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The effect of some polyene macrolides on absorption from the small intestine in the rat

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The effect of three polyene macrolides, candicidin, amphotericin B and nystatin on the absorption of [^{3}H]cholesterol was studied in the rat by using the *in situ* gut loop perfusion technique. Chronic treatment with candicidin and its presence at various concentrations in the gut loop perfusion experiments inhibited [^{3}H]cholesterol absorption although a smaller effect was also obtained with amphotericin B and nystatin at higher concentrations. A similar but much less pronounced action of candicidin was also observed on the absorption of [^{3}H]corticosterone and [^{14}C]phenytoin.

The group of compounds known collectively as the polyene macrolides has long been recognized as possessing antibiotic activity against fungi and yeast. It has been reported that several of the polyene macrolides, given orally at concentrations of 5–20 mg kg^{-1} day^{-1} for periods of up to one month, produce varying degrees of reduction in the size, and changes in the texture, of both the normal and hyperplastic canine prostate (Gordon & Schaffner, 1968; Schaffner, 1972). A similar effect has recently been observed after treatment with candicidin in the rat and the hamster (Fu, 1974; Wang, 1974). Studies with candicidin have shown that it is not absorbed from the gut (Schaffner, 1972) and there are no signs of histopathologic toxicity in the prostate itself. It seems probable, therefore, that candicidin could effect the prostate secondarily to its action in the gut. It has been postulated (Schaffner & Gordon, 1968; Schaffner, 1972) that candicidin binds with exogenous cholesterol in the lumen of the gut, thereby impairing its intestinal absorption and that as a result, the size of the body cholesterol pool is reduced, especially effecting any accumulation of cholesterol in the prostatic tissue. While the role of exogenous cholesterol in the development and progress of benign prostatic hyperplasia in man is far from clear the high concentration of cholesterol in the prostatic tissue is not in doubt (Swyer, 1942; Cannon, personal communication).

In order to establish whether candicidin influences the absorption of cholesterol and other compounds *in vivo*, we have used the *in situ* gut loop technique (Doluisio, Billups & others, 1969) in the rat.

MATERIALS AND METHODS

[4-^{14}C]Cholesterol (sp.act. 6 mCi mmol^{-1}); [7-^{3}H]cholesterol (sp.act. 9·4 Ci mmol^{-1}) and [1,2-^{3}H]corticosterone (sp.act. 30 Ci mmol^{-1}) were purchased from the Radiochemical Centre, Amersham and [^{14}C]phenytoin (sp.act. 5 mCi mmol^{-1}) from NEN Chemicals, Germany. The radioactive materials were diluted with the non-radioactive compound so that approximately 1 mg contained 1 μCi in the case of cholesterol and corticosterone and 0·25 μCi in the case of phenytoin. Corticosterone, cholesterol and glycocholic acid were purchased from Sigma Chemical Co. Ltd., oleic acid from Hopkin and Williams, and butyl PBD [2-(4′-t-butylphenyl)-5-(4″-biphenylyl)-

1,3,4-oxadiazole] from Intertechnique Ltd. Amphotericin B and nystatin were gifts from E.R. Squibb and Sons Ltd., and candicidin from Pharmax Ltd. All other chemicals were Analar grade reagents from BDH Ltd.

Male Wistar albino rats, 300–350 g, were starved for 18 h before being anaesthetized with an intraperitoneal injection (60 mg kg^{-1}) of pentobarbitone (Abbot). When they were deeply anaesthetized an incision (3″) was made along the ventral midline. The duodenum was ligated below the bile duct and a small slit made below the ligature. A plastic cannula (2·5 mm i.d.) attached to a 20 ml plastic syringe full of 0·9% saline at 37° was inserted into the duodenum and held in place with a double ligature. The distal gut was ligated above the ileocaecal junction and cut cephalad to the ligature. The intestinal lumen was cleared of particulate matter by washing with the saline in the syringe at the duodenal end. A second cannula attached to a syringe with the plunger removed was then inserted into the ileal end of the intestine and secured in place with a double ligature. The saline remaining in the gut was expelled into the duodenal syringe by pushing air through the lumen from the second syringe. 10 ml of the perfusion mixture containing 1 mg (1·0 or 0·25 μCi) of the compound under investigation, 300 mg glycocholic acid, 100 mg glucose and 0·4 ml oleic acid in 0·9% saline and maintained at 37% was rapidly introduced into the intestine through the duodenal syringe. At appropriate intervals (15 and 60 min for 2 and 4 h perfusions respectively) the gut loop contents were emptied alternately into the two syringes and 0·1 ml aliquots were taken for isotope counting. Any reduction in the volume of the perfusate (0·6–1 ml over 2 h) which could not be accounted for by removal of the counting aliquots was made good by addition of 0·9% saline at 37°. Anaesthesia was maintained by further small injections of pentobarbitone as necessary.

Faeces samples were homogenized in 2 volumes of distilled water using a Polytron blender and soaked overnight at 4°. They were then extracted twice with 2 volumes of light petroleum (60–80°) and the radioactivity in both the ether extract and the aqueous phase determined. Blood radioactivity was measured in 50 μl samples, taken from the tail vein, which were treated by dropwise addition of 100 vol hydrogen peroxide until colourless, and then counted. The total amount of blood in the body was assumed to be 7% of the body weight.

The scintillation fluid used was a 2:1 mixture of toluene and Triton X-100 containing 0·6% butyl PBD and the counter used was a Packard Model No. 2425. The external standard ratio method was used for quench correction.

The intestinal half-life ($t_{\frac{1}{2}}$) and the absorption rate constant (Ka min^{-1}) were calculated from the semilog plot of time versus the radioactivity remaining in the rat intestinal lumen expressed as a percentage of the amount at zero time.

RESULTS

The rate of disappearance of cholesterol from the rat gut loop was greatly increased by the presence of glycocholic acid. When this bile acid was excluded from the perfusion medium only 14% of the [^{3}H]cholesterol was absorbed in 4 h compared with 59% when it was present. Candicidin reduced the absorption of cholesterol at 2 h and the effect was directly related to the amount of the drug present (Table 1). The results at 4 h were similar; when the amount of candicidin was increased to 20 mg the absorption of cholesterol in 4 h was reduced to 9%. A smaller impairment of cholesterol absorption from the rat gut at both 2 and 4 h was also observed with amphotericin B and nystatin but only at the higher concentration.

Table 2 shows the effect of chronic administration of candicidin (20 mg kg^{-1} day^{-1}; for 6 weeks) on concentrations of radioactivity in the faeces after a single oral dose of 1 mg cholesterol containing 1 μCi[^{14}C]cholesterol. No candicidin was given on the day of the experiment. Blood concentrations of radioactivity were significantly reduced ($P < 0.01$) 5½ and 7 h after the [^{14}C]cholesterol administration in the candicidin-treated animals compared with the control group. The opposite pattern was observed in the excretion of radioactivity in the faeces during the first 24 h. Twenty-two percent of the radioactivity administered was recovered in the light petroleum extractable fraction of the faeces from the candicidin-treated rats against only 7% in the controls.

The *in situ* absorption experiments with [^{3}H]corticosterone and [^{14}C]phenytoin show that, unlike cholesterol, these two compounds were absorbed very rapidly from the intestine (Table 1). 80% of the [^{3}H]corticosterone and 90% of the [^{14}C]phenytoin

Table 1. *The effect of some polyene macrolides on the intestinal absorption kinetics in the rat.*

Intestinal absorption of	Polyene macrolide added (mg)	Amount absorbed in 2 h (%)	Intestinal half life ($t_{\frac{1}{2}}$, min)	Absorption rate constant (Ka $min^{-1} \times 10^2$)
[^{3}H]Cholesterol	—	40 ± 4		
	Candicidin 1	35 ± 1		
	,, 2	33 ± 1		
	,, 5	29 ± 2		
	,, 10	20 ± 3		
[^{3}H]Corticosterone	—	80 ± 2	46 ± 3	1·5 ± 0·1
	Candicidin 1	76 ± 1	56 ± 1	1·2 ± 0·1*
	,, 2	76 ± 1	59 ± 0**	1·2 ± 0 *
	,, 5	71 ± 1	68 ± 1**	1·0 ± 0 **
	,, 10	62 ± 5	88 ± 5**	0·8 ± 0·1**
	Amphotericin B 2	76 ± 3	48 ± 4	1·5 ± 0·1
	,, 10	74 ± 6	53 ± 9	1·4 ± 0·3
	Nystatin 2	77 ± 2	55 ± 2	1·3 ± 0·1
	,, 10	80 ± 1	39 ± 3	1·5 ± 0·1
[^{14}C]Phenytoin	—	90 ± 1	34 ± 1	2·1 ± 0·1
	Candicidin 10	81 ± 1	48 ± 2**	1·5 ± 0·1**

The disappearance of 1 mg (1 μCi) of the compound concerned was followed by the standard gut loop technique. Each value is the mean ± s.e.m. from four experiments. Student's *t*-tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. *The changes in the excretion of radioactivity in the faeces after an oral dose of [^{3}H]cholesterol in rats which had received candicidin for 6 weeks.*

	Percentage of radioactive dose excreted			
	Ether extract		Water soluble	
Day	Control	Candicidin treated	Control	Candicidin treated
1	7·3 ± 1·9	21·7 ± 3·9	1·4 ± 0·6	0·7 ± 0·2
2	12·2 ± 2·1	5·0 ± 0·7	2·2 ± 0·4	0·9 ± 0·1
3	4·5 ± 0·9	1·2 ± 0·1	1·1 ± 0·2	0·7 ± 0·3

Candicidin and cholesterol were given at concentrations of 20 mg kg^{-1} day^{-1} and 4 mg kg^{-1} respectively. The last dose of candicidin was administered 24 h before the [^{3}H]cholesterol. Each value is the mean ± s.e.m. from four animals.

were absorbed during a 2 h period giving intestinal $t_{\frac{1}{2}}$ values of 46 $\pm$ 3 and 34 $\pm$ 1 min respectively. Amphotericin B, nystatin and candicidin at low concentrations produced little change in corticosterone absorption. Larger doses of candicidin (10 mg) reduced ($P < 0{\cdot}01$) corticosterone absorption over 2 h, from 80 to 62% and had a small though still significant ($P < 0{\cdot}01$) effect on phenytoin absorption.

DISCUSSION

Bile acids promote the absorption of cholesterol *in vivo* (Siperstein, Chaikoff & Reinhardt, 1952) and also in the *in situ* gut loop technique suggesting that the latter may provide a useful experimental model for cholesterol absorption studies. Candicidin, at all concentrations, inhibited cholesterol absorption; similar but smaller effects were also obtained with two other polyene macrolides, amphotericin B and nystatin at higher concentrations.

In agreement with Borgström, Lindhe & Wlodawar (1958) we found that 7 h after oral [^{14}C]cholesterol only a very small proportion (9·6 $\pm$ 0·4%) of the radioactivity administered was recoverable from the blood.

The lower blood concentrations and increased faecal excretion of radioactivity in the candicidin-treated rats in the first 24 h after a dose of [^{14}C]cholesterol suggest that absorption of cholesterol was impaired even in the absence of freshly administered candicidin. It cannot be ascertained from the present study whether this reduction was due to candicidin accumulation in the gut or to an interaction between the drug and the intestinal mucosa temporarily changing its permeability and absorptive capacity. That candicidin action is not exclusively due to its physico-chemical interaction with exogenous cholesterol in the lumen of the gut as had been suggested (Schaffner, 1972), is shown by its effect at high concentration on corticosterone and phenytoin absorption.

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