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The use of phenothiazines to enhance the rectal absorption of water-soluble compounds

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The ability of phenothiazines to enhance the rectal absorption of sodium cefoxitin and gentamicin sulphate from aqueous formulations was examined in rats. In the absence of absorption-promoting adjuvants, sodium cefoxitin and gentamicin sulphate bioavailabilities from the rectal compartment were less than 5% of the corresponding intravenous administration. In aqueous microenemas containing 20 mg ml^{-1} phenothiazine, sodium cefoxitin bioavailability increased to 16–62%, while gentamicin sulphate bioavailability increased to 74–146%. The absorption-promoting potential of chlorpromazine and perphenazine was concentration-dependent, with significant increases in gentamicin sulphate absorption occurring with 1 mg ml^{-1} chlorpromazine or 2·5 mg ml^{-1} perphenazine. Maximal gentamicin sulphate bioavailability and serum concentrations were achieved with 10 mg ml^{-1} chlorpromazine or 20 mg ml^{-1} perphenazine. The findings indicate that the phenothiazines, which are well absorbed rectally, also significantly enhance the rectal absorption of water-soluble, poorly absorbed compounds.

The use of the rectal compartment as a site for systemic drug absorption has been largely limited to well-absorbed compounds whose oral use is precluded for a variety of reasons. In recent years, considerable effort has been directed toward identifying agents which increase the rectal absorption of poorly absorbed compounds. Surface-active agents (George et al 1977; Yamasaki et al 1981), chelating agents (Cassidy & Tidball 1967), salicylates (Nishihata et al 1980, 1981a, b), anti-inflammatory drugs (Yaginuma et al 1981a) and basic amino acid salts (Yaginuma et al 1981b) have been shown to increase gastrointestinal permeability to a variety of compounds.

Although the mechanisms of action of these absorption promoters is unknown, direct effects on the biological barrier membrane is a distinct possibility. As such, compounds whose pharmacological activity involves alterations in cellular membrane function may be candidates as promoters of drug absorption across biological membranes.

The phenothiazines, a class of antipsychotic and antiemetic compounds, are well absorbed rectally (Moolenaar et al 1981) and are available in a number of rectal dosage forms (Byck 1975). Chlorpromazine, a classical phenothiazine, has been shown to modulate various membrane functions, including alterations in hepatocyte membrane permeability (Tsao et al 1982) and reversal of cholera toxin induced intestinal secretions (Holmgren et al 1978). Because of the observed membrane effects of various phenothiazines, the potential of chlorpromazine and related compounds to enhance the rectal absorption of poorly absorbed compounds has been examined.

Gentamicin sulphate and sodium cefoxitin were used as target drugs. Both are water-soluble antibiotics which exhibit negligible rectal absorption. Eight phenothiazine compounds were examined for absorption-promoting potential and the concentration-dependence of two representative phenothiazines was determined.

Materials and methods

Animal preparation

Adult male Sprague-Dawley rats (200–250 g) were fasted overnight with free access to water. Anaesthesia was induced by intramuscular injections of 0·5 ml 43% (w/v) ethylcarbamate per 100 g.

Table 1. Effect of phenothiazines (20 mg ml^{-1}) on the rectal absorption of sodium cefoxitin and gentamicin sulphate.

	Per cent bioavailability (mean ± s.d.)			
	Gentamicin sulphate		Sodium cefoxitin	
Phenothiazine	Bio-availability %	Serum peak (μg ml^{-1})	Bio-availability %	Serum peak (μg ml^{-1})
None	1 ± 0·5	0·8 ± 0·2	3 ± 2·1	1·4 ± 0·7
Perphenazine	100 ± 14·3	16·6 ± 1·3	62 ± 8·1	16·6 ± 2·2
Chlorpromazine	107 ± 10·7	20·1 ± 4·4	—[a]	—[a]
Thioridazine	115 ± 19·9	18·9 ± 1·7	38 ± 6·6	14·4 ± 3·2
Triflupromazine	146 ± 25·8	23·7 ± 3·6	58 ± 13·9	19·8 ± 4·4
Fluphenazine	143 ± 8·0	25·6 ± 2·7	49 ± 8·7	14·0 ± 2·4
Promazine	76 ± 6·0	11·7 ± 1·0	41 ± 11·7	13·5 ± 3·7
Trifluoperazine	74 ± 11·5	14·3 ± 1·9	68 ± 1·1	18·2 ± 0·3
Prochlorperazine	76 ± 18·9	18·2 ± 4·7	16 ± 6·4	6·7 ± 2·7

[a] Insoluble.
n = 3 animals.

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Materials

Chemicals, used without further purification, were from: Merck Sharp & Dohme (Mefoxin, perphenazine); Sigma Chemical Co. (gentamicin sulphate, chlorpromazine); Smith Kline & French Labs (prochlorperazine edisylate, trifluoperazine dihydrochloride); E. R. Squibb & Sons (triflupromazine hydrochloride, fluphenazine dihydrochloride); and Wyeth Laboratories (promazine hydrochloride).

Dosage form and sampling procedures

Rectal microenemas (250 µl) were administered at an intrarectal depth of 2·5 cm with a blunt-tip syringe. All microenemas were buffered to pH 6 at a total ionic strength of 0·15 and contained either sodium cefoxitin or gentamicin sulphate at a concentration of 10 mg ml^{-1}. Phenothiaziones were included in the formulations as indicated in the text and tables. Serum samples were collected over 90 min and assayed for gentamicin or cefoxitin. The area under the serum concentration curve (AUC) was calculated by a summation of trapezoid areas. Per cent antibiotic bioavailability was determined by comparing the individual rectal AUC with a mean intravenous AUC based on 2·5 mg antibiotic injections for n = 6 animals. Student's *t*-test was used for statistical comparisons.

Gentamicin sulphate and sodium cefoxitin assays

Gentamicin sulphate in serum samples was assayed by microbiological techniques using *Bacillus subtilis* (ATCC #6633) (Sabath et al 1971). The precision of the gentamicin assay was 98 ± 10·1% for n = 10 replicates at the 7·50 µg ml^{-1} *concentration*, with a lower detection limit of 0·5 µg ml^{-1}. Sodium cefoxitin in serum samples was assayed by reverse phase high pressure liquid chromatography with sodium cefmetazole as the internal standard. Sodium cefmetazole (100 µg ml^{-1} aqueous cefmetazole, prepared daily) was added to each 100 µl serum sample. The serum samples and standards were deproteinized by precipitation with 75 µl of 10% trichloroacetic acid. Separation was performed with a Brownlee 10 µm RP-8 column (3·9 × 250 mm). The eluting solvent consisted of 100 ml pH 6·86 buffer (Fisher Gram-Pak), 110 ml methanol and 10 ml acetonitrile diluted to 1000 ml with water. At a flow rate of 3·0 ml min^{-1}, cefoxitin and cefmetazole in the eluents were detected using absorbance at 254 nm. A linear relation existed between standard concentrations in serum and drug peak heights, with virtually complete separation of the cefoxitin and cefmetazole peaks. The lower limit of the cefoxitin assay was 0·1 µg ml^{-1} with a precision of 103 ± 6·04% for 10 samples at a standard concentration of 6·25 µg ml^{-1}.

Results

Absorption studies

Rectal gentamicin sulphate and sodium cefoxitin absorption, which was negligible from aqueous formulations in the rat model, was significantly increased ($P < 0·05$) in the presence of a series of phenothiazine compounds at a concentration of 20 mg ml^{-1} (Table 1). With each of the eight phenothiazines examined, gentamicin sulphate absorption was increased to a greater extent than sodium cefoxitin absorption ($P < 0·05$). Data were not obtained for chlorpromazine and sodium cefoxitin since, at the concentrations employed, an insoluble suspension was formed.

Concentration dependence

The concentration dependence of the chlorpromazine and perphenazine potentiation of rectal absorption was examined using gentamicin sulphate as the target compound (Table 2). Significant ($P < 0·05$) increases in gentamicin sulphate absorption were observed with 1 mg ml^{-1} chlorpromazine or 2·5 mg ml^{-1} perphenazine. Maximal gentamicin sulphate bioavailability (100%) and serum concentration were achieved with 10 mg ml^{-1} chlorpromazine or 20 mg ml^{-1} perphenazine.

Discussion

The ability of various phenothiazines to strongly enhance the rectal absorption of two water-soluble, poorly absorbed compounds has been clearly demonstrated in this study. The absorption-promoting potential of chlorpromazine and perphenazine was concentration dependent, with significant increases in absorption occurring at phenothiazine concentrations as low as 1–2·5 mg kg^{-1} body weight in the rat model. The fact that the absorption of both basic (gentamicin) and acidic (cefoxitin) compounds was enhanced by phenothiazine treament may indicate a general increase in mucosal membrane permeability.

The greater absorption of gentamicin sulphate relative to sodium cefoxitin in response to phenothiazine treatment may be due to differences in membrane permeability intrinsic to each compound, acidic versus basic properties of the two antibiotics, or relative antibiotic solubilities in the presence of the phenothiazines. The formation of an insoluble suspension with chlorpromazine and sodium cefoxitin may indicate decreased cefoxitin solubility in the presence of certain phenothiazines. This in turn could lead to reduced absorption relative to that observed with gentamicin sulphate. The possibility of physicochemical interactions between the phenothiazines and the target compounds being involved in the absorption process cannot be assessed from the data.

Gentamicin sulphate bioavailability in the presence of triflupromazine and fluphenazine significantly exceeded 100%. As a function of the rate and extent of absorption, bioavailability obviously cannot exceed 100%. The bioavailabilities in response to these two phenothiazines probably indicate inaccuracy in the microbiological assay at the high end of the concentration range. However, the possibility that the pheno-

Table 2. Concentration dependence of chlorpromazine and perphenazine potentiation of rectal gentamicin sulphate absorption.

Phenothiazine (mg ml^{-1})	Gentamicin sulphate bioavailability: Chlorpromazine Bioavailability %	Chlorpromazine Serum peak (μg ml^{-1})	Perphenazine Bioavailability %	Perphenazine Serum peak (μg ml^{-1})
0	1 ± 0·5	1·1 ± 0·4	1 ± 0·5	0·8 ± 0·2
1	30 ± 10·1	6·8 ± 2·3	16 ± 18·6	2·3 ± 1·9
2·5	44 ± 9·9	8·3 ± 2·0	31 ± 13·8	5·5 ± 0·6
5	60 ± 4·4	11·4 ± 0·8	49 ± 11·7	8·7 ± 0·9
10	103 ± 3·1	17·5 ± 0·4	86 ± 35·8	13·1 ± 0·4
20	107 ± 10·7	20·1 ± 4·4	100 ± 14·3	16·6 ± 2·2

n = 3 animals (mean ± s.d.).

thiazine in the rectal formulation might alter kidney or liver clearance of gentamicin sulphate cannot be excluded.

Chlorpromazine has been shown to inhibit cyclic AMP-mediated intestinal secretion in mice (Holmgren 1978). The antisecretory effect of chlorpromazine was observed following hormonal or cholera toxin-induced increases in adenylate cyclase activity. Apparently, chlorpromazine inhibited both adenylate cyclase and protein kinase activities of the intestinal mucosal membrane. Since chlorpromazine affected only intestinal fluid secretion, but not fluid absorption (Holmgren 1978), it is not evident whether its effects on adenylate cyclase or protein kinase could be involved in promoting its absorption in the present study. However, in view of the potent effects of adenylate cyclase and protein kinase activity on membrane function and permeability (Kimberg 1974; Greengard 1976), their possible involvement in intestinal drug transport cannot be precluded.

The membrane stabilizing effect of chlorpromazine and other phenothiazines (Seeman 1972) is apparently responsible for many of the non-specific membrane alterations associated with phenothiazine treatment, especially alteration of membrane-bound enzyme activity. The importance of this membrane stabilizing response in modifying drug transport across intestinal epithelial cells is unknown.

Calcium ions serve as important regulators of many membrane transport systems (Frizzel 1977; Ilundain & Naftalin 1979). Chlorpromazine, known to interact with membrane-bound and intracellular calcium (Seeman 1972), may influence intestinal drug absorption through alterations in calcium-dependent membrane processes.

Although a mechanism of action for the phenothiazines in promoting rectal drug absorption cannot be identified from the present data, it should be noted that a relatively general effect is observed in that all eight phenothiazines significantly increased drug absorption. This probably indicates the existence of a unifying mechanism which the phenothiazines exert on the mucosal cell layer.

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