ELSEVIER

International Journal of Pharmaceutics 144 (1996) 233–239

international journal of pharmaceutics

Note

Interactions between food components and drugs. Part 4: Influence of pectins and bile salts on propranolol absorption

Gerhard Dongowski[a], Reinhard Neubert[b], Heike Haase[b], Beate Schnorrenberger[a,b]

[a]*German Institute of Human Nutrition Potsdam-Rehbrücke, Arthur-Scheunert-Allee 114-116, D-14558 Bergholz-Rehbrücke, Germany*

[b]*Institute of Pharmaceutics and Biopharmaceutics, Martin-Luther-University, Wolfgang-Langenbeck-Straße 4, D-06120 Halle/Saale, Germany*

Received 13 May 1996; revised 26 August 1996; accepted 4 September 1996

Abstract

Influence of dietary fiber components on drug absorption was studied in vitro using artificial membranes and mucosa preparations from guinea pig in 2-compartment model systems (permeation model and equilibrium dialysis). Well defined pectin preparations with different structural properties were used as food components and propranolol (P) as basic model drug. The retardation of drug was increased with decreased degree of esterification (DE) of pectin. Pectins with a blockwise distribution of free carboxyl groups possessed a more intensive effect than pectins with a random arrangement. It was found that P transport across the artificial lipid membrane was significantly decreased by pectins with a blockwise ($DE \leq 54\%$) or statistical ($DE = 36\%$) distribution of free COOH. Pectins with lower molecular weight giving low viscosities in the medium showed only a small effect on permeation of the drug. Furthermore, the influence of bile acids without and with pectins on P absorption was studied. The bile salts did only influence P transport when they were applied above the critical micellar concentration (CMC). P transport across the lipid membranes increased slightly when pectins were additionally used to the bile salts above the CMC. Transport of P across the guinea pig mucosa was less than the permeation through the artificial lipid membranes. However, the transport of P across the mucosa was significantly reduced by glycocholic acid (GC), by pectin BL-3 as well as by BL-3 and GC in the same way as found using the artificial lipid membranes.

Keywords: Drugs; Propranolol; Food components; Pectin; Dietary fiber; Interactions; Transport; Artificial lipid membranes; Guinea pig mucosa

PII S0378-5173(96)04738-2

1. Introduction

The bioavailability of an orally administered drug is influenced by its properties and by the conditions in the gastrointestinal tract. Food components can act on the release from the dosage form as well as on the absorption of drugs. There are only a few investigations on the mechanisms of influence of standard meals or food components on drug absorption (Pletscher and Peretti, 1990; Neubert et al., 1993). It is necessary to estimate the potential interactions between drugs and the individual food components such as proteins, lipids or carbohydrates as well as the consequences of these interactions with effective in vitro methods.

Dietary fibers consist mainly of plant cell wall polysaccharides. They are not hydrolyzed by the enzymes of the small intestine. Dietary fiber can act on lipid and carbohydrate metabolism, mineral absorption and colon cancer (Vahouny and Kritchevsky, 1986; Kritchevsky and Bonfield, 1995). Some dietary fiber components interact with bile acids and act as native cation exchanger. Dependending on its macromolecular and structural properties dietary fiber can principally influence the absorption of drugs in small intestine. The investigations were studied with pectin as dietary fiber (Neubert et al., 1992, 1995).

In this study the influence of soluble pectins on the permeation behaviour of the basic drug propranolol (P) across artificial lipid membranes and guinea pig mucosa was investigated. Pectins with different structural parameters were used in these experiments. Furthermore, the influence of bile salts (BS) on the transport process of P in absence and presence of pectin was also investigated.

2. Materials and methods

2.1. Pectins

Pectins with random or blockwise distribution of free carboxyl groups and different degree of esterification (DE) as well as pectins with different molecular weight were used. Pectins ST-2 and ST-3 were purified high- and low-esterified pectins (DE 70.8 and 36.0%) without additives (Copenhagen Pectin A/S, Lille Skensved, Denmark) possessing an extensive random (statistical) distribution of free and esterified COOH groups. To prepare the very highly esterified pectin ST-1, selected high-esterified citrus pectin was further esterified using MeOH/conc. H_2SO_4 (12 d) at 4°C. The preparation of pectins BL-1, BL-2 and BL-3 with a blockwise distribution of free COOH was conducted using very highly esterified pectin, which was gradually de-esterified in aqueous solution at pH 7.50 and 30°C under pH-stat conditions with pectin esterase from oranges (Sigma, St. Louis, USA) until calculated amounts of NaOH were consumed (Dongowski, 1995). Pectin series with different molecular weights was prepared by mechanolysis of a high-esterified citrus pectin up to 50 h in dry state in the vibrating mill Vibratom (Siebtechnik GmbH, Mühlheim, Germany) (Bock et al., 1977). The intrinsic viscosity $[\eta]$ of the preparations was between 1078 and 55 ml/g galacturonan. Characterization of pectins is described by Dongowski (1995).

2.2. Bile salts and propranolol

The sodium salts of glycocholic acid (GC) and glycochenodeoxycholic acid (GCDC) were obtained from Sigma Chemie GmbH, Deisenhofen, Germany and taurocholic acid (TC) was purchased from Fluka, Buchs, Switzerland.

The β-blocking agent propranolol was obtained from COM-Pharma-Handel GmbH, Hamburg, Germany.

2.3. Permeation model system

The in vitro permeation model system (Neubert and Fürst, 1989) used consists of donor (DC) and acceptor compartment (AC) separated by an artificial lipid membrane with collodium as matrix and dodecanol as lipid. The experiments were carried out at pH 7.2 (Sörensen phosphate buffer) and 37°C (pectin: 0.5% galacturonan; drug: 1–1.5 mmol/l). The concentration of P was measured in the AC.

Table 1
Effect of statistical (ST) and blockwise (BL) distribution of free carboxyl groups and of degree of esterification of pectin on the permeation of propranolol across artificial lipid membranes

Pectin preparation	Characteristics of pectin			Propranolol permeated after 3 h
	Degree of esterification (%)	Galacturonan, AG (%)	Intrinsic viscosity, [η] (ml/gAG)	(%)
ST-1	92.6	72.81	471	19.58 ± 1.55
ST-2	70.8	65.79	692	17.76 ± 1.64
ST-3	36.0	74.38	395	12.63 ± 1.05
BL-1	71.6	72.73	390	17.03 ± 0.91
BL-2	54.2	71.47	390	12.65 ± 0.59
BL-3	34.5	73.87	337	10.06 ± 0.95
Without pectin				23.85 ± 1.74

Mean ± S.D.; $n = 6$.

2.4. Experiments with mucosa of guinea pig

Small intestine of cerebral dislocated Charles River guinea pigs (weight 300–400 g) was prepared and stored in modified Krebs-Ringer bicarbonate buffer pH 7.2. The experiments were done immediately after preparation of the mucosa with a micro 2-compartment model under oxygen treatment, containing the mucosa on the donator side. The DC contained Sörensen phosphate buffer pH 7.2 and in the AC the Krebs-Ringer buffer pH 7.2 was present.

2.5. Analytical assays

P was assayed spectrophotometrically at 320 nm using spectrometer UV-120-02 from Shimadzu, Duisburg, Germany and using HPLC (Kontron Instruments, Neufahrn, Germany), with pump 323, Knauer-Nucleosil RP 18 column (35 cm, without pre-column) and DAD 440 detector (290 nm) (mobile phase: acetonitril, phosphoric acid and water, 35:0.2:63.8).

3. Results and discussion

3.1. Influence of pectins on the transport of P across lipid membranes

The transport of P was investigated using a 2-compartment model system. Under the experimental conditions the macromolecular pectin was not able to permeate across the lipid membrane. The influence of degree of esterification of pectin and of the distribution of free carboxyl groups of pectin molecules in the DC on the permeation of P is shown in Table 1. The results were compared with experiments without pectin. Generally, the presence of pectin caused a decrease of propranolol amount transported into the AC. Structural parameters of the pectins such as DE or viscosity influence this effect. The drug permeation was lowered only to a relatively small extent in the presence of practically fully esterified pectin ST-1. The high-esterified pectin ST-2 with about 30% free COOH in a random distribution acts in similar manner. On the other hand, in the presence of low-esterified pectin ST-3 (DE 36.0%) a distinct effect on P permeation was found. A similar effect was determined using preparation BL-2 although its degree of esterification was only 54.2%. The reason for this behaviour was found in the different distribution of free COOH in both pectins. Generally, pectins with a blockwise arrangement of free COOH causes the strongest retardation of drug transport. In these linear macromolecules free carboxyl groups exist in blocks beside regions with $COOCH_3$ groups. The consequence is an appearance of continuous negatively charged and polar regions in the pectin molecules.

Table 2
Influence of intrinsic viscosity of pectin on the permeation of propranolol across artificial lipid membranes

Pectin preparation	Characteristics of pectin			Propranolol permeated after 2 h
	Degree of esterification (%)	Galacturonan, AG (%)	Intrinsic viscosity, [η] (ml/gAG)	(%)
M-0	72.8	67.19	1078	8.6 ± 0.7
M-1	72.3	68.23	853	9.9 ± 0.6
M-2	70.7	65.59	720	9.8 ± 0.9
M-5	70.8	68.26	453	11.4 ± 0.8
M-10	70.8	64.99	349	10.6 ± 0.5
M-25	70.8	67.06	161	10.8 ± 0.5
M-50	71.9	66.94	55	10.9 ± 0.3

The number after M shows the time of grinding during preparation of the series.
Mean $\pm$ S.D.; $n = 6$.

Since electrostatic effects are obviously included in these interactions between P and pectins, it was suspected that the non-esterified pectic acid should show the most intensive influence on P transport across lipid membranes. But these experiments could not be evaluated exactly due to the poor or incomplete solubility of pectic acid.

The influence of the molecular weight (viscosity is related to MW) of pectins on drug permeation was also investigated. Only pectins with high intrinsic viscosities [η] (720–1078 ml/g AG) caused a significant retardation of the drug transport. Pectins with a lower molecular weight that means preparations giving low viscosities in the medium of DC showed smaller effects on P in permeation model (Table 2). Additionally to the electrostatic effects caused by free carboxyl groups there is an influence of viscosity of pectin on the interactions with the drug in the medium. The characteristic property of macromolecular pectin, the viscosity, obviously had little influence on the permeation of P across artificial lipid membranes.

3.2. Influence of bile salts and pectin on the transport of P across lipid membranes

An important phenomenon of BS is the critical micellar concentration (CMC) (Small, 1971). Properties and effects of BS are significantly different below or above this concentration. Gasco et al. (1984) found an increase in transport of P across lipid membranes by ion-pair formation in presence of TC. In contrast, below the CMC, BS (1 mM) did not significantly influence the permeation of P in our experiments (Fig. 1).

Furthermore, the transport of P across the lipid membranes in the presence of both pectin and BS was also determined. Two pectins with blockwise distribution of free carboxyl groups (BL-1 and BL-2) and BS had, below the CMC, no effect on transport of P. Only the presence of BL-2 and GC caused a significant increase of the P amount in AC (Fig. 2). Interactions between pectin and BS were found in several studies (Falk and Nagyvary, 1982; Hoagland and Pfeffer, 1987; Pandolf and Clydesdale, 1992; Judd and Truswell, 1985). The most intensive interaction occurred in vitro between BS and pectins with the highest DE (Dongowski, 1995). This may be the reason for the additional effect of BS in the presence of esterified pectins on the permeation of P.

The permeation of P is drastically reduced in the presence of BS above the CMC. This reduction was more than 50% (see Fig. 1). The strongest decrease of permeation of the drug was found in the presence of GCDC, a dihydroxy BS. TC showed a more intensive effect on the P transport than GC (Fig. 3). BS form micelles with a negative charge because of their ionic groups. It appears to be possible that P cations can associate with the acid groups on the surface of the micelles.

Investigations with pectin ST-1 possessing the highest DE and the BS, TC, and GC in concen-

trations above the CMC were also carried out. Under these conditions the amount of permeated drug increased in comparison to the experiments in the presence of BS (Fig. 3). Evidently, the formation of the micelles is disturbed in presence of pectin (Furda, 1990). This led to a reduced association between bile acids and the drug. On the other hand the chosen pectin preparations showed the most intensive interaction with BS in vitro (Dongowski, 1995).

3.3. Transport of P across guinea pig mucosa

Furthermore, the influence of GC and/or pectin BL-3 on P transport across guinea pig mucosa was studied. The transport across the mucosa preparation after 180 min is reduced in comparison to that with the lipid membrane; whereas about 24% of P was transported across the artificial membrane, 6.1% of P were able to pass the guinea pig mucosa. Addition of 12 mM GC led only to a marked decrease in permeation (1.3%). The permeation of P across mucosa was also significantly reduced by 2.3% resp. 2.4% when BL-3 resp. pectin and GC were used. However, the decrease in P transport caused by BL-3 and by BL-3/GC was less in comparison to the reduction of P transport caused by GC alone. The reason for this result could be the ability of biomacromolecules such as pectins to disturb the formation of micelles and, therefore, to reduce the interaction between P and GC (BS) micelles.

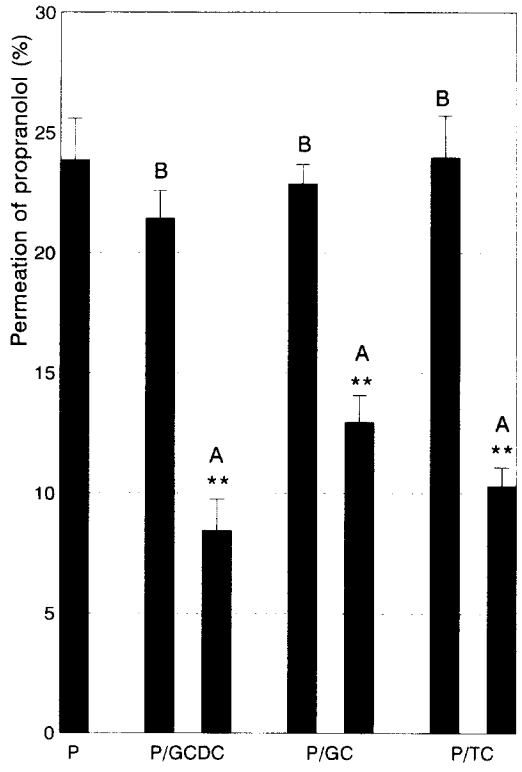

Fig. 1. Effect of bile acids below (B) and above (A) the CMC on permeation of propranolol (P) across artificial lipid membranes (GCDC, glycochendeoxycholic acid; GC, glycocholic acid; TC, taurocholic acid).

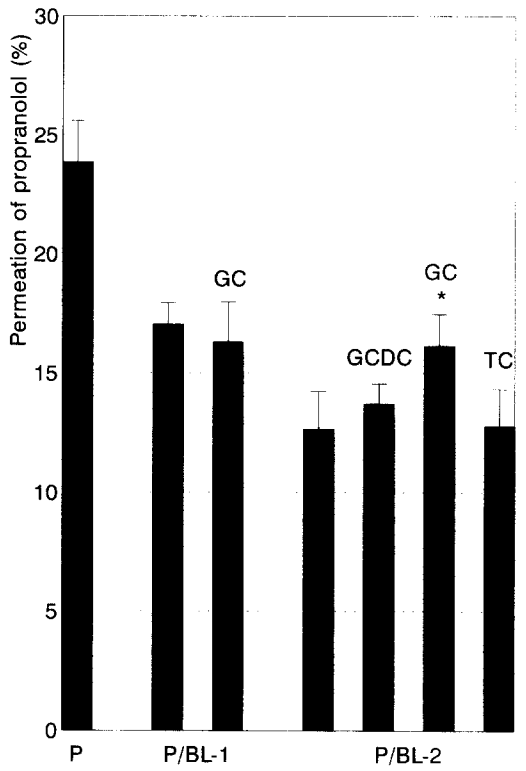

Fig. 2. Transport of propranolol (P) across artificial lipid membranes in presence of bile acids and pectins below critical micellar concentration (BL-1, BL-2, pectins with blockwise distribution of free carboxyl groups; GCDC, glycochenodeoxycholic acid; GC, glycocholic acid; TC, taurocholic acid).

4. Conclusions

Transport and in consequence absorption of drugs are influenced by the conditions in the intestinal tract. It was shown that in addition to the structural properties of the tested pectins and the physicochemical conditions in the milieu, the type and properties of membranes also influence the measurements. Different effects of well characterized food components and different in vitro models were tested in the experiments. The polysaccharide pectin gives viscous solutions, particularly when it is used in an isolated soluble form. The viscosity depends on the molecular weight and the concentration in the system. Furthermore, pectin is a polyelectrolyte. The ion-exchanger properties vary depending on the degree of esterification of the carboxyl groups with methanol as well as on the distribution of free and esterified COOH in the macromolecule (Kohn et al., 1968). Therefore, different interactions with drugs appears to be possible, particularly when drugs have cationic character. Furthermore, a hindrance of drug transport by viscosity of the medium must also be taken into account. It is well known that pectin can interact with bile salts resulting in the appearance of higher BS amounts in colon and faeces (Kay and Truswell, 1977). The mechanism for this effect may be action on the formation of or due to disturbing of the mixed micelles formed during digestion of fat. Drug transport and absorption is also influenced by bile acids and micelle formation because of the lipophilic character of some drugs. It must be taken into consideration that BS above a definite concentration (CMC) forms micelles alone due to changes in the physicochemical properties.

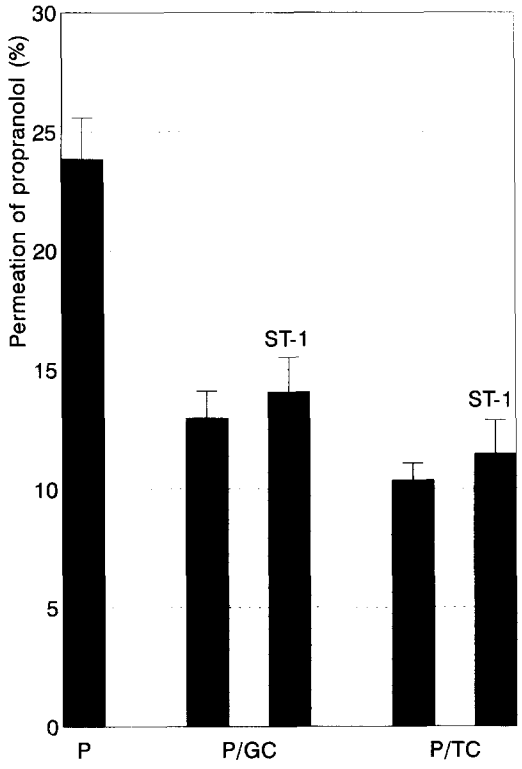

Fig. 3. Transport of propranolol (P) across artificial lipid membranes in presence of bile acids and pectins above the critical micellar concentration (ST-1, pectin with statistical distribution of free carboxyl groups; GC, glycocholic acid; TC, taurocholic acid).

It is possible to study interactions between BS, pectins and drugs using the transport model systems applied. The transport across artificial lipid membranes can be used as a screening method. A natural transport system such as guinea pig mucosa is needed to confirm the results obtained with the artificial lipid membrane.

Further research work has to be focused on two directions. First, it is necessary to investigate these interactions on the molecular level. Secondly, the influence of pectins and bile salts on the pharmacokinetics of drugs such as P has to be studied.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) project NE 427/4-1.

References

Bock, W., Anger, H., Kohn, R., Malovikova, A., Dongowski, G. and Friebe, R., Charakterisierung mechanolytisch abgebauter Pektinpräparate. *Angew. Makromol. Chem.*, 64 (1977) 133–146.

Dongowski, G., Influence of pectin structure on the interaction with bile acids under in vitro conditions. *Z. Lebensm. Unters. Forsch.*, 201 (1995) 390–398.

Falk, J.D. and Nagyvary, J.J., Exploratory studies of lipid–pectin interactions. *J. Nutr.*, 112 (1982) 182–188.

Furda, I., Interaction of dietary fiber with lipids—Mechanistic theories and their limitations. In Furda, I. and Brine, C. (Eds.), *New Developments in Dietary Fiber. Physiological, Physicochemical, and Analytical Aspects*, Plenum Press, New York, 1990, pp. 67–81.

Gasco, M.R., Trotta, M. and Eandi, M., The influence of bile salts on the absorption in vitro and in vivo of propranolol. *J. Pharm. Biomed. Anal.*, 2 (1984) 425–439.

Hoagland, P.D. and Pfeffer, P.E., Cobinding of bile acids to dietary fiber. *J. Agric. Food Chem.*, 35 (1987) 316–319.

Judd, P.A. and Truswell, A.S., The hypocholesterolemic effects of pectin in rats. *Br. J. Nutr.*, 53 (1985) 409–425.

Kay, R.M. and Truswell, A.S., Effect of citrus pectin on blood lipids and fecal steroid excretion in man. *Am. J. Clin. Nutr.*, 30 (1977) 171–175.

Kohn, R., Furda, I. and Kopec, Z., Distribution of free carboxyl groups in the pectin molecule after treatment with pectin esterase. *Coll. Czechoslov. Chem. Commun.*, 33 (1968) 264–269.

Kritchevsky, D. and Bonfield, C. (Eds.), *Dietary Fiber in Health and Disease*, Eagan Press, St. Paul, 1995.

Neubert, R., Fritzsch, B. and Dongowski, G., Wechselwirkungen zwischen Nahrungsbestandteilen und Arzneistoffen. *Pharmazie*, 48 (1993) 723–728.

Neubert, R., Fritzsch, B. and Dongowski, G., Interactions between food components and drugs. Part 3. Interactions between pectin and propranolol. *Pharmazie*, 50 (1995) 414–416.

Neubert, R., Mäder, C. and Dongowski, G., Einfluß von Ballaststoffen auf die Arzneistoffverfügbarkeit aus einer Verapamil-Retardformulierung. *Pharmazie*, 47 (1992) 714–716.

Neubert, R. and Fürst, W., In vitro-Untersuchung des Arzenistofftransportes—Methoden, Möglichkeiten, Grenzen. *Pharm. uns. Zeit*, 18 (1989) 112–122.

Pandolf, T. and Clydesdale, F.M., Dietary fiber binding of bile acids through mineral supplementation. *J. Food Sci.*, *57* (1992) 1242–1245.

Pletscher, W. and Peretti, E., Beeinflußung der Arzneimittelwirkung durch Nahrungseinnahme. *Pharm. Acta Helv.*, 65 (1990) 210–214.

Small, D.M., The physical chemistry of cholanic acids. In Nair, P.P. and Kritchevsky, D. (Eds.), *The Bile Acids. Chemistry, Physiology, and Metabolism. Vol. 1: Chemistry*. Plenum Press, New York, London, 1971, pp. 249–355.

Vahouny, G.V. and Kritchevsky, D. (Eds.), *Dietary Fiber, Basic and Clinical Aspects*. Plenum Press, New York, 1986.