electrostatic interaction. The ΔS changes probably result from hydrophobic binding (O'Reilly 1969).

If the binding of piroxicam involves both hydrogen and hydrophobic bonds at multiple binding sites, we suggest that the drug binds strongly and that the dose required might be such that only a relatively small amount will be available in plasma to achieve this therapeutic effect. It is therefore not surprising that the amount of piroxicam in plasma following a single oral dose of 20 mg produces a good therapeutic effect at plasma level as low as 5 $\mu g\ ml^{-1}$ (Mäkisara & Nuotio 1978) compared with other drugs used for arthritis. In addition, the high protein binding nature of the drug in human plasma has been documented and was confirmed by our observation (Table 2) where about 90% of the drug was bound to the albumins at $0{\cdot}15 \times 10^{-5}$ M at 5 °C.

From Table 1 the progressive reduction in the fraction of piroxicam bound as its free concentration increases (with the amount of protein unchanged) reflects the saturable nature of the binding sites by the drug at high concentrations.

REFERENCES

Bezkorovainy, A. (1963) Biochemistry 2: 10–16

Brogden, R. N., Pinder, R. M., Speight, T. M., Avery, G. S. (1977) Drugs 13: 241–265

Ishizaki, T., Nomura, T., Abe, T. (1979) J. Pharmacokin. Biopharm. 7: 369–387

Klotz,. I. M., Huntson, D. L. (1971) Biochem. 10: 3065–3069

Mäkisara, P., Nuotio, P. (1978) Piroxicam: Proceedings of the Royal Society of Medicine, London, Academic Press, pp 25–30

Nuotio, P., Mäkisara, P. (1977) Piroxicam Symposium: XIV International Congress of Rheumatology, San Fransisco, Calif. June 26–July 1, 1977, abst. p 221

O'Reilly, R. A. (1969) J. Clin. Invest. 48: 193–202

O'Reilly, R. A. (1973) Ann. N.Y. Acad. Sci. 226: 293–308

Scatchard, G. S. (1949) Ibid. 51: 660–672

Wiseman, E. H. (1977) Piroxicam Symposium, XIVth International Congress of Rheumatology, San Fransisco, Calif., June 26–July 1, abst. p 221

J. Pharm. Pharmacol. 1984, 36: 834–836
Communicated June 15, 1984

Inhibition of lipid absorption by non-ionic hydrophobic surfactant Pluronic L-81, and its benzoyl esters

WIESLAW J. BOCHENEK*, REGINA SLOWINSKA, BARBARA KAPUSCINSKA, MIROSLAW MRUKOWICZ, *Department of Cardiology, Medical Academy, Wroclaw, Poland*

The benzoyl ester of Pluronic L-81 (BEP) has been purified on an aluminium oxide column and fractionated on the basis of its mobility characteristics into three fractions, BEP I, II and III. Absorption studies were made with rats fed by gavage a lipid preparation containing [^{14}C]triolein and [^{3}H]cholesterol (control animals) and one of the fractions or Pluronic L-81, or crude BEP. After 4 h the amount of lipid absorbed and transported was calculated as the difference between the dose fed and the amount of lipid recovered from the gastric and intestinal luminal contents and intestinal mucosa. Pluronic L-81 was the most effective in inhibiting absorption of triolein but it did not inhibit absorption of cholesterol. BEP-II was almost as effective in inhibiting the absorption of triolein and also inhibited absorption of cholesterol. Crude BEP was less effective and BEP-I and III had only limited activity. Inhibition of triolein absorption by Pluronic L-81 may be partly related to its delaying action on gastric emptying. As purified BEP preparations had practically no effect on gastric emptying, their inhibiting activity involved intestinal mechanisms of lipid absorption.

We have previously shown that the benzoyl ester (BEP) of the hydrophobic poloxalene, Pluronic L-81, is an effective hypolipaemic agent comparable in activity to the parent compound but less toxic and much better tolerated by laboratory animals (Kapuscinska et al 1982b; Bochenek & Rodgers 1977). Used on a chronic basis in rabbits fed atherogenic diet, BEP decreased serum cholesterol (Kapuscinska et al 1982a). The mechanism by which BEP exerts its hypolipaemic effect is thought to be the same as other hydrophobic surfactants composed of polypropylene oxide and polyethylene oxide which during absorption of fat cause an accumulation of lipid in the intestinal mucosal cells (Brunelle et al 1979; Bochenek et al 1983). This was shown to be associated with decreased secretion of chylomicrons into intestinal lymph (Tso et al 1981). The observed effects are readily reversible in as little as 30 min after discontinuation of surfactant (Bochenek et al 1983).

BEP does not have a uniform structure and experiments have been designed to examine the activities of crude BEP and fractions derived from it and compare these with Pluronic L-81 in absorption studies of lipids in rats.

Materials and methods

Radioactive chemicals, glycerol tri [1-^{14}C] oleate and [1,2(n)-^{3}H] cholesterol were purchased from Amersham Corp., Arlington Heights, IL, and were purified by thin layer chromatography (tlc) before use. Triolein and cholesterol were from ICN Pharmaceuticals, Cleve-

* Correspondence and present address: Division of Gastroenterology, Department of Medicine, Albany Medical College, Albany, New York 12208 USA.

land, OH. Sodium taurocholate was supplied by Calbiochem-Behring, San Diego, CA. Pluronic L-81 was kindly donated by Dr I. Scholka of BASF Wyandotte, MI. The benzoyl ester of Pluronic L=81 (BEP) was synthesized by esterification of Pluronic L-81 as described by Kapuscinska et al (1982a). Aluminum oxide neutral was purchased from Fluka AG, Buchs SG, Switzerland. Other reagents were obtained from POCh, Gliwice, Poland.

Fractionation of BEP. Crude BEP preparation was fractionated on columns using aluminum oxide as a stationary phase and a mixture of benzene–ethyl ether–methanol (25 : 20 : 10) saturated with water as eluent. Crude BEP (25 g) was applied to a column 4·5 × 70 cm packed with fresh aluminum oxide and washed with eluent and eluted at 30 ml h^{-1}. The initial 200 ml of eluent was discarded. Then 10 ml fractions were collected and pooled as follows: Preparation BEP-I, fractions 28 through 42; BEP-II, fractions 43–66; and BEP-III, fractions 67 through 88. The solvent was removed and the preparations were analysed by tlc on aluminum oxide using the same solvent system as above. The hydroxyl group content of Pluronic L-81 and the preparations of BEP was determined by infrared spectroscopy at 2·75–3·1 nm and ester bonds by the hydroxylamine method.

Lipid preparation for absorption studies. Labelled and unlabelled triolein and cholesterol were mixed to provide a specific activity of [^{14}C]triolein of 0·058 μCi m mol^{-1} and of [^{3}H]cholesterol of 0·58 μCi m mol^{-1}. This preparation was used in the control experiments and supplemented with either Pluronic L-81 or one of its ester preparations to provide 50 mg per dose in tests. In the absorption studies lipid mixtures were administered to rats in volumes 0·5–0·58 ml to provide 500 mg of triglycerides and 20 mg of cholesterol. The amounts of lipids administered were calculated separately for each batch of lipid using standards which were analysed for cholesterol and triolein content.

Absorption studies. Buffalo strain rats of either sex, 150–200 g, were fasted for 18 h overnight and then given by gavage one of five lipid preparations. After 4 h the rats were anaesthetized with diethyl ether and the stomach ligated at the oesophago-gastric junction and at the pylorus, and the small bowel at its terminal portion. The stomach was separated from the small bowel contents, which were transferred to Erlenmeyer flasks, as were the colonic contents. The small bowel was rinsed with 10 ml of 4 mm sodium taurocholate in 0·9% NaCl and the intestinal mucosa was scraped off on chilled glass plates. The washings and the mucosa were pooled with the intestinal contents. Lipids from the stomach and intestine were extracted by the procedure of Folch et al (1957). The contents of the colon were extracted using the Van de Kamer method (1949). Samples of the extracts were placed in scintillation vials and the solvent evaported under nitrogen. After addition of 10 ml of a mixture of 6 g PPO and 75 mg POPOP $litre^{-1}$ toluene, the radioactivity was counted with a quench correction determined by external standard ratio. The quantity of lipid was calculated by comparing radioactivity of standards in which triolein and cholesterol contents were determined enzymatically*.

The amount of lipid absorbed and transported out of the intestine was calculated by subtraction of the total amount found in the stomach and intestine from the dose of lipid administered. The results were evaluated statistically by analysis of variance and the significance of difference from the control group was calculated using Duncan's multiple range test.

Table 1. Hydroxyl group and ester bond content in Pluronic L-81 and its ester preparations.

Substance	Hydroxyl groups mmol g^{-1}	Ester bond μmol g^{-1}
Pluronic L-81	1·36	0·00
BEP-I	0·34	1·02
BEP-II	0·28	1·08
BEP-III	0·52	0·84

Results

Esterification of Pluronic L-81 decreased the amount of free hydroxyl groups (Table 1). Of the three purified fractions of crude BEP, BEP II had the fewest hydroxyl groups, and was esterified with an efficiency reaching 80%. BEP-I was esterified to approximately 75%, and BEP-III to 62%. These preparations showed differing mobilities on tlc, Pluronic L-81 being the least mobile, and BEP-I the fastest (Pluronic L-81 < BEP-III < BEP-II < BEP-I). None of the preparations migrated as one spot. Recoveries of ^{14}C-lipid (referred to as triolein) are in Table 2. Four hours after intragastric administration of the lipid mixture, animals treated with Pluronic L-81 retained approximately 36% of this

Table 2. Recoveries of ^{14}C-lipid.

Group	Stomach (mg)	Intestinal lumen and mucosa (mg)	Absorbed and transported (mg)
Control (6)†	30·7 ± 12·5	101·5 ± 40·6	365·5 ± 30·0
Pluronic L-81 (6)	185·5 ± 47·9**	107·8 ± 74·5	224·8 ± 53·1**
BEP (7)	78·6 ± 25·7	178·7 ± 41·4	275·2 ± 39·4
BEP-I (6)	35·2 ± 13·3	123·4 ± 49·3	341·5 ± 38·9
BEP-II (5)	44·4 ± 10·8	208·9 ± 47·9	249·5 ± 27·9*
BEP-III (6)	42·14 ± 13·0	159·9 ± 29·2	308·9 ± 28·5
	$P < 0·001$	NS	$P < 0·01$

Values represent means ± s.d.
† Number of animals per group.
*$P < 0·05$ and **$P < 0·01$: Tested against the control group by Duncan's multiple range test.

* Enzymatic kits for determination of cholesterol were donated by Dr J. Bellm of Boehringer-Mannheim.

material in the stomach, an amount significantly more than controls which retained only 6%. The remaining groups did not differ statistically from the controls, though the average for rats receiving crude BEP was almost twice as high as for the control group.

The intestinal content of triolein, which includes triolein present in the lumen and mucosa, also showed variations. The least amounts were present in controls while the BEP-II-treated animals had the highest values, but the difference was not statistically significant. Absorption and transport of triolein (hereafter referred to as absorption) differed significantly depending on treatment. The most effective absorption was by control rats which absorbed approximately 75% of the dose. The least amounts, 43% and 52% of the dose administered, were absorbed by rats receiving Pluronic L-81 and BEP-II respectively. BEP-I and III gave results similar to controls.

Table 3. Recoveries of ^{3}H-lipid.

Group	(mg)	Intestinal lumen and mucosa (mg)	Absorbed and transported (mg)
Control (6)†	1·38 ± 0·42	9·78 ± 1·33	11·03 ± 1·2
Pluronic L-81 (6)	7·52 ± 1·26**	4·16 ± 2·02**	10·24 ± 1·52
BEP (7)	4·41 ± 1·53*	11·86 ± 4·63*	7·53 ± 1·79
BEP-II (6)	1·57 ± 0·59	11·34 ± 1·77	9·08 ± 1·12
BEP-II (5)	1·78 ± 0·44	11·9 ± 2·44*	6·43 ± 1·35*
BEP-III (6)	2·07 ± 0·59	7·78 ± 1·65*	13·69 ± 2·02
	$P < 0·001$	$P < 0·001$	$P < 0·005$

Values represent means ± s.d.
† Number of animals per group.
*$P < 0·05$ and **$P < 0·01$: Tested against control group by Duncan's multiple range test.

Recoveries of ^{3}H-lipid (cholesterol) are in Table 3. The values for retention in the stomach were essentially as for triolein; however intestinal and mucosal contents of cholesterol were significantly affected by various treatments. Crude BEP and BEP-II-treated animals had significantly higher intestinal contents of cholesterol than the controls, while Pluronic L-81 and BEP-III groups had significantly less. Absorption of cholesterol was significantly different depending on the type of treatment. The control rats absorbed on average 50% (the expected value) while the animals receiving BEP-II absorbed only 32% of the dose, which was a significant difference. Crude BEP also appeared to decrease cholesterol absorption (32% absorbed) but because of wide variations this inhibition was not significant. Absorption of cholesterol from the lipid emulsion containing BEP-III was more effective than in the controls, but did not reach statistical significance.

Discussion

This study showed greater gastric retention of lipid preparations containing Pluronic L-81 than those containing crude BEP which also slowed gastric emptying but to a much lesser extent. The purified BEP fractions had practically no effect on gastric emptying. Slow gastric release of lipid emulsions containing Pluronic L-81 may be partly responsible for the low amounts of triolein absorbed.

Inhibition of lipid absorption by hydrophobic nonionic surfactants of Pluronic type takes place in enterocytes causing accumulation of lipid within intestinal mucosa (Brunelle et al 1979). Inhibition of absorption by ester derivatives also acts at the cellular level. It was demonstrated by us recently that the mechanism involves inhibition of assembly, and secretion into lymph, of large chylomicrons, favouring secretion of small chylomicrons (Bochenek et al unpublished data). Of the three preparations of BEP, only fraction II produced a significant inhibitory effect on absorption of both triolein and cholesterol. As it is most probably the only active component of crude BEP, and also seems to be better tolerated, this or an equivalent fraction should be used in further studies.

REFERENCES

Bochenek, W. J., Rodgers, J. B. (1977) Biophys. Acta 489: 503–506

Bochenek, W. J., Rodgers, J. B. (1980) Exper. Mole. Pathology 33: 223–230

Bochenek, W. J., Kapuscinska, B., Kyriakides, E., Safarik, J., Peng, S. J., and Rodgers, J. B. (1983) Abstract VIII International Symposium on Drugs Affecting Lipid Metabolism, Philadelphia

Brunelle, C. W., Bochenek, W. J., Abraham, R., Kim, D. N., Rodgers, J. B. (1979) Am. J. Dig. Dis. 24: 718–725

Folch, J., Lees, M., Sloan-Stanley, G. H. (1957) J. Biol. Chem. 266: 297–509

Kapuscinska, B., Bochenek, W. J., Pikiewicz, K. (1982a) Atherosclerosis 45: 235–239

Kapuscinska, B., Bochenek, W. J., Slowinska, R., Szczepaniak, M., Unolt, J., Janik, R. (1982b) J. Pharm. Pharmacol. 34: 533–534

Kapuscinska, B., Bochenek, W. J., Kyriakides, E., Friday, S., Rodgers, J. B. (1983) Abstract VIII International Symposium on Drugs Affecting Lipid Metabolism. Philadelphia

Tso, P., Balint, J. A., Bishop, M. B., Rodgers, J. B. (1981) Am. J. Physiol. 241: G487–G497

Van de Kamer, J. H., Huinik, H. T. B., Weyers, H. A. (1949) J. Biol. Chem. 177: 347–355