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Gastric ulceration and the concentration of salicylate in plasma in rats after administration of ^{14}C-labelled aspirin and its synthetic triglyceride, 1,3-dipalmitoyl-2(2′-acetoxy-[^{14}C]carboxylbenzoyl) glycerol

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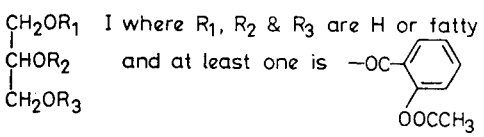
Triglycerides containing aspirin in place of one or more fatty acid residues of the molecule have been synthesized. Metabolism of the compound with the labelled (^{14}C) drug residue introduced specifically into the 2-position of the triglyceride is reported. Plasma salicylate concentrations with this synthetic glyceride were determined and compared with those obtained with commercially available aspirin labelled with the ^{14}C-isotope. Both compounds gave a therapeutic concentration of salicylate in the plasma after ingestion. The 1,3-di-fatty acyl-2-aspirin glyceride was absorbed through the intestine as 2-aspirin monoglyceride, some 20% of which was transported through the thoracic-duct chyle and about 30% through the portal system. Whereas pronounced ulceration of the rat stomach occurred with free aspirin, the above fatty acyl glyceride of aspirin produced no ulceration.

Many workers have shown that by the use of enteric coated aspirin or various salts of aspirin, the hazard of stomach ulceration is reduced (of Levy, 1961).

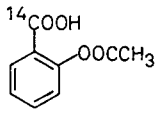
Synthesis of glycerides of aspirin was undertaken, where one, two or three fatty acids in a triglyceride structure were replaced by the active drug moiety as indicated in I. The object was to incorporate water-soluble drugs into the lipid structure whereby the organic drug acid would be rendered fat soluble.

The present paper discusses the chemical synthesis and the plasma and lymph drug concentration obtained by feeding the single radio-labelled [^{14}C] compound (II) and plasma concentrations of salicylate after feeding 2-acetoxy [^{14}C]benzoic acid (III). It also shows that stomach ulceration in the rat is avoided by the use of the 2-aspirin glyceride (II) instead of aspirin. Based on the analogy with the absorption of triglycerides it was felt that the pancreatic lipase would cleave the 1 and 3 fatty acids from the glyceride and the aspirin would be absorbed as a 2-aspirin monoglyceride. That this is the mode of absorption of the compound and that the 2-monoglyceride entering the blood is cleaved within the mucosa and/or the blood itself to free aspirin and salicylate was suggested by the findings; a part of the monoglyceride is transported through the thoracic duct chyle.



II $R_1 = R_3 = OC_{16}H_{31}$

R_2 = 2′-acetoxy[^{14}C]carboxylbenzoyl



III

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MATERIALS AND METHODS

(a) *1,3-Dipalmitoyl 2(2′-acetoxy-[^{14}C]carboxyl benzoyl) glycerol (II, ^{14}C-2-ASATG)*

Acetylsalicylic acid (250 μCi, spec. act. 22 mCi ml mol^{-1}) and carrier acetylsalicylic acid (3 g, 16·65 mmol) in dry benzene (20 ml) were converted to the acid chloride with oxalyl chloride (5 ml) by heat (60°–70° for 2½ h). The solvents were removed and the acid chloride (1·09g, 5·48 mmol) dissolved in chloroform (20 ml) added to 1,3-dipalmitin (2·6 g, 4·57 mmol) in dry chloroform (25 ml) and pyridine (2 ml). The reaction was at 0° for 2 h and overnight at room temperature (20°). After working up in the usual manner crystallization from light petroleum (40° to 60°) at 4° gave colourless crystals of II. These were dissolved in a minimum of light petroleum and chromatographed on a column of silicic acid (32 g, column diameter 2·5 cm). The column was eluted stepwise with ether and light petroleum (200 ml 1% v/v, 250 ml 5% v/v, 500 ml 8% v/v, 150 ml 25% v/v and 500 ml 100% v/v). The required product, found

in the 8% eluate, gave colourless crystals of II, m.p. 48° with a radiopurity of 99·8% (thin layer chromatography using two different solvent systems, (1) silica gel G plates impregnated with 5% boric acid, developed in chloroform–acetone, 96:4 v/v, (2) silica gel G plates developed in diethyl ether–benzene–ethanol–acetic acid, (40:50:2:0·2 v/v). Yield: 3·02 g, 90% yield based on dipalmitin, spec. act. 21·93 mCi mmol^{-1} [Found C, 72·14; H, 10·08; $C_{44}H_{74}O_8$ (mol. wt = 730·99) requires C, 72·30; H, 10·19%].

(b) 2-*Acetoxy*[^{14}*C*]*benzoic acid* (*III, ASA*)

Acetylsalicylic acid (250 μCi, spec. act. 22 mCi mmol^{-1}, Radiochemical Centre, Amersham) and carrier acetylsalicylic acid (2 g) were co-crystallized from anhydrous ether (50 ml) with the addition of light petroleum 40° to 60° (95 ml). The radioactivity of the test feeds was regulated to 4 to 10 $\times$ 10^6 d min^{-1} by the addition of respective non-labelled carrier compounds.

Male Wistar rats (200 to 300 g) in metal cages were fasted overnight but allowed free access to drinking water. The amount of drug required per rat was suspended in 1·5 ml 0·9% w/v sodium chloride, 0·5 ml egg albumin was added and the mixture was sonicated in a vial for 15 s. The emulsion was transferred into a 2 ml plastic syringe and fed to the rat, under very light anaesthesia, with a rubber catheter. Radiopurity of the compound was checked by t.l.c. before and after sonication. The emulsion was freshly prepared each time just before feeding.

The amount of drug prepared was based on the weight of the rat before starving but the animals were again weighed just before feeding. The residual radioactivity in the catheter vial and syringe was extracted into chloroform–methanol (2:1) (Folch, Lees & Sloane-Stanley, 1957) after the addition of hydrochloric acid (6 M, 0·25 ml) and the amount of radioactivity fed to the starved animal was recorded as d min^{-1} kg^{-1}. The actual weight in mg of drug fed was calculated from the d min^{-1} fed and the specific activity.

The rats were killed alternately at different intervals after feeding from 15 min to 24 h. Blood was withdrawn from the abdominal aorta into an ice cold tube containing EDTA (1 mg ml^{-1}) and sodium fluoride (2·5 mg ml^{-1}). The plasma was obtained by centrifugation at 4° at 1500 *g* for 20 min and then stored at −20° until analysed. Plasma (0·5 ml) was used directly for ^{14}C counts in a Packard Scintillation Spectrometer (Tri-Carb-3380). Lipid extracts, after removal of solvent were counted in a scintillation fluid (15 ml) consisting of 2,5-diphenoxyloxazole (5 g), 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (0·1 g) dissolved in methanol (50 ml) and made up to 1 litre with scintillation grade toluene. Plasma samples (0·5 ml) were counted in Instagel (10 ml) (Nuclear Enterprises Ltd). The c min^{-1} were converted to d min^{-1} by the use of efficiency curves obtained by the use of an external standard (Davies & Hall, 1969).

RESULTS

(a) *Blood concentrations after feeding aspirin (III) and the triglyceride analogue (II)*

Starved rats (62) were divided into groups, 32 rats were given ^{14}C-ASA (III, 250 mg kg^{-1}) and 30 were given ^{14}C-2-ASATG (1·014 g kg^{-1} equivalent to 250 mg ASA). Animals were killed alternately at various times and the blood was immediately drawn for ^{14}C counts.

The radioactivity of the feed after taking into account the recovery from the catheter varied from 8·3 to 9·6 $\times$ 10^6 d min^{-1}. The recovery of counts from plasma ranged from 6·5 $\times$ 10^6 d min^{-1} at the peak of absorption to 1·9 $\times$ 10^6 d min^{-1} at 24 h after feeding and the specific activities of the compound fed ranged from 15·9 to 17·1 $\times$ 10^4 d min^{-1} mg^{-1}. The percentage of ^{14}C label found in plasma varied from 71 to 19% at the peak of digestion and 24 h after, respectively. The mg of salicylate in the plasma were calculated from the data as follows: mg salicylate derived from ASATG = [(Mol wt salicylic acid)/(Mol wt ASATG)] $\times$ mg ASATG fed, and in the case of salicylate derived from aspirin feeding, mg salicylate = [(Mol wt salicylic acid)/(Mol wt fed aspirin)] $\times$ mg aspirin fed.

Radioactive compounds were necessary to establish the nature of the metabolites presented to the intestine and those found in the blood. Although salicylate could have been measured fluorometrically or spectrometrically, lipid metabolites of salicylate are not easily estimated. Hence the label in conjunction with t.l.c. successfully established the identification and quantitation of lipid metabolites (Table 1).

Although it is not the primary object to describe metabolism in this paper, Table 1 gives typical results of identification of the metabolites in the intestinal lumen and in plasma at an early digestion period of 1 h and at the peak of digestion (8 h). The main metabolite presented to the intestinal mucosa is the 2-monoglyceride of aspirin (Table 1 and Billimoria & Kumar 1976) and this appears almost completely as free salicylate in the plasma.

A comparison of plasma concentration of salicylate after feeding 2-ASATG and free aspirin was obtained

Table 1. *Percentage of radioactivity found in various lipid fractions of stomach, intestine and plasma after feeding ^{14}C-2-ASATG.*

	PL	MG	Free salicylate	DG	TG
Stomach					
1 h	0·7	0·4	1·2	3·5	94·1
8 h	0·9	0·8	3·5	1·8	94·9
Intestine					
1 h	0·7	1·6	5·3	3·7	88·7
8 h	8·4	27·9	12·8	4·3	46·6
Plasma					
1 h	0·6	8·5	90·1	—	0·8
8 h	1·5	2·5	95·7	1·0	1·3

PL: phospholipids; MG: Monoglycerides; DG: diglycerides; TG: triglycerides.

by measuring concentrations of radioactivity in plasma at the respective peak hours of absorption, after feeding the same salicylate concentration in the form of triglyceride and free aspirin. Thus after feeding the equivalent of 174 mg kg^{-1} of salicylate, the 2-ASATG compound produced a concentration of 17 mg salicylate per 100 ml plasma at 8 h, whereas aspirin produced a concentration of 30 mg/100 ml, 2 h after feeding the compound (Fig. 1).

These experiments suggest that the triglyceride containing the active drug at the same concentration as that of aspirin gave a peak plasma concentration which was just over half that given by aspirin.

Dose response

Approximately 50 to 450 mg kg^{-1} of ^{14}C-ASA, corresponding to 38 to 350 mg salicylate and 800 mg to 3·5 g of the ^{14}C-2-ASATG, corresponding to 150 mg to 700 mg salicylate were fed to animals in various groups. The approximate time for the drug to reach peak plasma concentration was obtained from the previous results; for aspirin a 2 h digestion period was allowed and for triglyceride the period was 6 h. The animals were killed alternately and the concentration of salicylate per 100 ml of plasma was calculated.

The mean concentration of salicylate in mg/100 ml plasma at 2 and 6 h after feeding various amounts of 14C-ASA and 2-ASATG to rats are given in Fig. 2. Up to a fed dose of aspirin corresponding to 160 mg kg^{-1} salicylate a linear relation existed between the dose fed and the plasma salicylate concentrations thereafter no appreciable increase in plasma salicylate was observed up to 330 mg kg^{-1}. Similarly a linear relation existed between the fed dose and plasma salicylate concentration up to a fed dose of 2·6 g of 14C-2-ASATG (corresponding to 500 mg kg^{-1} salicylate fed). Thereafter an increased dose did not give rise to higher plasma concentrations of salicylate.

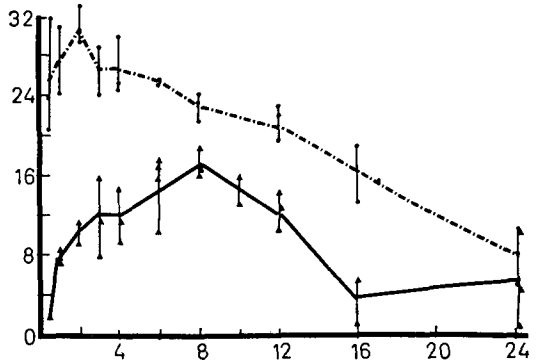

FIG. 1. Total salicylates (mg/100 ml) (ordinate) in plasma after feeding 250 mg kg^{-1} 2-acetoxy[^{14}C]-benzoic acid (III, ^{14}C-ASA) and 1·014 g kg^{-1} 1,3-dipalmitoyl 2(2′-acetoxy[^{14}C]carboxyl benzoyl) glycerol (II, ^{14}C-2-ASATG). After recoveries from the catheter were calculated the actual dose kg^{-1} of 2-ASATG was equivalent to 174 mg kg^{-1} salicylate. ^{14}C-ASA ●— . —●; ^{14}C-2-ASATG ▲——▲. Abscissa: Time (h).

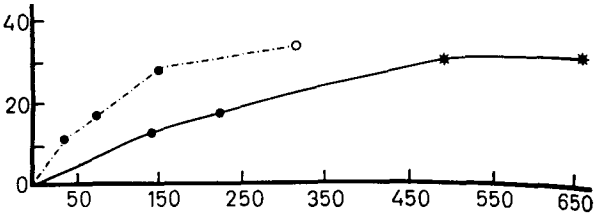

FIG. 2. Dose response of feeding various concentrations of 2-acetoxy[^{14}C]benzoic acid (III, ^{14}C-ASA) and 1,3-dipalmitoyl 2(2′-acetoxy[^{14}C]carboxyl benzoyl) glycerol (II, ^{14}C-2-ASATG). ^{14}C-ASA – – – – –; ^{14}C-2-ASATG ———. Mean values of 2 rats ●; Mean values of 3 rats ○; Mean values of 5 rats *. Ordinate: mg salicylate in rat plasma (100 ml). Abscissa: mg total salicylate fed kg^{-1}.

Booster dose

When an average dose equivalent to 180 mg salicylate kg^{-1} derived from 2-ASATG was fed to animals, a mean plasma salicylate concentration of 12 mg/100 ml was observed at 4 h (Fig. 3). At this time a group of animals was given a booster dose (equivalent to another 180 mg salicylate kg^{-1}) and the plasma concentrations of the boosted animals rose to approximately 29 mg/100 ml after 12 h whereas the unboosted animals gave a peak value of 15 to 17 mg/100 ml between 6 and 8 h and at 12 h about 12 mg/100 ml.

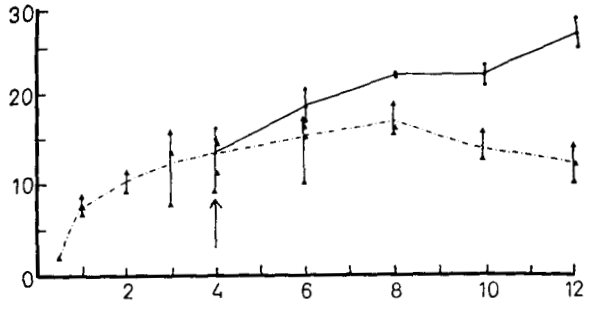

FIG. 3. Total salicylate concentration found in plasma (up to 12 h) (mg/100 ml) (ordinate) after feeding 1,3-dipalmitoyl 2(2′-acetoxy[^{14}C]carboxyl benzoyl) glycercol (II, ^{14}C-2-ASATG) (180 mg) (▲— . —▲) followed by a second booster dose (180 mg) after 4 h (●——●). Arrow indicates booster dose. Abscissa: Time (h).

Thoracic duct chyle

Thoracic ducts of rats (2) were cannulated by the modified method of Bollman, Cain & Grindley (1948). The corn oil emulsion of the test compound was fed by gastric intubation under light anaesthetic. The rats were subsequently kept in the restraining cages and allowed to drink freely from a solution of 5% glucose in Krebs-Ringer saline. Serial collection of lymph was carried out every 2 h for 24 h. Total activity in each sample was determined by counting directly 0·5 ml of the lymph with 10 ml of the Instagel (Packard Instruments Ltd) in a scintillation spectrometer. The total radioactivity recovered (Table 2) in the individual lymph collections at various times differed widely (0·5 to 3·5% of the fed dose). The maximum activity of the ^{14}C isotope occurred in the 6 to 8 h lymph samples. The overall recovery of the ^{14}C-isotope indicated that over 10% of the ingested isotope was voided into the lymph (Table 3). Since only 50% of the fed drug is absorbed compared with free aspirin the result implies that some 20% of the absorbed drug is transported via the chyle.

Table 2. *Recovery of ^{14}C isotopes in the lymph of rats pre-fed with corn oil emulsion of* 1,3-*dipalmitoyl* 2-(2′-*acetoxy*-[^{14}C]*carboxylbenzoyl) glycerol* (2-*ASATG*) *at varying time intervals.*

Lymph sample	Time after feeding (h)	% Total isotope activity recovered
1	0–2	0·50
2	2–4	1·02
3	4–6	2·10
4	6–8	3·50
5	8–10	2·00
6	10–12	1·03
7	12–24	0·12

Actual d min^{-1} obtained from chyle ranged from $\cdot 2 \times 10^4$ to $8{\cdot}4 \times 10^4$ between 1 and 12 h.

Table 3. *Total recovery of ^{14}C-isotope in lymph collection (24 h) after feeding* 1,3-*dipalmitoyl* 2(2′-*acetoxy* [^{14}C-]*carboxyl benzoyl) glycerol* (2-*ASATG*).

Total d min^{-1} fed	Total d min^{-1} recovered up to 24 h	% d min^{-1} recovered
$2{\cdot}4 \times 10^6$	$2{\cdot}5 \times 10^5$	10·3

Induction of gastric lesions with aspirin and its triglyceride analogue (2-ASATG)

Female Wistar rats (24), ~150 g, in metal cages were fasted overnight but had free access to drinking water. Aspirin and its triglyceride (2-ASATG, non-radioactive) were suspended in 0·7% aqueous methyl cellulose solution by ultrasonic irradiation. The rats were divided into four groups. A single dose of aspirin (250 ml kg^{-1}) was fed to one group of 6 rats. Varying concentrations of aspirin triglyceride (equivalent to 250 mg to 500 mg kg^{-1} of aspirin) were fed to two groups, each of 6 rats. Saline and methyl cellulose were fed to 3 rats as control.

The rats were killed at selected intervals of 1, 2 and 4 h, by injection of pentobarbitone. The ventral surface was opened and the pylorus ligatured, freed from the intestine and the whole stomach cleared of surrounding mesentery. Finally the oesophagus was cut to remove the stomach from the body. The stomach was inflated with 10 ml of 0·5% saline and allowed to stretch for a minumum of 10 min and then opened along the greater curvature. After the mucosal folds had been gently stretched, stomachs were pinned onto the cork mats and examined.

Aspirin fed animals killed at 1 h showed marked mucosal erosions, whereas no erosions were noticed in the 2-ASATG fed animals, or controls killed at 1 to 4 h.

DISCUSSION

The preparation of a number of naphthylacetic acid derivatives of glycerol have been recorded in a Syntex patent (Zaffaroni, 1972). However, the procedure of preparation of the compounds especially the 2-substituted glycerides is different from the present procedure.

As far as we are aware, the above patent, a Schering Corporation patent (Sherlock, 1972) [1,2-

diacetone 3(2'-acetoxy benzoyl) glycerol], Abbot Laboratories (Paris & Garmaise, 1976), our own patent (Billimoria, 1974) and the work of Ciampa, Vittoria, & Manna (1968) is the only literature available on these compounds. Furthermore, isotopically labelled compounds have not previously been described by any of the workers, nor have the blood concentrations obtained by feeding such compounds been recorded.

The data from the radio-labelled compounds suggests that after feeding 2-ASATG, no breakdown of the compound occurs in the stomach. The metabolite presented to the mucosa is mainly the 2-monoglyceride (2-ASAMG) of aspirin. This 2-ASAMG is absorbed as such and is cleaved in the mucosa and the blood to free salicylate where 90 to 95% of the absorbed compound (depending on the time of digestion) appears in the blood as free salicylate. Since aspirin is absorbed directly from the stomach, the peak of absorption occurs at 1½ to 2 h which is in agreement with the earlier finding of Crabtree, Data & Christian (1956), where a concentration of 28 mg% at 2 h has been obtained in rat blood. However, after feeding the triglyceride analogue of aspirin (2-ASATG), the relatively late peak of plasma salicylates observed at 6 to 8 h, after feeding, and a complete lack of breakdown of the compound in the stomach suggests that the absorption of the 2-ASATG compound occurs from the small intestine.

It could be seen from the comparative dose response of ^{14}C-ASA and ^{14}C-2-ASATG and the additional booster dose of aspirin triglyceride after 4 h that the effective therapeutic concentration of 25 mg% of plasma salicylate can readily be obtained; these values are known to be therapeutically effective in rheumatic fever (Ansell, 1963).

The results from analysis of the serially (postprandial) collected lymph samples up to 24 h after feeding a single dose of labelled 2-ASATG compound to rats, showed that up to 10% of the counts fed were transported by the thoracic duct chyle. This represents approximately 20% of the absorbed compound, since only 50% of the fed triglyceride was being absorbed through the intestinal lumen.

While with ingested aspirin, ulceration of the stomach is a major common hazard, evidence obtained on the basis of visual inspection of the rat stomach, after feeding various dosages of 2-ASATG compound indicated complete absence of such ulceration. No obvious ulceration of the small intestine was observed when the intestines were removed for chemical estimation but detailed histology was not done on the intestine as it was felt that results from such experiments would only be conclusive after long term feeding.

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