoxon test with Bonferroni correction was used to assess differences between data sets.

3. Results

The plot of mean plasma EE concentration as a function of time after oral administration of 1 mg kg^{-1} for the three groups of rabbits studied is shown in Fig. 1. This figure shows that the mean plasma concentrations of EE were higher, and the t_{max} reached later in the rabbits that had received the fiber in enteric capsule form than in the other two groups.

The values of the pharmacokinetic parameters are given in Table 1.

As bioavailability reflects the extent and the rate at which a drug reaches blood, we compared the most important parameters representing both aspects: AUC (extent), C_{max} (extent and rate) and t_{max} (rate).

When comparing AUC, we can see that the mean value is 7.8 and 4.1 times higher, respectively, when glucomannan was administered in enteric capsules (4884±949 ng min mL^{-1}) than when the fiber was administered dispersed in water (626±148 ng min mL^{-1}) or EE was given alone (1203±204 ng min mL^{-1}); the fraction of dose absorbed after it was ingested as a solution relative to the capsules is 12.8%.

When C_{max} is compared, this is also higher (10.6 and 7.8 times, respectively) when the fiber was administered in enteric capsules (118±17.7 ng mL^{-1}) than when glucomannan was administered dispersed in water (11.1±2.3 ng

Table 1

Pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after oral administration of 1 mg kg^{-1} EE alone (control) and in the presence of glucomannan (dispersed in water or enteric capsules)[a]

Parameters	Control	Dispersed in water	Enteric capsules
λ (min^{-1})	0.0071±0.0002	0.0095±0.0013[b]	0.0062±0.0016[c]
AUC$_{0-t}$ (ng min mL^{-1})	1001±171	584±133[b]	4168±710[b,c]
AUC$_{t-\alpha}$ (ng min mL^{-1})	202±34.8	42.8±19.5[b]	717±406[b,c]
AUC$_{0-\alpha}$ (ng min mL^{-1})	1203±204	626±148[b]	4884±949[b,c]
Cl/F (l kg^{-1} min^{-1})	0.850±0.133	1.68±0.41[b]	0.211±0.040[b,c]
V_a/F (l kg^{-1})	120±20.8	178.8±49.4[b]	36.0±10.9[b,c]
$t_{1/2\lambda}$ (min^{-1})	97.6±2.76	73.8±9.71[b]	120±43.0[c]
C_{max} (ng mL^{-1})	15.2±2.48	11.1±2.32	117±17.7[b,c]
t_{max} (min)	10.0	10.0	20.0

[a] Values are the mean standard±deviation for six rabbits.

[b] Significant differences with control.

[c] Significant differences with dispersed in water.

mL^{-1}) or EE was given alone (15.2±2.48 ng mL^{-1}), with significant differences.

t_{max} reflects that the EE absorption is faster when glucomannan is administered dispersed in water (10 min) or the estrogen is given alone (10 min) than when the fiber is given in enteric capsules (20 min).

4. Discussion

Pharmacokinetic interactions have been studied by many authors, but the assays carried out with dietary fiber are scarce. In addition, the results of these studies are variable, reporting increases, decreases or even no modification in the drug concentrations after the fiber administration [15–24]. Even when the same drug and dietary fiber were used, the data obtained can be contradictory [25–28]. We found only one study about the interaction of glucomannan with other drugs [29]. In that study, it was shown that glucomannan decreased glibenclamide absorption.

With regard to interactions between fiber and EE, Fernandez et al. [15] showed that the extent of EE absorbed (represented by C_{max} and AUC) decreased between 29% and 35%, respectively, when it was administered with two commercial fibers containing 76.5% and 2% (fiber A) and 65% and 2.2% (fiber B) insoluble and soluble fibers, respectively, without modifying the rate of absorption (represented by t_{max} and k_a). In this study, it was concluded that the adsorption of EE to these fibers was the most probable interaction mechanism. The same mechanism has been proposed by Garcia et al. [16] to describe the action of guar gum, a soluble dietary fiber, on EE bioavailability. These authors found a decrease in the extent of EE absorbed (AUC and C_{max} decreased, respectively, 35.6 and 32.2%) in the presence of guar gum without any modification in t_{max} (10 min). However, in the study, authors demonstrated that psyllium had a different effect on EE bioavailability: the extent of EE absorbed did not change (C_{max} decreased 24.1%, AUC increased 4.5%), but there was a delay in the rate of absorption (t_{max}=18.30 min).

When comparing the results obtained after the administration of EE with glucomannan dispersed in water with those obtained after the administration of this estrogen without fiber, we can see that C_{max} is 1.4 times lower, AUC 1.9 times lower and that t_{max} is identical. These results indicate that there is a reduction in the extent of EE absorbed. Due to the low levels of EE used in oral contraceptives, this decrease may lead to ineffective therapeutic concentrations.

However, the data obtained after the administration of fiber in enteric capsules indicate that AUC and C_{max} are higher (4.1 and 7.8 times, respectively) than when the estrogen was administered alone, and also, we can appreciate that there is a delay in t_{max} (20 min).

The changes in the pharmacokinetic parameters of EE after its administration with glucomannan dispersed in water could be explained, in agreement with Fernandez et al. [15]

and Garcia et al. [16], as follows: in the stomach, where this drug is well absorbed [32,33], the fiber forms a highly viscous solution, trapping EE inside it, and therefore, resulting in decreased drug absorption and lower values for C_{max}. This phenomenon will also take place in the upper portions of the intestine, where glucomannan forms a gel that prevents drug access to mucosal surfaces, resulting in a lower AUC.

The data obtained after the ingestion of EE with 1.5 g of glucomannan dispersed in water are very similar to those reported for 3.5 g of guar gum [16] and 4 g of two fibers [(A) wheat bran (76.5%), fiber fruit (12%) and guar gum (2%); (B) P. ovata seeds (65%) and P. ovata seed cuticles (2.2%)] [15].

The results obtained when EE was administered with the fiber contained in enteric capsules were very different to those expected: AUC was much higher (4.1 times) and the rate of absorption was lower (t_{max}=20 min) than when EE was administered alone.

The explanation for this behavior can be based on the following facts:

1. The small intestine has the ability to metabolize drugs by numerous pathways involving both phase 1 and phase 2 reactions [39]. The gut wall metabolism is an important factor in the low systemic bioavailability of EE when it is orally administered [40].
2. Dietary fiber can modify intestinal enzymatic activity, both the gut wall enzymes [41] as well as those of the intestinal content [42]. When the enteric capsule reaches the intestine, the coating begins to dissolve. After its liberation, glucomannan would form a highly viscous solution that would prevent, as in the stomach, EE access to mucosal surface. On the other hand, there could be a reduction of EE metabolism in the intestinal wall that not only compensates the EE trapped by glucomannan, but also results in a higher estrogen absorption.

Another aspect that could have contributed to the results obtained with the enteric capsules could be an increase in the paracellular absorption of EE across the gut wall, avoiding possible degradation by the enzymes located inside the cells of the gut wall [43].

The presence of fiber could increase the paracellular transport of EE, which would contribute to a higher absorption of the unaltered drug. In vitro studies with Caco-2 cells [44] revealed that the relative contribution of the transcellular pathway correlated well with the lipophilicity of the drugs.

In conclusion, we think that when glucomannan is administered in enteric capsules, there is a significant reduction in the intestinal first-pass effect of EE and, therefore, a much higher bioavailability is reached. If further studies confirm this fact, the administration of fiber in enteric capsules could be used to improve the absorption of

drugs with an important intestinal first-pass effect, like EE, for example.

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