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Age related changes in the clearance and oral absorption of sodium cromoglycate in the developing albino rat

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A dual radioisotope method was used to investigate the clearance and oral absorption of sodium cromoglycate. Radiolabelled sodium cromoglycate was administered orally at a dose of 100 mg kg $^{-1}$ (^{14}C-labelled) and simultaneously subcutaneously at a dose of 2 mg kg^{-1} (^{3}H-labelled) to rat pups 5, 9, 14, 20, 29 and 75 days old. Blood concentrations of ^{14}C and ^{3}H were measured at intervals for 24 h after dosing. Since the compound is not metabolized the blood concentrations of ^{14}C were taken as a measure of the sodium cromoglycate absorbed orally and the blood concentrations of ^{3}H as a measure of the subcutaneously administered material. Using the area under the oral ^{14}C blood curve (AUC) as an index of bioavailability, the calculated bioavailability of sodium cromoglycate (692·9 – 945·9 min μg ml^{-1}) in 5, 9 and 14 day old pups was 4—8 times greater than that observed (61·0 – 118·8 min μg ml^{-1}) in 20, 29 and 75 day old pups. The blood clearance of sodium cromoglycate was increased four-fold in 75 day old animals (43·9 ml min^{-1} kg^{-1}) and three-fold in 20 and 29 day old pups when compared to the clearance in 5, 9 and 14 day old pups. The clearance in 5, 9 and 14 day old pups was relatively constant (10·8 – 9·9 ml min^{-1} kg^{-1}). In rats less than 14 days old the systemic absorption of sodium cromoglycate after oral administration was 2—3 times greater (6·8—9·2%) than in rats aged 20, 29 or 75 days old (2·7—3·3%). The reduction in oral bioavailability of sodium cromoglycate as the pups grew older was, therefore, due to both an increased blood clearance and a decreased absorption of the compound.

Sodium cromoglycate (the disodium salt of 1,3-bis [2-carboxy chromon-5-yloxy]-2-hydroxyl propane) is a compound effective in the treatment of asthma. For allergic indications in the lung the compound is inhaled as a fine aerosol powder. Absorption of sodium cromoglycate is rapid and complete from the lung (Moss & Ritchie 1970). Similarly absorption is both rapid and complete after subcutaneous administration. Experiments conducted in these laboratories have shown that, following subcutaneous administration of sodium cromoglycate (2 mg kg^{-1}) to adult (160 g) rats, only 1·0 ± 0·4% of the dose was present at the injection site after 6 h. Similar results were demonstrated for experiments in neonatal animals. For instance in 8 day old rat pups 1·9 ± 0·1% of the dose remained at the site after 6 h. Sodium cromoglycate is poorly absorbed, however, from the gastrointestin altract in adult animals (Moss et al 1970; Ashton et al 1973). Recently, the compound has been found to be effective in a variety of allergic conditions of the gastrointestinal tract when administered orally at dose levels substantially higher than those used for inhalation. These include proctitis (Heatley et al 1975), ulcerative colitis (Mani et al 1976), food allergy (Esteban et al 1977) and milk allergy (Freier 1977). The last indication clearly applies principally to young infants. Preclinical safety evaluation studies were, therefore, carried out to support administration of the compound to the young by the oral route. During this work we observed that the bioavailability of sodium cromoglycate after oral administration to rats aged 10 days old was greater than when the compound was administered to rats aged 21 and 41 days old. We report experiments that investigate the factors responsible for this change in oral bioavailability in young rats. Since sodium cromoglycate is rapidly and completely absorbed after administration by the subcutaneous route, this route was chosen for comparison with oral dosing. This obviated the practical problems of systemic administration to very young animals.

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MATERIALS AND METHODS

Sodium cromoglycate. ^{3}H-Labelled sodium cromoglycate, with a specific activity of 40·9 μCi mg^{-1} was

synthesized by the Radiochemical Centre (Amersham, Bucks., U.K.). ^{14}C-Labelled sodium cromoglycate, with a specific activity of 10·9 μCi mg^{-1} was synthesized in these laboratories by Mr A. E. Kitson. Both materials had a radiochemical purity greater than 98%. Pure sodium cromoglycate was also used.

Oral doses

Sodium [^{14}C]cromoglycate was prepared as an aqueous solution at a concentration of 10 mg ml^{-1}. Each pup, irrespective of age, received 10 μCi of sodium [^{14}C]cromoglycate. The radioactive material was diluted with a suitable weight of non-radioactive material in a ratio determined by the mean weights of the animals immediately before dosing. Oral doses were administered at approximately 100 mg kg^{-1} (90–120 mg kg^{-1}). Individual doses were calculated precisely by reference to dosing solution volume and pup weight.

Subcutaneous doses

Sodium [^{3}H]cromoglycate was prepared as an aqueous solution at a concentration of 2 mg ml^{-1}. Each pup, irrespective of age, received sodium [^{3}H]cromoglycate with a specific activity of 40·9 μCi mg^{-1}. Subcutaneous doses were administered at 2 mg kg^{-1}. The oral and subcutaneous dose levels were chosen to give comparable blood concentrations of the drug.

Animals

Pregnant CR/CD rats were supplied by Charles River (Manston, Kent). Parturition occurred within the same 24 h period for all the mothers. Two days after birth, all of the pups were pooled, and five male and five female pups were randomly returned to eight foster mothers. This process ensured randomization of each litter. No rejection of a pup occurred by the foster mothers. Six litters were deemed experimental litters and pups from these litters were used in blood sampling experiments, two further litters were used as maintenance litters. When a pup from an experimental litter was killed, it was replaced with one of a similar weight and of the same sex, from a maintenance litter. The replacement pup was marked and not used for blood sampling experiments. Twenty-one days after birth the pups were weaned. They were then removed from their 'mothers' and the sexes were housed separately.

Dosing of animals

Absorption was evaluated in pups, 5, 9, 14, 20, 29 and 75 days after parturition. Three male and three female pups, one from each experimental litter, were used on each occasion. These pups were then dosed orally with sodium [^{14}C]cromoglycate using a ball-ended dosing needle (14 g × 3·5 cm) and immediately afterwards subcutaneously with sodium [^{3}H]cromoglycate via a fixed needle 100 μl microsyringe. After dosing pre-weaning pups were returned to their 'mothers'.

Collection of blood samples

Blood samples were collected from each pup at 0·25, 0·5, 1, 1·5, 2, 3, 4 and 6 h after dosing. Blood samples were collected from the tip of the tail into a uniform diameter heparinized glass capillary tube ('Hemocap'—Drummond Scientific, Broomall, Pa., U.S.A.). Between each blood sample the pups below 21 days of age were returned to their 'mothers'. Additional blood samples were collected, 24 h after the drug had been given, by cardiac puncture into a heparinized disposable syringe under diethyl ether anaesthesia. These pups were then killed.

Determination of blood radioactivity

The volume of the blood sample was calculated from the filled length of the capillary tube. The blood was then washed into a paper cone ('Combusto-Cone'—Packard, Downers Grove, Illinois, U.S.A.) with heparinized 0·9% NaCl (saline). Each sample was then combusted in the Packard B306 Sample Oxidizer. The level of ^{14}C and ^{3}H in each sample was then determined by liquid scintillation spectrometry.

Treatment of results

The areas under the ^{14}C and ^{3}H blood concentration curves (AUC's) between 0 and 24 h were calculated for each pup using the trapezoidal method (Gibaldi & Perrier 1975). Absorption was assumed to be complete after subcutaneous administration. Clearance was calculated from the relationship: dose (subcutaneous)/AUC (subcutaneous). The fraction of the oral dose absorbed was calculated from the relationship: AUC oral/AUC subcutaneous × dose (subcutaneous)/dose (oral), where AUC oral is defined by AUC ^{14}C and AUC subcutaneous is defined by AUC ^{3}H.

Estimation of biliary excretion

Rat pups were dosed subcutaneously at 4, 8, 15, 21 and 41 days of age with sodium [^{14}C]cromoglycate (10 mg kg^{-1}). Four hours after administration, the

pups were killed and the radioactivity present in the gastrointestinal tract, and the remaining organs and carcass was determined. Analysis of the tissues was accomplished by digesting them in normal sodium hydroxide solution at 30 °C for 48 h. Samples of the digest were neutralized and the radioactivity present assayed by liquid scintillation spectrometry using Fisofluor m.p.c. liquid scintillation cocktail (Fisons Scientific Apparatus, Loughborough, U.K.).

RESULTS

There was no detectable sex difference in any of the parameters measured and results obtained in male and female animals are therefore combined. The mean blood concentrations of ^{3}H versus time in rats of different ages are shown in Fig. 1. The ^{3}H concentrations are a measure of the subcutaneously administered sodium cromoglycate. In all age groups peak blood concentrations of ^{3}H were observed at 15 min, the time of the first sample. The concentrations at this time range from 3·08 in the 9 day old rats to 0·50 μg ml^{-1} in the 75 day old rats. The blood concentrations declined rapidly and consequently were very low (<0·02 μg ml^{-1}) at 24 h. The concentrations of ^{3}H in the blood were used to calculate the blood clearance of sodium cromoglycate. The change in blood clearance of sodium cromoglycate with age is detailed in Table 1. In younger pups (5, 9 and 14 days old) the blood clearance was relatively constant (9·9–10·8 ml min^{-1} kg^{-1}). The clearance increased in 20 day old animals and reached a value four-fold higher than the younger animals in 75 day old animals (43·9 ml min^{-1} kg^{-1}).

The mean blood concentrations of ^{14}C versus time in rat pups of different ages are also illustrated in Fig. 1. The ^{14}C concentrations are a measure of the orally administered sodium cromoglycate. In the 5, 9 and 14 day old pups the blood concentrations of ^{14}C rose, within 30 min, to a plateau which was then sustained for at least the next 6 h. The blood concentrations at plateau for these rats were approximately 1 μg ml^{-1}. By 24 h the blood concentrations of ^{14}C had fallen to 0·10 μg ml^{-1} in the 5 day old rats and 0·06 μg ml^{-1} in both the 9 and 14 day old rats. The blood concentrations of ^{14}C rose to an early peak in the older rats, but in contrast to those in the younger animals, the concentrations then declined rapidly. The changes in blood concentration profile with age were mirrored in the calculated AUC for each age group. The 5, 9 and 14 day old rats had mean AUC's (692·9–889·3 min μg ml^{-1}) substantially greater than those calculated for older animals (61·0–118·8 min μg ml^{-1}) (Table 1).

Table 1. Values (n = 6) of oral AUC, blood clearance and oral absorption calculated for rats of different ages after administration of sodium cromoglycate

Age (days)	AUC Oral ± s.e.m. min μg^{-1} ml^{-1}	Clearance ± s.e.m. ml min^{-1} kg^{-1}	Absorption ± s.e.m. %
5a	889·3 ± 141·8	10·8 ± 1·0	9·2 ± 0·9
9	692·9 ± 91·5	10·2 ± 0·7	6·8 ± 0·6
14	945·9 ± 115·5	9·9 ± 0·6	8·9 ± 0·8
20	118·8 ± 30·4	26·2 ± 2·3	2·7 ± 0·4
29	109·0 ± 17·1	31·3 ± 3·8	3·3 ± 0·5
75a	61·0 ± 9·7	43·9 ± 2·4	2·6 ± 0·3

a — only five animals used on day 5 and four on day 75.

The ^{3}H and ^{14}C data were used to calculate the percentage absorption after oral administration of sodium cromoglycate (Table 1). The 5, 9 and 14 day old rats absorbed a greater proportion (6·8–9·2%)

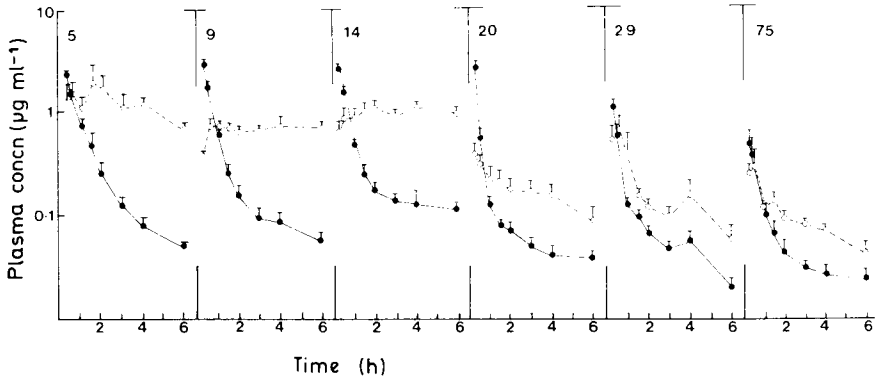

FIG. 1. Blood concentrations (± s.e.m.) of ^{3}H (●——●) and ^{14}C (○——○) after simultaneous administration of oral sodium [^{14}C] cromoglycate and subcutaneous sodium [^{3}H]cromoglycate to rats of varying ages. The figures above each curve indicate the age of the animals.

of the administered dose than did the older rats (2·7–3·3%).

The fraction of the dose which was estimated to have been excreted in the bile after subcutaneous administration remained approximately constant throughout development of the rat (4–41 days of age, Table 2). Little of the administered dose (3·0 ± 0·7% at 4 days old, 1·1 ± 0·1% at 41 days of age) was detectable in the carcass and remaining tissues at any age.

Table 2. Biliary excretion of sodium cromoglycate by rats (n = 4) at 4, 8, 15, 21 and 41 days of age. Sodium cromoglycate was administered subcutaneously and biliary excretion calculated by % of the dose present in the gastrointestinal tract 4 hours after administration.

Age (days)	% of dose present in the gastrointestinal tract ± s.e.m.
4	38·0 ± 4·6
8	28·5 ± 9·5
15	26·8 ± 7·9
21	38·9 ± 4·0
41	46·4 ± 3·6

DISCUSSION

Sodium cromoglycate is not metabolized in any animal species studied including the rat (Ashton et al 1973). Thus the concentrations of radioactivity determined in blood are a direct measure of sodium cromoglycate concentration.

The ^{14}C area under the blood curve (AUC) identifies the orally absorbed sodium cromoglycate, and is a measure of bioavailability. This was several times higher in the younger rats (5, 9 and 14 days old) than in the older ones (20, 29 and 75 days old). The factors which influence the oral bioavailability of sodium cromoglycate are the fraction of the dose absorbed (F) and the blood clearance of the compound (clearance = F × dose/AUC). The blood clearance of subcutaneously administered sodium cromoglycate increases as the rats grow older. In the 20, 29 and 75 day old rats the blood clearance was 2·5–4 times more rapid than in the 5, 9 and 14 day old pups. This increase can be explained by the maturation of both biliary and renal excretion processes. Both have been shown to develop with age for other compounds (Hunter & Klaassen 1975; Horster & Lewy 1970). The blood clearance represents the total clearance which is the sum of all clearance processes. Sodium cromoglycate is cleared by renal and biliary excretion and hence blood clearance is the sum of renal and biliary clearance. The biliary and renal excretory systems for sodium cromoglycate therefore appear to develop at approximately equal rates in the rat since there is a constant proportion of a subcutaneously administered dose excreted into the gastrointestinal tract at each age investigated. That this value is a valid estimate of biliary excretion is indicated by the small amount of sodium cromoglycate remaining in the carcass and the low reabsorption of compound that could occur.

After oral administration of sodium cromoglycate to adult rats, the absorption did not exceed 4% (Moss et al 1970). The 20, 29, 75 day old rats show a percentage absorption with a similar value to that reported. In contrast the 5, 9 and 14 day old rats absorb 2–3 times more of an oral dose. The differences in absorption are likely to be due to changes in the structure or absorptive area of the gastrointestinal tract. Reduced absorption due to the presence of solid food in the gut cannot be discounted, however. The difference in absorption cannot be ascribed to a first pass effect since clearance by the biliary system represents a constant proportion of total clearance in the developing rat. An age related decrease in absorption by the gastrointestinal tract of the growing rat occurs with materials ranging from immunoglobulin G to cadmium metal (Morris & Morris 1974; Sasser & Jarboe 1977). Both of these examples appear to involve changes in permeability of the gastrointestinal tract. A decrease in the area of the gastrointestinal tract of the rat relative to body weight during development has also been indicated in the literature (Younoszai & Ranshaw 1974; Morselli 1976). To our knowledge, an increased absorption of a pharmaceutical in young animals or children has not been reported. Although increased bioavailability in neonates, compared with adults, has been demonstrated for the acid labile pencillins, this has been ascribed to the achlorhydria of the neonate. Most studies on the effect of age on absorption have tended to report either no change or a decrease (Morselli 1976; Wallin et al 1974). These reports dealt with compounds that are normally well absorbed in young animals or children and presumably cross the intestinal membranes by virtue of their lipophilic nature. Sodium cromoglycate is highly polar and other mechanisms, such as passage between absorbtive cells (Fromter & Diamond 1972; Turnberg 1978) as postulated for particulate matter (Volkheimer 1975) may be involved to explain its absorption. The parameters relating to the absorption of polar compounds will not necessarily relate to absorption of more lipo-

philic materials. Age-related changes in absorption rate have been previously reported for sodium cromoglycate from sites other than the gastrointestinal tract. Hemberger & Schanker (1979) found, under conditions in which diffusion predominates as the mechanism for absorption, that sodium cromoglycate was absorbed twice as rapidly from the lung in rats aged 1–12 days than from rats of 18 days or older.

In conclusion age-related changes in both clearance and oral absorption have been demonstrated for sodium cromoglycate in the developing rat. Whilst the bioavailability of sodium cromoglycate is consequently much greater in the young than the older rat the compound can still be considered to be poorly absorbed after oral administration.

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