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The fate of [^{14}C]disodium cromoglycate in man

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The fate of [^{14}C]disodium cromoglycate (DSCG) has been examined in 12 asthmatic patients. Maximum plasma concentrations (mean 9·2 ng/ml) were obtained within 15 min of inhaling DSCG (20 mg) and the average plasma half-life was 81 min. Although absorption from the lung is rapid, most of the inhaled dose is swallowed. Only 2·0% of the dose was excreted in the urine, and 84% was recovered from the faeces. DSCG is poorly absorbed from the gastrointestinal tract, only 0·4% of an oral dose (20 mg) appeared in the 24 h urine and 83% was recovered from the faeces. Intravenous administration of DSCG resulted in approximately equal amounts (30–50% of dose) being excreted via the urine and faeces. No metabolites of DSCG were detected chromatographically.

Disodium cromoglycate (DSCG, Intal), the disodium salt of 1,3-bis(2-carboxychromon-5-yloxy)-2-hydroxypropane, is used in the treatment of bronchial asthma (Howell & Altounyan, 1967; Kennedy, 1967). The animal pharmacology of this compound has been reported by Cox, Beach & others (1970). DSCG has no bronchodilator activity or steroid-like activity in animals. It inhibits mast cell disruption and the passive cutaneous anaphylactic reaction.

In man, inhalation of this drug inhibits antigen-induced bronchospasm (Pepys, Hargreave & others, 1968) and exercise-induced bronchoconstriction (Davies, 1968).

In monkeys, rabbits and rats, only 1, 2 and 4% of an oral dose of DSCG was absorbed, respectively (Moss, personal communication). However, after its intratracheal administration, DSCG was rapidly absorbed from the lung in these three species (Moss & Ritchie, 1970). Intravenous studies (Cox, 1970) have shown that DSCG is rapidly excreted via the bile and urine in the mouse, rat, hamster, rabbit, dog, monkey and baboon, the amounts excreted by either route varying with the species. Examination of the urine, bile and faeces from the rat, rabbit and monkey, by Sephadex chromatography, electrophoresis and thin-layer chromatography, in solvent systems known to separate closely related DSCG analogues and hydrolytic breakdown products of chromones, indicated that all the recovered radioactivity was unchanged DSCG (Moss, Jones & others, 1970).

In the present study the absorption, metabolism and excretion of [^{14}C]DSCG has been examined in asthmatic patients. This drug is given by inhalation therapeutically, but its fate was also examined after oral and intravenous administration. A preliminary communication of the metabolic fate of DSCG in man has already been published (Walker, Richards & Paterson, 1971).

METHODS

Subjects

Twelve asthmatic patients, previously treated with a variety of drugs, including corticosteroids, were given the drug with their full consent. Measurements of

pulse, blood pressure, FEV_1 (forced expiratory volume in 1 s), and FVC (forced vital capacity) were made.

Inhalation study. Six subjects received a 20 mg dose of [^{14}C]DSCG (33·4 μCi), as a micronized powder (more than 50% w/w being between 2 and 6 μm), from a Spinhaler (Fisons). All other therapy, with the exception of steroids, was discontinued throughout the first 24 h of the study after which previous treatment was resumed. At appropriate times for the first 6 h after inhalation of [^{14}C]DSCG, venous blood samples (10 ml) were taken. Urine samples were collected for 24 or 48 h, and faeces were collected for 3 days.

Oral study. Four subjects were given 20 mg of [^{14}C]DSCG (8·5 μCi) by mouth. Urine was collected for 24 h and faeces for 3 days; no blood samples were taken.

Intravenous study. Two subjects were given [^{14}C]DSCG (3·85 mg, 5·6 μCi; 3·75 mg, 5·5 μCi). Blood samples (10 ml) were taken during the first 4 h. Urine was collected for 24 h and faeces for 3 days The electrocardiogram was monitored continuously for the first 60 min.

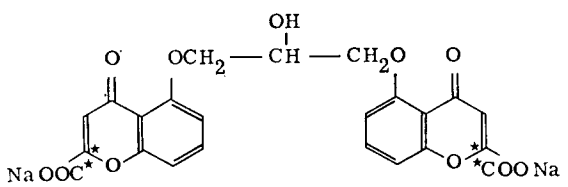
Before the intravenous metabolic study, non-radioactive DSCG was given in doses of 0·5, 1, 2, 4 mg, each injection being made over 30 s. Any effects noticed by the patient were recorded and in addition the electrocardiogram, heart rate, and blood pressure were monitored.

Urine and faeces were examined for total ^{14}C-activity and also for metabolic products of DSCG.

Physical properties

Materials. Disodium cromoglycate and [^{14}C]disodium cromoglycate (specific activity 1·67 μCi/mg) labelled on the carboxylic acid groups and in the '2'-position on the benzophenone ring (I) were prepared by Fisons Pharmaceuticals Ltd., Loughborough, Leicestershire.

I



Chromatography. The R_F values of DSCG in the following solvent systems were obtained by descending chromatography (16 h) using Whatman 3 mm paper. A. Butan-1-ol–glacial acetic acid–water (4 : 1 : 2 by vol) R_F = 0·29. B. Propan-2-ol–ammonium hydroxide (sp. gr. 0·88) (7 : 3 by vol) R_F = 0·45. C. Butan-1-ol–ammonium hydroxide (sp. gr. 0·88)–water (10 : 1 : 1 by vol) R_F = 0·00. D. Butan-1-ol–ethanol–water (9 : 4 : 7 by vol) R_F = 0·10. E. Chloroform–methanol–water (45 : 45 : 10 by vol) R_F = 0·66. F. Propan-2-ol–ammonium hydroxide (sp. gr. 0·88)–methanol–water (20 : 1 : 2 : 2 by vol) R_F = 0·10.

Quantitative estimation of [^{14}C]DSCG

Scintillation counting. The total ^{14}C-activity in a sample was measured in a Tri-Carb Liquid Scintillation Spectrometer (Model No. 3375; Packard). All counting was done at 4°. Urine (1 ml) was counted in Instagel (Packard) (9 ml). Faeces were homogenized with water and an aliquot (0·5 ml) of the supernatant was counted

in Instagel (9 ml). Blood was collected into lithium heparin tubes and spun at 2000 rev/min for 10 min. Plasma (2 ml) was counted in Instagel (18 ml). The counting efficiency of any sample was determined from a standard curve by the automatic external standard method. Some faecal samples gave a very high initial counting rate probably due to chemiluminescence, however, a dark adjustment period of 24 h at 4° allowed the true counting rate of the sample to be determined.

Radiochromatogram scanning. Urine collected from patients given [14C]DSCG (0·1–1 ml) was banded (2·5 cm band) on a strip (4 cm wide) of Whatman 3 mm paper. The strip was chromatographed in one of the solvents A–F. Strips with a reference spot of [14C]DSCG were chromatographed at the same time. The strips were scanned, using a Panax Radiochromatogram Scanner (RTLS–1A) with the paper attachment (RCMS–3), at a rate of 60 cm/h, using a time constant of 10 s at 1·2 KV (for best signal/noise ratio, the gas being 98% argon–2% propane). To detect any metabolites (<1% of recovered radioactivity) radiochromatograms were cut into sections (2 cm long) and the sections cut into small pieces and counted in Instagel. Where the urinary activity was low, the urine was freeze dried and the residue extracted with methanol (95–100% recovery). This was then chromatographed as described above.

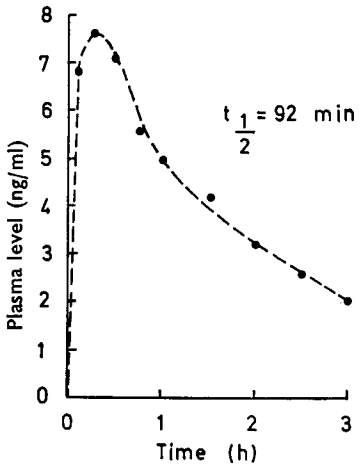


FIG. 1. Plasma concentrations of [14C]DSCG after inhalation of 20 mg by one subject (III).

Table 1. *Maximum plasma concentrations, urinary and faecal recovery of [14C]-disodium cromoglycate in patients after inhalation of 20 mg.*

Patient	Sex	Maximum plasma level DSCG (ng/ml)	Plasma $t_{\frac{1}{2}}$ (min)	% dose recovered in faeces (3 days)	% dose excreted in urine 24 h	48 h
I	M	12·1	56	87	2·5	—
II	M	6·5	71	80	1·3	—
III	M	7·7	92	—	2·6	2·7
IV	M	9·8	90	—	3·1	3·2
V	F	11·9	106	—	1·6	—
VI	F	6·9	71	—	0·7	—
Mean ± s.e.		9·2 ± 1·0	81 ± 7	84	2·0 ± 0·4	—

RESULTS

Inhalation study

DSCG appeared rapidly in the blood (Fig. 1) and a mean maximum plasma concentration of 9·2 ng/ml (6·5–12·1 ng/ml) was obtained within 15 min after inhalation (Table 1). The mean plasma half-life was 81 min (range 56–106 min), and very little DSCG (<1 ng/ml) remained in the blood after 4 h. Between 0·7 and 3·1% of the dose inhaled appeared in the urine within 24 h (Table 1), whereas little of the dose was excreted during the 24–48 h period. The urinary excretion of the drug was rapid, 50% of the total amount excreted in 24 h was eliminated within 2 h (Fig. 2). In 2 patients 80–87% of the dose was recovered from the faeces during 3 days. We have found that some 5–10% of the dose remains in the inhaler after inhalation.

Oral study

Only 0·4% (range 0·3–0·5%) of the oral dose was excreted in the urine in 24 h (Table 2). Most of the dose was recovered from the faeces in the two subjects examined (81 and 84%). Absorption of DSCG from the gastrointestinal tract is much slower than absorption from the lungs, 50% of the total amount of the dose excreted in 24 h was eliminated within 5 h (Fig. 2).

Intravenous study

Approximately equal amounts of the drug were excreted in the urine and in the

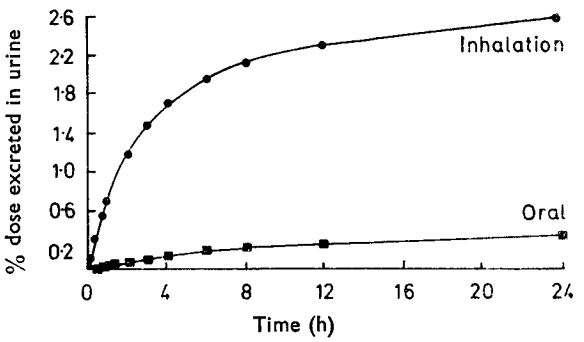


FIG. 2. Urinary excretion of [^{14}C]DSCG after inhalation (III) and oral (VIII) administration (20 mg).

Table 2. *Pattern of excretion after oral and intravenous administration of* [^{14}C]-*disodium cromoglycate to asthmatic patients.*

Patient	Sex	Route of administration	% dose excreted in urine (24 h)	% dose excreted in faeces (3 days)
VII	M	Oral	0·4	81
VIII	M	Oral	0·3	84
IX	F	Oral	0·5	—
X	F	Oral	0·3	—
XI	F	Intravenous	33·9	32
XII	M	Intravenous	52·5	38

faeces after intravenous administration (Table 2). DSCG was excreted rapidly in the urine of the two subjects (Fig. 3); most of the dose that was eliminated via this route was excreted within the first hour. The remainder was recovered from the faeces in 3 days. A semi-logarithmic plot of plasma concentration against time after administration of DSCG showed that during the first 10 min the plasma concentrations declined rapidly from ≈800 to ≈100 ng/ml, giving a distribution half-life of approximately 3 min. When after about 60 min the plasma concentration reached 12 ng/ml (the maximum obtained after inhalation) the second phase of the graph indicated a plasma elimination half-life of 76 min and 100 min, respectively for each subject. This is about the same as the plasma half-life obtained after inhalation of DSCG.

No pharmacological effects were observed after inhalation of DSCG (20 mg), the peak plasma concentration reached was 12·1 ng/ml. Three subjects given non-radioactive DSCG intravenously all had a sensation of heat. At the smaller doses this was localized in the chest, but as the dose was increased it spread to the throat and arms, and intense sweating also occurred. These symptoms appeared more rapidly and lasted longer the larger the dose, being present within 15 s of ending the 4 mg injection, and lasting from 5 to 6 min after that dose. In two subjects there

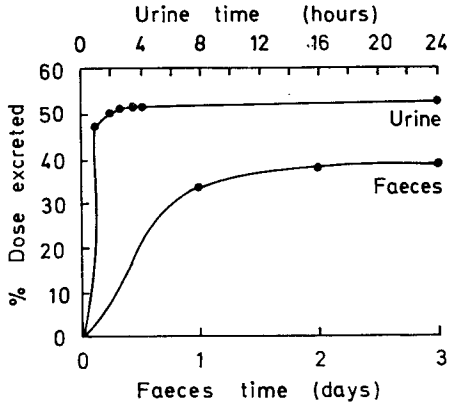


FIG. 3. Urinary and faecal excretion of ^{14}C-activity after an intravenous dose of [^{14}C]DSCG (3·75 mg.)

were associated rises in heart rate and blood pressure, which returned to baseline before the symptoms disappeared. Similar symptoms occurred in the two subjects given intravenous [^{14}C]DSCG. Plasma concentration measurements showed that the symptoms were at their highest at over 800 ng/ml of drug and had disappeared at 100 ng/ml.

All the samples of urine and faeces were examined chromatographically using a radiochromatogram scanner, but, as also reported for animals (Cox & others, 1970), no metabolic products of DSCG were found. All the radioactivity chromatographed as the parent compound.

DISCUSSION

Morrow, Gibb & others (1968) have suggested that absorption of foreign compounds from the lung resembles that from the gastrointestinal tract. However, we

have found DSCG to be rapidly absorbed from the lung after inhalation, but slowly absorbed from the gastrointestinal tract after oral administration to asthmatic patients. DSCG, being the disodium salt of a strong acid ($pK_1a = pK_2a = 1{\cdot}9$), is poorly absorbed from the gastrointestinal tract, only a small amount being absorbed by passive diffusion. Enna & Schanker (1969) have shown that relative to gastrointestinal mucosa, pulmonary epithelium possesses a high permeability to lipid insoluble molecules and ions, and this could explain the absorption of DSCG from the lung. The amount of DSCG excreted in the urine after buccal administration (0·5% of the dose given) was similar to that found after oral administration (Jones, personal communication).

Cox & others (1970) found in animals that renal excretion of DSCG was by glomerular filtration and also by active transport across the renal tubular epithelium. When the clearance rate of DSCG (208–423 ml/min found for the six patients inhaling the drug) is compared to that of creatinine (125 ml/min) our results indicate that DSCG is actively secreted via the renal tubules in man. DSCG, being a highly polar molecule with a molecular weight of 512, satisfies the criteria for biliary excretion (Millburn, Smith & Williams, 1967), and is excreted in the bile of rats (Moss & others, 1970). As our results show, it is also excreted in the bile in man,

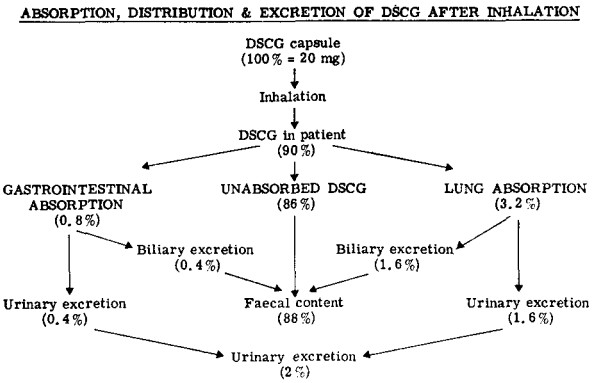


FIG. 4. Absorption, distribution and excretion of [^{14}C]DSCG after inhalation.

equal amounts of the drug being recovered from the urine and faeces after an intravenous dose.

The drug for inhalation is micronized in a size range providing more than 50% by weight between 2–6 μm (Cox, Bell & Hartley, 1969). Particles of DSCG in this range are highly cohesive and become agglomerated into masses. The dispersion of these agglomerates of powder depends upon the efficient use of the inhaler. At flow rates of air through it of less than 80 litre/min, up to 50% of the dose can be deposited in the mouth (Moss, Jones & others, 1971), above this flow rate much less powder is deposited in the mouth and a greater dispersion is achieved. Powder deposited in the mouth, together with any DSCG that may be washed back by ciliary action from the trachea or coughed up is then swallowed. The low percentage absorption reflects the difficulty of getting DSCG into the lungs.

Fig. 4 summarizes the fate of DSCG after inhalation using values calculated from our data. From the maximum amount of 90% of the dose that reaches the patient (since $\approx$10% remains in the inhaler), and from the urinary and faecal excretion data

we have calculated that 86% of the dose is unabsorbed. This calculated value agrees well with the faecal recovery of radioactivity (80–87%). However, only 4% of the inhaled dose is absorbed, and this is partly from the lung (3·2%) and partly from the gastrointestinal tract (0·8% calculated from the oral and intravenous results). The intravenous study indicated that urinary excretion accounts for half the total amount absorbed. After oral administration 0·4% of the dose was excreted in the urine and thus 0·8% of the dose must have been absorbed. Therefore, the total amount of DSCG absorbed by the lung and gastrointestinal tract after inhalation (4%) is excreted via the bile (2%) and urine (2%).

The mechanism by which intravenous DSCG produced heat and sweating with a rise in heart rate and blood pressure in man was not established. The animal pharmacology (Cox & others, 1970) revealed no cardiovascular effects from very high doses of DSCG except in two species. In conscious dogs, 0·05–0·1 mg/kg (equivalent to a total of 3·5–7 mg in a 70 kg man) produced transient collapse with a large decrease in heart rate and blood pressure, and the animals recovered within 10 min. The effect could be abolished by pretreatment with atropine. Tachyphylaxis occurred, and the authors thought the effect in the dog was by activation of chemoreceptors in the pulmonary and possibly the coronary circulation, initiating a reflex via the vagus. In contrast, intravenous injections of 0·001–0·02 mg/kg in the anaesthetized marmoset (equivalent to 0·07–1·40 mg in a 70 kg man), caused marked hypertension and tachycardia, probably by stimulation of the sympathetic nervous system at a post-ganglionic site but not directly on receptors.

It would seem that the changes observed in man are most closely related to those seen in the marmoset, and could certainly be explained by transient sympathetic stimulation. This only occurs at blood concentrations some ten times those observed after an inhalation dose.

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REFERENCES

Cox, J. S. G. (1970). In: *Disodium Cromoglycate in Allergic Airways Disease*, pp. 13–25. Editors: Pepys, J. & Frankland, A. W. Butterworths: London.

Cox, J. S. G., Beach, J. E., Blair, A. M. J. N., Clarke, A. J., King, J., Lee, T. B., Loveday, D. E. E., Moss, G. F., Orr, T. S. C., Ritchie, J. T. & Sheard, P. (1970). *Adv. Drug Res.*, **5**, 115–196.

Cox, J. S. G., Bell, J. H & Hartley, P. S. (1969). *Br. med. J.*, **2**, 634.

Davies, S. E. (1968). *Ibid.*, **3**, 593–594.

Enna, S. J. & Schanker, L. S. (1969). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **28**, 359.

Howell, J. B. L. & Altounyan, R. E. C (1967). *Lancet*, **2**, 539–542.

Kennedy, M. C. S. (1967). *Acta allerg. (Kbh.)*, **22**, 487–489.

Millburn, P., Smith, R. L. & Williams, R. T. (1967). *Biochem. J.*, **105**, 1283–1287.

Morrow, P. E., Gibb, F. R., Davies, H. & Fisher, M. (1968). *Toxic. appl. Pharmac.*, **12**, 372–396.

Moss, G. F., Jones, K. M., Ritchie, J. T. & Cox, J. S. G. (1970). *Ibid.*, **17**, 691–698.

Moss, G. F., Jones, K. M., Ritchie, J. T. & Cox, J. S. G. (1971). *Ibid.*, **20**, 147–156.

Moss, G. F. & Ritchie, J. T. (1970). *Ibid.*, **17**, 699–707.

Pepys, J., Hargreave, F. E., Chan, M. & McCarthy, D. S. (1968). *Lancet*, **2**, 134–137.

Walker, S. R., Richards, A. J. & Paterson, J. W. (1971). *Biochem. J.*, **125**, 27P.