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Toxicity and hypolipaemic activity of benzoyl ester of polyoxethylene-polyoxypropylene block copolymer (BEP) in rats

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In 1977 Bochenek & Rodgers reported that polyoxyethylene-polyoxypropylene block copolymers, non-ionic surface active agents (Pluronics)†, could affect intestinal absorption of cholesterol and neutral fat. The most effective surfactant in the series for inhibiting lipid absorption was Pluronic L-81, a highly hydrophobic surfactant with molecular weight of 2750, containing 90% polyoxypropylene and 10% polyoxyethylene components. Chronic treatment with this agent at a concentration of 1% of the diet by weight in swine fed a high cholesterol, high neutral fat diet resulted in a decrease of serum cholesterol (Brunelle et al 1979). Dose response studies in rodents indicated, however, that Pluronic L-81 given chronically has a low level of tolerance at higher doses resulting in poor food intake and diarrhoea. Therefore, attempts were made to develop a derivative that would be better tolerated while maintaining the basic activity of the original substance.

Material and methods

Pluronic L-81 was supplied by BASF Wyandotte Corp., Wyandotte, MI, U.S.A. Hydroxyl group content was determined by infrared spectroscopy at 2·75–3·1 μm. Acute toxicity was determined in mice (Swiss albino), 20–25 g and Wistar rats, 140–200 g of either sex. BEP suspended in 1% aqueous solution of methylcellulose was administered orally and intraperitoneally. LD50 values were calculated using the method of Litchfield & Wilcoxon (1949). Long-term studies with BEP were made with male Wistar rats, 175–250 g, housed in light tight cages with the light period 4 p.m.–4 a.m. Animals were randomly assigned to one of three groups. The control group (C) received regular rat chow enriched with 10% soy bean oil making triglyceride content in the diet 15%, and 1% cholesterol. The experimental groups were fed the same diets fed to controls with the supplement of BEP at a dose of 0·5% (group E_1) and 1·5% of the diet by weight (group E_2). These diets were offered freely for 4 weeks. The amount of food taken was measured and stools were collected over 3 days at the end of the study for determination of fat content.

After 4 weeks of feeding in the 6th hour of the dark period, rats were exsanguinated under light ether anaesthesia by puncture of the abdominal aorta. Blood samples were collected into heparinized tubes. At autopsy the entire liver was removed and weighed. Hepatic samples were extracted for lipids (Folch et al 1957). Plasma and hepatic cholesterol were determined by a ferrous oxide method (Zak 1957). Plasma triglycerides were measured enzymatically (using reagent kits donated by Boehringer Mannheim). Total lipid of liver extracts was determined gravimetrically and lipid phosphorus by modification of the Bartlett method (Bartlett 1959). Faecal fat was measured using the procedure of Van de Kamer et al (1949). Statistical evaluation of data was by using Student's *t*-test for non-paired data.

Synthesis of BEP. A mixture prepared by consecutive addition of Pluronic L-81, triethylamine and benzoyl chloride (molar ratio, 1:2·5:2·25) was maintained at 100 °C for 2 h with constant stirring. The resulting product was then diluted with ethyl ether filtered and the solvents removed in a rotary evaporator by increasing the vacuum to 5 mm Hg and the temperature to 150 °C. The esterification procedure was repeated twice more and the final purification included filtering through charcoal, kaolin and albumin oxide. The final product was a clear, odourless, light brown liquid. As determined by infrared spectroscopy, it contained 0·15 mmole of OH groups g^{-1} of ester. Considering the amount of OH groups in Pluronic L-81 (1·3 mmol g^{-1}) the efficiency of esterification procedure was approximately 90%.

Results

Acute toxicity. Table 1 presents the LD50 values for Pluronic L-81 and the ester. In mice the toxicity of BEP by mouth was 2 times lower and intraperitoneal administration 4 times lower than of Pluronic L-81.

Biological activity. During chronic BEP administration, the behaviour of rats and their condition remained stable. There was no difference in the amount of food consumed by the three groups of animals (Table 2). All rats increased

Table 1. Acute toxicity.

	LD50 mg kg^{-1} (mice)		LD50 mg kg^{-1} (rats)	
	Route of administration			
	Peritoneal	Oral	Peritoneal	Oral
Pluronic L-81	420	1830	1140	2300
BEP	1625	3000	>3000*	>5000*

* 100% survival at designated dose.

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Table 2. Body weight gain, food intake and plasma lipid concentration in control and experimental rats.

Group	Body weight gain (g/4 weeks)	Food intake (g/24 h)	Total faecal lipid (% of fat intake)	Plasma total cholesterol (mg/100 ml)	Plasma glycerides (mg/100 ml)
C n = 10	57·2 (7·5)	18·4 (3·4)	10·5 (3·7)	81·6 (13·6)	103·6 (27·2)
E_1 n = 7	34·2 (18·2)	19·1 (6·0)	21·6 (5·6)†	120·4 (12·9)†	125·0 (27·9)
E_2 n = 7	23·0 (25·7)†	16·5 (2·7)	34·9 (10·4)†	67·6 (4·9)*	80·2 (6·2)

Values represent mean (with s.d.).
C Control group taking normal rat chow + 10% soy bean oil + 1% cholesterol.
E_1 Group taking control group diet + 0·5% BEP.
E_2 Group taking control group diet + 1·5% BEP.
* $P < 0·01$.
† $P < 0·001$ in comparison with Control group.
n Number of animals.

Table 3. Liver weight and liver lipid content in control and experimental rats. (Key as in Table 2).

Group	Liver wt (g)	Total lipid (mg g^{-1} liver)	Total cholesterol (mg g^{-1} liver)	Phospholipids (mg g^{-1} liver)
C n = 10	8·8 (1·3)	107 (28·1)	3·5 (1·1)	18·4 (4·8)
E_1 n = 7	9·4 (0·7)	63·5 (29·9)*	1·7 (0·8)*	11·9 (3·4)†
E_2 n = 7	10·0 (1·4)	35·9 (8·3)†	1·2 (0·4)†	10·0 (2·2)†

their initial body weight in the course of the experiment, but rats given the higher dose of BEP had significantly lower weight gains than the controls. BEP was also associated with significantly greater faecal fat excretion in both experimental groups ($P < 0·001$).

The hypolipaemic activity of BEP was observed only in the E_2 group and was reflected mainly by reduction in plasma cholesterol (Table 2). In addition to change in blood lipids rats treated with BEP had less lipid deposited in the liver (Table 3). At autopsy the livers of the control animals appeared fatty while the livers of BEP-treated rats looked normal. Total liver lipid in the E_1 group was twice ($P < 0·01$) and in the E_2 group 3 times lower than in the controls ($P < 0·001$). Hepatic cholesterol and phospholipid contents were also decreased significantly by BEP treatment but increasing the dose from 0·5 to 1·5% did not result in as great a change as observed for total lipid.

Discussion

Esterification of Pluronic L-81 resulted in lowering of the toxicity of the parent compound. Parenteral toxicity was reduced more than oral toxicity indicating that the ester form is less toxic regardless of the route of administration. As expected, mice were more sensitive than rats, in which the LD50 of more than 3000 mg kg^{-1} for intraperitoneal and more than 5000 mg kg^{-1} for oral administration suggest that BEP has no significant acute toxic effects for rats.

Administration of BEP was well tolerated throughout the chronic experiment. Of the two experimental groups, the E_2 group animals had the greater faecal excretion of fat which probably explains why their weight gain was poor while the E_1 group did not differ significantly from control rats.

Plasma lipid concentrations were decreased only in rats given the 1·5% dose of BEP and statistical significance was reached only for plasma cholesterol. Rats given the 0·5% dose of BEP had higher plasma concentrations of cholesterol and triglycerides than the controls. It is unlikely that the slight increase of food intake in these animals would account for the rise in blood lipids. In view of their increased faecal fat excretion it was also thought unlikely that increased efficiency of fat absorption played a role.

Another parameter of lipid metabolism examined was the amount of various lipids in the liver. Total hepatic lipid was significantly decreased in the BEP-treated animals and there seems to be a dose response relationship. Cholesterol and phospholipid content decreased significantly at the 0·5% dose of BEP but increasing the dose to 1·5% brought only a slight additional effect.

Data collected so far indicate that BEP decreases the utilization of dietary fat by interfering with its absorption. Although hypolipaemic effects were observed only at 1·5% dose, the lower dose (0·5%) of BEP still prevented accumulation of lipids in the livers of the experimental animals. Discrepancies of plasma and hepatic lipid values between the two experimental groups suggest that mechanisms other than effects taking place in the gastrointestinal tract influence lipid metabolism.

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