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The absorption and excretion of carbenoxolone in man

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Blood, urine and bile concentrations of carbenoxolone have been measured after oral dosage to patients. The compound is rapidly absorbed attaining high blood concentrations and, as with rats, is excreted mostly in the bile, with only traces (2% of the dose) appearing in the urine. Blood concentrations of the drug generally exhibit two maxima, at 1–2 and 3–6 h after dosage, which are taken to indicate enterohepatic circulation of the biliary-excreted conjugates. As absorption of orally administered carbenoxolone is so rapid and does not occur when the gastric contents have pH >2, it is inferred that the major site of absorption is the stomach. The high blood concentrations of carbenoxolone (60% of the dose present in the blood) are probably due to a high degree of binding of the drug to the plasma proteins. In contrast to the rat, in which carbenoxolone is largely hydrolysed to β-glycyrrhetic acid before absorption, in man carbenoxolone is absorbed largely unchanged and is excreted in the bile as the glucuronide. Gastric absorption of carbenoxolone may be necessary for the increased production of gastric mucus observed with the drug and hence is necessary for its gastric ulcer-healing activity.

Carbenoxolone, a drug used in the treatment of gastric and duodenal ulcer (Robson & Sullivan, 1968), has a mode of action which appears to involve the production of increased amounts of gastric mucus (Goodier, Horwich & Galloway, 1967). The drug labelled with ^{14}C is absorbed from the gastrointestinal tract in the rat and metabolized to conjugates of carbenoxolone and β-glycyrrhetic acid, the hydrolysis product of carbenoxolone. Most of the drug and its metabolites are excreted in the bile of rat and only negligible amounts are excreted in the urine (Iveson, Parke & Williams, 1966).

The extent and site of absorption, and the rate and route of excretion, of the drug have now been studied in patients with gastric ulcers to determine whether these parameters are similar to those observed in the rat and thus to ascertain whether the mode of action of the drug is topical or systemic.

EXPERIMENTAL

Determination of carbenoxolone

The concentration of total carbenoxolone, namely carbenoxolone and any of its known conjugates and metabolites, present in whole blood (heparinized), urine, gastric contents and bile, collected at intervals from patients with gastric ulcer receiving carbenoxolone, was determined spectrophotometrically (Coleman & Parke, 1963). The carbenoxolone sodium was taken as a single dose of 200 mg (50 mg tablets) with a little water on an empty stomach.

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Determination of carbenoxolone and its metabolites excreted in bile

Patients with T-tube drainage after cholycystectomy were given a single oral dose of 100 or 200 mg of carbenoxolone sodium on an empty stomach and the total carbenoxolone of the bile collected by drainage determined (Coleman & Parke, 1963).

The bile was also examined for metabolites by chromatography on thin-layer plates (0·25 mm) of fluorescent silica gel HF 254 (Merck) developed in the solvent system A: acetic acid–1,2-dichloroethane–n-butanol–water (4:4:1:1 by vol.). Carbenoxolone and its metabolites (see Table 1 for Rf values) were detected by their characteristic quenching of the background fluorescence when viewed under ultraviolet light (Chromatolite lamp). Glucuronide conjugates were detected by spraying the chromatograms with a mixture of 0·2% w/v naphthoresorcinol in acetone and 9% v/v aqueous phosphoric acid (5:1 by volume) and heating at 120° for 10 min; glucuronides appeared as violet spots on a buff background. The C-30 ester glucuronide of carbenoxolone and the 3-*O*-sulphate ester of β-glycyrrhetic acid were identified by comparison with authentic synthetic materials (Iveson & others, 1966; Iveson & Parke, 1970).

Determination of carbenoxolone and β-glycyrrhetic acid in mixtures

Carbenoxolone and β-glycyrrhetic acid were quantitatively determined in mixtures by a modification of the method of Coleman & Parke (1963). Solutions of mixtures in chloroform were chromatographed on thin-layer plates (0·25 mm) of silica gel HF245 in solvent system B: n-butanol–aqueous ammonia solution (s.g. 0·88) (5:1 by volume) (see Table 1 for Rf values). The bands corresponding to carbenoxolone

Table 1. *Rf values of carbenoxolone and its metabolites.* Compounds were chromatographed on thin-layer plates (0·25 mm) of silica gel HF 254 developed in the solvent system A: acetic acid–1,2-dichloroethane–n-butanol–water (4:4:1:1 by vol); or solvent system B: n-butanol–aqueous ammonia (s.g. 0·88) (5:1 by vol)

Compound	Rf value in Solvent A	Rf value in Solvent B
Carbenoxolone	0·95	0·15
Carbenoxolone-30-glucuronide	0·85	—
β-Glycyrrhetic acid	0·95	0·40
β-Glycyrrhetic acid-3-sulphate	0·75	—
β-Glycyrrhetic acid-30-glucuronide	0·80	—
β-Glycyrrhetic acid diglucuronide	0·60	—

and β-glycyrrhetic acid were located by reference to authentic materials under ultraviolet light (Chromatolite lamp) and were separately excised, transferred to tapered centrifuge tubes and the triterpenoids quantitatively eluted from the silica gel by shaking with 3·0 ml of ethanol. The silica gel was deposited by centrifugation and the extinction of the solutions of carbenoxolone and β-glycyrrhetic acid was determined at 248 nm (Coleman & Parke, 1963).

Recoveries of known amounts of carbenoxolone and β-glycyrrhetic acid (5–50 μg) separately, and in various mixtures of both, were all 100 ± 5% of the added amounts.

Effect of gastric pH on absorption of carbenoxolone

A single oral dose of carbenoxolone sodium (150 mg) was administered together with 120 ml 0·05M sodium citrate buffer, pH 8·5, to three normal subjects. Samples (5 ml) of gastric contents were aspirated at intervals and samples of blood (heparinized) were taken simultaneously. The total carbenoxolone content of the blood was determined as previously described.

The pH values of the samples of gastric aspirates were measured, and the concentration of carbenoxolone and its hydrolysis product, β-glycyrrhetic acid, were quantitatively determined by the method previously described for mixtures of these two terpenoids. Gastric aspirates (3 ml) were adjusted to pH <1 with 2N HCl and the total carbenoxolone plus β-glycyrrhetic acid was extracted with 2 × 2·5 ml portions of chloroform. The bulked chloroform extracts from each sample of gastric aspirate were dried over anhydrous sodium sulphate, filtered and concentrated to 1·0 ml. Samples of 0·2–1·0 ml of the concentrated chloroform extract were applied to thin-layer plates of silica gel HF 254 together with reference spots (50 μg) of authentic carbenoxolone and β-glycyrrhetic acid. The two compounds were then separated and quantitatively determined.

RESULTS

Concentration of carbenoxolone in blood, urine and gastric contents

The concentration of total carbenoxolone present in the blood at various time intervals after oral dosage of patients with carbenoxolone sodium is summarized in Fig. 1. The characteristic pattern of blood concentration shows an initial maximum

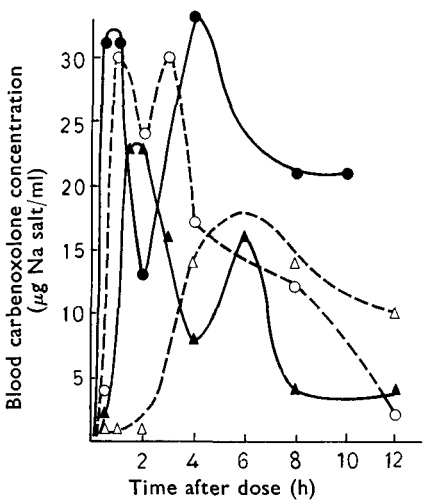

FIG. 1. Concentration of carbenoxolone sodium in whole blood of patients I (male) (●—●), II (female) (○ - - - ○), III (female) (▲—▲) and IV (male) (△ - - - △) at different periods of time after oral administration of 200 mg carbenoxolone sodium.

at 1–2 h and a second maximum occurring at 3–6 h after dosage. Furthermore, from some of the blood concentration curves it could be inferred that a progression of decreasing concentration maxima may even occur (see patients I and II in Fig. 1) In two patients (see patient IV in Fig. 1) there was a delay in attaining significant

blood concentrations and these never reached those exhibited by the other patients. This delayed absorption may have been due to the premature taking of food. In 10 patients the first maximum had a mean value of 24 μg carbenoxolone sodium/ml of blood within the range of 13–35 μg/ml; the corresponding values for the second maximum were 20 μg/ml (3–32 μg/ml) and at 12 h after dosage were 4 μg/ml (<1–10 μg/ml). However, there were cases (e.g. patient I in Fig. 1) where the second maximum was greater than the first. The mean value of 24 μg/ml for the first maximum, for a blood volume of 5 litres, corresponds to a total of 120 mg carbenoxolone sodium, equivalent to 60% of the dose. The maximum value of 35 μg/ml similarly corresponds to 88%, of the dose.

The concentration of carbenoxolone and its metabolites in the urine samples were always lower than the corresponding concentrations in the blood (see Table 2). In patients with high blood concentrations, the maximum urine concentrations of drug and metabolites occurred immediately after the blood maxima. In the two patients not showing high blood maxima urine concentrations of the drug were negligible. Assuming an average output of 50 ml of urine per hour, the total carbenoxolone excreted in 12 h would be equivalent to a maximum of 4 mg of carbenoxolone sodium, or 2% of the dose administered. The chemical identity of the material excreted in the urine was not determined.

The highest concentration of carbenoxolone in solution in the gastric contents was 130 μg/ml at 0·5 h after dosage in patient IV who exhibited delayed absorption of the drug (see Fig. 1). In the other patients the maximum concentration of drug was always <50 μg/ml and progressively declined with time, although trace amounts of the drug (<10 μg/ml) were still present 10 h after dosage (see Table 2). These low concentrations of carbenoxolone in solution were due largely to its insolubility at the acid pH of the stomach contents. As the total volume of the gastric contents, and other factors such as the rate of gastric emptying, the extent of duodenal reflux, and the rate of disintegration of the carbenoxolone tablets, were unknown, concentration of the drug in the gastric contents was not of great significance.

Table 2. *Concentration of carbenoxolone in the blood, urine and gastric contents of patient I*

Time after dosage (200 mg Na salt) (h)	Concentration of carbenoxolone (μg Na_2 salt/ml) in Blood	Urine	Gastric contents
0·5	31	3	34
1	31	5	9
2	13	3	5
4	33	4	<2
8	21	10	7
10	21	7	9

Excretion of carbenoxolone and its metabolites in bile

The concentration of carbenoxolone and its metabolites in bile was determined in four cholycystectomized patients who had subsequently received carbenoxolone sodium. Two of these patients did not show signs of jaundice and the total amount of carbenoxolone excreted in their bile in 48 h was 20·6 and 19·7% of the dose. In

both, the highest concentrations (79 and 125 μg/ml) occurred 6–12 h after dosage. Details of the first of these two cases are given in Table 3. The other two patients did show signs of jaundice and the total carbenoxolone excreted in the bile in 48 h was 14·8 and 4·8% of the dose; the highest concentrations were 62 μg/ml in the 18–24 hour period in the first of these two subjects and 33 μg/ml in the 6–12 h period in the second subject. The lower rates observed in the jaundiced patients may be indicative of impaired hepatic conjugation and excretion, or could simply be due to differences in the extents of drainage.

Table 3. *Excretion of carbenoxolone and its metabolites in the bile**

Time after dosage (200 mg Na salt) (h)	Volume of bile collected (ml)	Carbenoxolone		
		Concentration (μg Na_2 salt/ml)	Total amount (mg Na_2 salt)	% Dose
0–6	136	38	5·1	2·6
6–12	150	79	11·9	5·9
12–18	135	73	10·1	5·1
18–24	132	46	6·1	3·1
24–30	144	26	3·8	1·9
30–36	124	16	2·2	1·1
36–42	114	13	1·4	0·7
42–48	96	3	0·3	0·2
				20·6

* This patient was not jaundiced

With T-tube drainage of the biliary tree not all of the bile secreted is collected, and an unknown and variable amount by-passes the drainage tube and enters the duodenum. The total amount of the drug excreted in the bile of the non-jaundiced patients, about 20%, of the dose, is therefore a fraction of the total biliary excretion. The total volumes of bile collected in 48 h from these two patients were 1·03 and 0·82 litres. Assuming a normal average excretion of bile of 1·2 litres/day it is likely that only 30 to 40% of the total bile secreted was collected, so that the total amount of carbenoxolone excreted by this route, in 48 h, could be 50–70% of the dose.

Chromatography of the bile on thin-layer plates of silica gel revealed one major metabolite with the Rf value and characteristic naphthoresorcinol colour reaction of carbenoxolone-30-glucuronide, together with possible traces of other metabolites, particularly β-glycyrrhetic acid-3-sulphate.

Effect of pH of gastric contents on absorption of carbenoxolone

The simultaneous oral administration of an alkaline buffer solution of sodium citrate with carbenoxolone sodium had the anticipated effect of neutralizing the normal gastric acidity, raising the pH of the gastric contents and therefore increasing the proportion of the drug present in the ionized form. In one subject VI (see Table 4), the pH of the stomach contents, initially 7·5–8, fell steadily over 2 h to 3·1. Only traces of carbenoxolone were found in the blood during this period. With the second subject, V (see Table 4), the pH of the stomach contents fell during the first hour from the initial value of 7·5–8 to 1·9 and then to 1·3 in the second hour. The blood concentrations of carbenoxolone during the first hour were negligible but

Table 4. *Effect of pH of gastric contents on absorption of carbenoxolone.* The sodium salt (150 mg) was administered orally simultaneously with 120 ml 0·05M sodium citrate buffer pH 8·5

	Subject V (male)				Subject VI (male)			
		Gastric contents				Gastric contents		
Time after dosage (h)	Blood concn of carbenoxolone (μg/ml)	pH	Carbenoxolone concn (μg/ml)	β-Glycyrrhetic acid concn (μg/ml)	Blood concn of carbenoxolone (μg/ml)	pH	Carbenoxolone concn (μg/ml)	β-Glycyrrhetic acid concn (μg/ml)
0	<1	7·4	260	13	<1	7·5	75	5
0·25	—	7·9	195	7	—	6·9	70	5
0·5	<1	5·4	65	3	<1	8·2	82	7
0·75	—	2·2	52	3	—	6·3	95	6
1·0	<1	1·9	90	3	<1	6·0	72	5
1·25	—	1·7	25	1	—	5·9	38	3
1·5	9	1·5	25	1	1	5·8	24	1
1·75	—	1·5	39	1	—	4·5	50	2
2·0	12	1·3	25	1	1	3·1	18	<1

during the second hour reached 12 μg/ml. The third subject was similar to the second, the pH falling from 7·3 to 2·0 after 1 h and to 1·5 after 2 h; blood concentrations of carbenoxolone were <1 μg/ml during the first hour but reached 10 μg/ml at the end of the second hour. Appreciable concentrations of carbenoxolone therefore do not appear in the blood until the pH of the stomach contents falls below 2.

Determination of the content of carbenoxolone and β-glycyrrhetic acid present in the stomach contents shows that at pH 7·5–8 the concentrations of carbenoxolone in solution (75 and 260 μg/ml) are higher than those observed at lower pH values or in patients not receiving the alkaline buffer (see Table 2). The concentrations of the hydrolysis product, β-glycyrrhetic acid, are some 5% of the carbenoxolone concentration and do not increase with time. Carbenoxolone sodium in solution normally contains some 5% of β-glycyrrhetic acid, so that there is no evidence of further hydrolysis of carbenoxolone to β-glycyrrhetic acid after incubation in the stomach with gastric contents, either at acid or neutral pH values.

DISCUSSION

Many acidic drugs are absorbed from the stomach, the rate of absorption being dependent primarily on the dissociation constant of the drug and the lipid-solubility of its unionized molecule (Schanker, Shore & others, 1957; Schanker, 1964; Kakemi, Arita & others, 1967). Carbenoxolone is a weak acid (pK′, 6·7; pK″, 7·1) that is highly lipid soluble in its non-ionized state. The distribution coefficients for carbenoxolone at 24° between chloroform and aqueous buffers are 2 at pH 7·4 and >100 at pH 1·0, and between n-octanol and aqueous buffers are 9 at pH 7·4 and >100 at pH 1·0 (Lindup, personal communication). It would therefore be reasonable to expect carbenoxolone to be readily absorbed from the stomach, where normally it would not be significantly ionized (0·0002% at pH 1·0). However as β-glycyrrhetic acid, the parent substance, was found to be largely excreted unchanged in the faeces when administered orally to man, and hence was assumed to be largely unabsorbed (Carlat, Margraf & others, 1959), it was thought that carbenoxolone also would be largely unabsorbed and that its action in the healing of gastric ulcers would be essentially topical. These views were questioned when studies using isotopically-labelled β-glycyrrhetic acid and carbenoxolone showed both compounds to be readily

absorbed in the rat after oral administration (Parke, Pollock & Williams, 1963; Iveson, Parke & Williams, 1966).

The present results show that carbenoxolone is extensively and rapidly absorbed in man after oral administration and suggest that the principal site of absorption is the stomach. The rapid attainment of high blood levels, an average of 24 μg/ml equivalent to 60% of the dose present in the blood within 1 h of dosage, suggests that most of the drug is absorbed even before gastric emptying occurs. Furthermore, where the stomach contents were buffered to pH 7·5 (85% of carbenoxolone ionized), the normal rapid absorption of the drug, as indicated by high blood concentrations, did not occur. As the gastric acidity was restored and the stomach contents attained pH <2 ($<0{\cdot}002\%$ of carbenoxolone ionized) the drug appeared in the blood. Thus it is when the drug is largely non-ionized that absorption occurs most readily, and the major part from the stomach.

With aspirin, a typical acidic drug, absorption in dog and man is increased at low pH values because of decreased ionization of the drug, and is also increased at high pH values by virtue of the increased solubility of the drug (Truitt & Morgan, 1964). Further, the pH-dependency of the absorption of barbiturates from the stomach of the rat is marked only with those compounds, such as hexobarbitone, which, in their molecular form, have a high lipid solubility and hence are rapidly absorbed (Kakemi & others, 1967). Carbenoxolone is not readily soluble in aqueous media, even at neutral pH values, and in the unionized state is highly lipid-soluble, so that its absorption would be expected to be markedly affected by the intra-gastric pH, as has been observed. Moreover, the high degree of binding of carbenoxolone to plasma protein would have an effect on the equilibrium of the drug between the gastric contents and the blood that would result in an acceleration of the absorption process.

Analysis of the stomach contents showed that over 2 h no appreciable hydrolysis of carbenoxolone to β-glycyrrhetic acid occurs, even at pH 8, which favours the hydrolysis of the drug. Moreover, it was present in the blood unchanged and mostly excreted in the bile as the glucuronide. Thus, in contrast to the rat in which it is largely hydrolysed to β-glycyrrhetic acid before absorption (Iveson & others, 1966), in man carbenoxolone is absorbed largely unchanged.

The high blood concentrations obtained in these absorption studies suggests that most of the drug is in the circulating blood, so that although carbenoxolone is readily absorbed it does not appear to equilibrate readily with the remaining tissues of the body. This could indicate a high degree of binding of the drug to the plasma proteins. Preliminary experiments *in vitro* with human blood have confirmed this; at a plasma concentration of 25 μg/ml all the carbenoxolone was bound to plasma proteins and no unbound drug could be detected. Sulphachloropyridazine, a drug highly bound to plasma proteins, at an oral dose of 1 g gave a maximum plasma concentration in man of 100 μg/ml, which is equivalent to some 40% of the dose being present in the plasma (Newbould & Kilpatrick, 1960). With carbenoxolone, the peak blood concentrations were equivalent to nearly twice this, namely 60–70% of the oral dose present in the blood.

In man, as in the rat, only traces of carbenoxolone and its metabolites were found in the urine, and the total amount of the drug excreted by this route is unlikely to exceed 5% of the dose. The principal route of excretion of carbenoxolone in man,

as in rat, has been shown to be via the bile, but whereas in rat bile the principal metabolites are the glucuronide and sulphate conjugates of β-glycyrrhetic acid, in man the major biliary excretion product is carbenoxolone glucuronide. It would thus appear that the ester linkage of carbenoxolone is stable in man, not only in the gastro-intestinal tract before absorption, the site of its hydrolysis to β-glycyrrhetic acid in the rat, but also during the transport of the drug in the blood and its passage through the liver into the bile. The high excretion of carbenoxolone and its metabolites in the bile is more likely to be due to their high molecular weight (carbenoxolone mol. wt, 571) (see Williams, Millburn & Smith, 1965) than to the high degree of their binding to plasma proteins, since the protein-bound sulphachloropyridazine (mol. wt = 285) is readily excreted in the urine (Newbould & Kilpatrick, 1960), and indomethacin (mol. wt = 358), also highly protein-bound, is excreted approximately equally in both urine and bile (Hucker, Zacchei & others, 1966).

Drugs that are excreted in the bile as conjugates may undergo hydrolysis in the small intestine followed by reabsorption of the drug which is then excreted again in the bile, thus giving rise to an enterohepatic circulation of the drug (Williams & others, 1965). It is therefore possible that carbenoxolone, which is almost exclusively excreted in the bile, may also undergo enterohepatic circulation. Because of the rapid absorption of the drug and its confinement to the blood plasma compartment any reabsorption of drug following the secretion of bile into the intestine should be reflected in an immediate rise in the blood levels of carbenoxolone. Furthermore, the periodic emptying of the gall-bladder would be expected to give rise to a parallel periodicity in the blood concentration. Such a periodicity has been observed, resulting in maximum blood concentrations of the drug at approximately one and four h after dosage. This is strongly indicative of the enterohepatic circulation of carbenoxolone. An alternative explanation of this periodicity is that it could be attributed to the sequential absorption of the drug, first from the stomach and then of the remainder from the small intestine after gastric emptying. This is unlikely, since the peak blood concentrations are each equivalent to approximately 60% of the dose and in some cases the second peak was even greater than the first one.

It is suggested that the ulcer-healing action of carbenoxolone is the result of an increase in gastric mucus (Goodier & others, 1967). This could be due to increased secretion or to an increased rate of biosynthesis of glycoprotein, but either mechanism would probably require the passage of the carbenoxolone into the cells of the gastric mucosa, which is likewise the first requirement for the absorption of the drug. Absorption of carbenoxolone from the stomach may therefore be a prerequisite for its gastric-ulcer healing activity.

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