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The fate of Drotaverine - Acephyllinate+ in rat and man I. Absorption, distribution and excretion in the rat

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SUMMARY

Two different labelled forms were used for the pharmacokinetic investigations: the carbon 1 in the isoquinoline ring (Drotaverine-^{14}C-Acephyllinate) and the carboxyl group of theophylline-7-acetic acid (Drotaverine-Acephylline-^{14}C-ate).

Drotaverine-^{14}C-Acephyllinate was rapidly absorbed from duodenal and ileal segments. Biliary excretion was substantial after oral administration and radioactivity was excreted mostly in the feces.

Absorption of Drotavenine-Acephylline-^{14}C-ate from the gastrointestinal tract was very poor and radioactivity was therefore excreted for the most part in the feces.

The results of the study were confirmed by whole body autoradiography.

INTRODUCTION

Benzyl-isoquinoline derivatives (Paraverine, Ethaverine, Drotaverine) are well known for their excellent smooth muscle relaxant and cardiovascular properties. Drotaverine-Acephyllinate (Fig. 1), (theophylline-7-acetate of 6,7,3,4-tetraethoxy-1-benzyl-3, 4-dihydro-isoquinoline) is a potential new smooth muscle relaxant and geriatric synthesized by Szentmiklósi and Mészáros (1). The clinical studies showed a positive peripheric vasodilating effect (2).

Comparative pharmacology indicated that the duration of action of Drotaverine-Acephyllinate was greater than that of Drotaverine HCl (No-Spa® Chinoin) and at the same time was 1.6 times less toxic which made it therapeutically more advantageous.

Whole body autoradiography of Drotaverine--1-^{14}C HCl was carried out on mice by Magyar (3), absorption and excretion were studied by Simon (4) in the rat.

Comparative blood level studies of Drotaverine--1-^{14}C HCl and Drotaverine-^{14}C-Acephyllinate performed by Szentmiklósi (5) on mice showed higher values for Depogen after intravenous as well as oral administration.

Strolin-Benedetti (6) examined the absorption and excretion of theophylline-7-acetic acid (acepiphylline) in rats and dogs and found that absorption was very poor, the compound being eliminated for the most part in the feces.

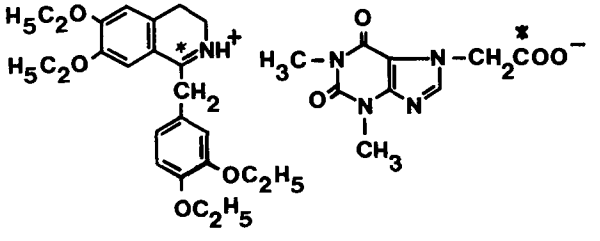

Fig. 1 : The chemical structure of Drotaverine-Acephyllinate (positions of ^{14}C label).

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+ Depogen® (Chinoin, Budapest).

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MATERIALS AND METHODS

Radiochemicals

Drotaverine-1-^{14}C HCl was synthesized by Koltai (7) and this compound was used for the preparation of Drotaverine-^{14}C-Acephyllinate (spec.act.: 0.266 GBq/g, 7.255 mCi/g; 169.1 MBq/mM, 4.57 mCi/mM).

Drotaverine-Acephylline-^{14}C-ate was synthesized at the Institute for Drug Research, Budapest (spec. act.: 0.107 GBq/g, 2.88 mCi/g; 67.7 MBq/mM, 1.83 mCi/mM).

Radiochemical purity of the labelled compounds proved to be higher than 95 per cent as checked by thin layer chromatography using the LB 2723 Berthold scanning system.

Experimental animals

For the whole body autoradiographic and balance studies Wistar-H/Riop and for the measurements of absorption and biliary elimination, CFY male albino rats weighing 180-200 g were used. On a small number of animals control studies were carried out and the experimental results proved that there were no strain differences.

Absorption studies by in vivo loop technique

On the small intestine of narcotized animals (40 mg/kg i.p. Nembutal) following upper laparotomy two loops were isolated, the first in the duodenum (8 cm long), the second in the ileum (10 cm long).

Radioactive Drotaverine-Acephyllinate dissolved in 0.3 ml distilled water was injected into the sacs and the abdominal wall was temporarily closed. At the end of the experimental period the sacs were opened, rinsed with 10 ml distilled water and the wall the loop was homogenized. Radioactivity of the rinsing water and homogenate was measured and the missing part was considered to have been absorbed.

Whole body autoradiography

Whole body autoradiography was carried out according to the method described by Ullberg (8) using PMV-450 MP Cryomicrotome (LKB). The X-ray film copies were evaluated by Telechrom OE-976 (Chinoin) videodensitometer.

Biological sampling

After administration of the drug, the animals were placed in individual metabolic cages, urine and feces were collected separately. During the experimental period the rats were given pellet food and water ad libitum. $^{14}CO_2$ content of the expired air was absorbed in Carbo-Sorb® II. (Packard). At the end of the experimental time the animals were killed by chloroform anaesthesia and total radioactivity of the carcass was measured.

For collecting bile samples the bile duct of Nembutal-narcotized animals was cannulated.

Measurement of radioactivity

200 µl sample of the intestinal wall homogenate, rinsing water or bile was diluted with 800 µl distilled water and 9 ml 1:2 V/V Triton-X 100- toluene cocktail (6 g PPO, 0.4 g POPOP in 1000 ml toluene) was added.

Radioactivity of 50 µl urine samples was measured by adding 10 ml Bray cocktail (4 g PPO, 0.2 g POPOP, 60 g naphthalene, 100 ml methanol, 20 ml ethylene glycol, 1000 ml dioxane).

The feces were dried to constant weight over P_2O_5 and homogenized, combusted by Oxiscint equipment (9) according to Gács (10). The radioactive CO_2 was absorbed in 2 ml Carbo-Sorb II., 13 ml cocktail was added (4 g PPO, 0.25 g POPOP in 1000 ml toluene).

For the measurement of the expired $^{14}CO_2$ the above system was used.

The carcass was dissolved in 200 ml conc. sulphuric acid (4-5 days, preliminary experiments proved that no radioactivity bubbled out of the system), 50 mg sample taken, 0.4 ml H_2O_2 added, after 3 hours 6 ml ethylene glycol monomethyl ether and 10 ml toluene cocktail (6 g PPO, 1000 ml toluene).

Radioactivity was measured by LKB Wallac 8100 equipment, quench correction made by external standardization.

RESULTS

Absorption from intestinal loops

Absorption of Drotaverine-^{14}C-Acephyllinate from duodenum and ileum as a function of time is shown in Table I (dose: 80 µg and 600 µg per sac). Drotaverine-Acephyllinate was quickly absorbed from the sacs with high absorption capacity; there was no difference between the two intestinal segments.

Table I : Absorption of Drotaverine-^{14}C-Acephyllinate from duodenal and ileal sacs expressed in percentages of the administered dose.

	80 µg/sac				600 µg/sac
	10 min	12.5 min	20 min	30 min	15 min
duodenum	76.3 ± 5.9 n = 7	77.6 ± 8.0 n = 6	82.3 ± 10.3 n = 6	88.5 ± 2.9 n = 7	77.2 ± 8.9 n = 8
ileum	68.0 ± 3.3 n = 5	82.1 ± 6.2 n = 6	84.2 ± 4.6 n = 7	92.4 ± 3.4 n = 7	84.0 ± 5.1 n = 8

Whole body autoradiography

The studies were made with both labelled forms of the compound. The animals were administered 15 mg/kg Drotaverine-Acephyllinate dissolved in 1.0 ml distilled water through a gastric tube or in 0.1 ml distilled water injected into the tail vein.

The autoradiograms showed that 2 hours after oral Drotaverine-^{14}C-Acephyllinate treatment there was a high level of radioactivity in the stomach and small intestines, medium in the lacrimal glands, hypophysis, oesophagus and liver (Fig. 2). 24 hours following treatment total radioactivity of animals decreased, there was a significant level in the stomach and the radioactivity was very characteristic in the large intestines indicating fecal excretion. 72 hours after administration, the drug and/or its metabolites were almost completely excreted from the rat, a low level remaining only in the large intestines.

5 minutes after iv administration of Drotaverine-^{14}C-Acephyllinate the radioactivity entered the central nervous system showing a medium level in the brain and spinal cord. Medium level in the wall of stomach and small intestinal lower level in large intestines indicated that the compound and/or its metabolites was excreted through the mucous membranes. There was a high level of radioactivity in the adrenals (Fig. 3). 2 hours following iv administration the radioactivity of stomach and small intestines was high which meant a biliary excretion in addition to the above mentioned mucosal transport. There was no radioactivity in the central nervous system and considerably decreased radioactivity in the other organs, e.g. adrenals.

After Drotaverine-Acephylline-^{14}C-ate oral administration the major part of the radioactivity was always found in the gastrointestinal tract showing the poor absorption of theophylline-7-acetic acid. The low level of radioactivity of the renal pelvis (2 hours) and urinary bladder (24 hours) indicated slight absorption (Fig. 4).

5 minutes after iv administration of Drotaverine-Acephylline-^{14}C-ate there was a high level of radioactivity in the kidney and urinary bladder showing the quick urinary elimination of theophylline-7-acetic acid. Considerable activity of small intestines, medium level of stomach and liver indicated mucosal and biliary excretion (Fig. 5). 30 minutes after administration the radioactivity of renal pelvis, urinary bladder and small intestines was high, it was lower in the renal cortex. 2 and 6 hours after administration there was radioactivity only in the intestines and urinary bladder.

Excretion

Table II shows the biliary excretion of radioactivity after an 800 µg/kg oral dose of Drotaverine-^{14}C-Acephyllinate. The biliary excretion was high (15.22 D% within 150 minutes) but lower than after equimolar oral dose (500 µg/kg) of Drotaverine-1-^{14}C HCl (4) (Table III).

Table IV shows the elimination of radioactivity in urine, feces and expired air after oral administration of Drotaverine-^{14}C-Acephyllinate. The experimental results show that the major part of the radioactivity was excreted in the feces, there was practically no $^{14}CO_2$ in the expired air. The part of radioactivity found in the carcass was mostly due to the feces remaining in the rectum.

Table V shows the excretion of radioactivity after oral administration of 15 mg/kg Drotaverine-Acephylline-^{14}C-ate. The elimination was complete in 72 hours.

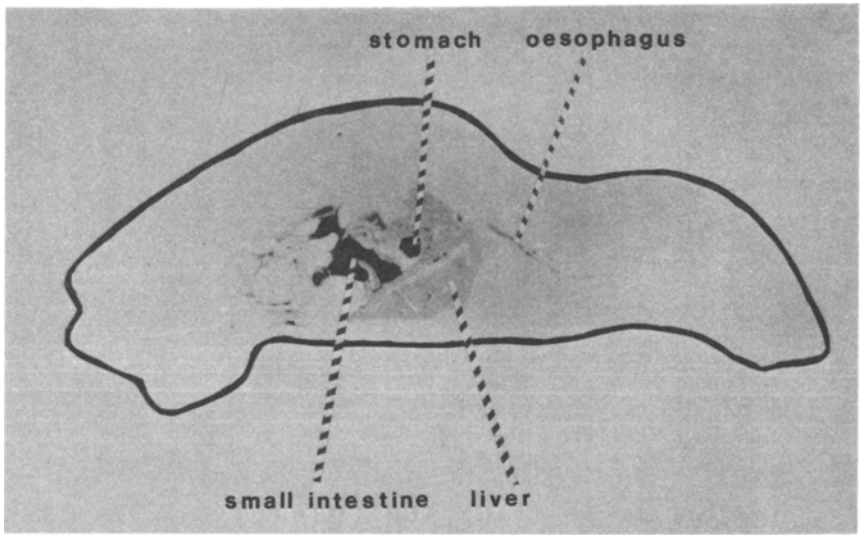

Fig. 2 : Distribution of radioactivity 2 hours after oral administration of Drotaverine-^{14}C-Acephyllinate.

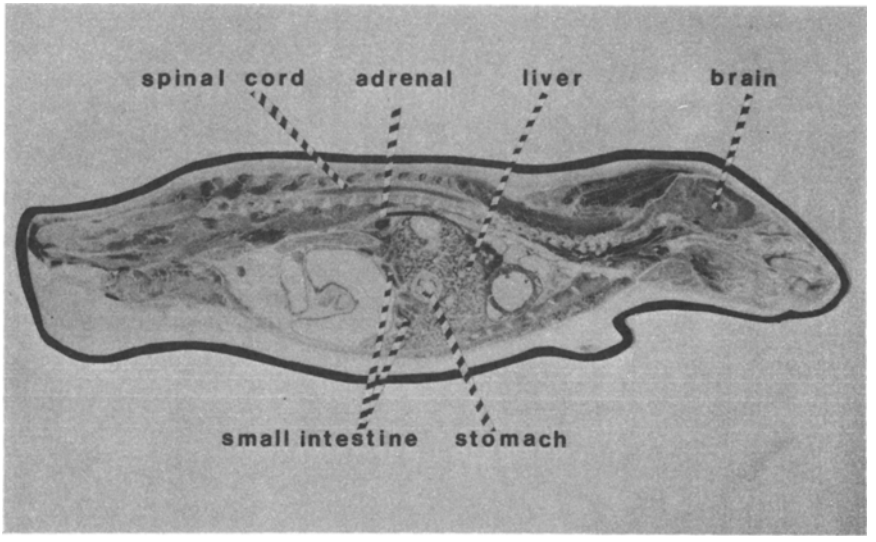

Fig. 3 : Distribution of radioactivity 5 minutes after intravenous administration of Drotaverine-^{14}C--Acephyllinate.

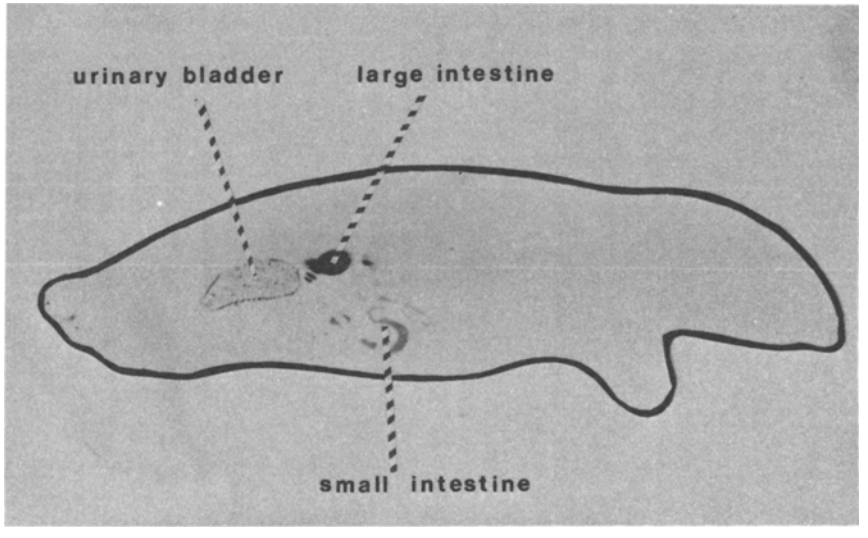

Fig. 4 : Distribution of radioactivity 24 hours after oral administration of Drotaverine-Acephylline-^{14}C-ate.

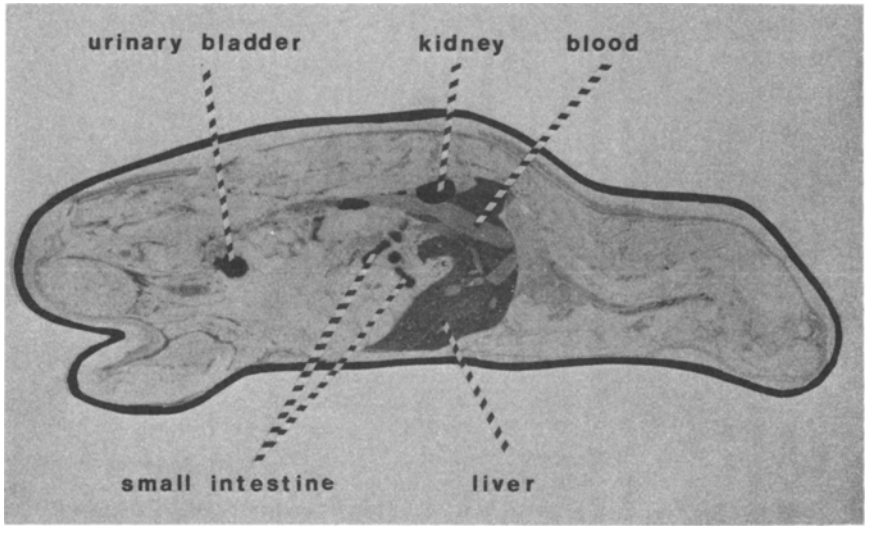

Fig. 5 : Distribution of radioactivity 5 minutes after intravenous administration of Drotaverine-Acephylline-^{14}C-ate.

Table II : Biliary excretion of radioactivity after oral administration of Drotaverine-^{14}C-Acephyllinate expressed in D% (800 µg/kg).

	0 - 30 min	30 - 60 min	60 - 90 min	90 - 120 min	120 - 150 min	0 - 150 min
$\bar{x}$	1.82	3.60	3.79	2.88	2.13	15.22
S.D.	± 1.24	± 1.77	± 3.43	± 1.06	± 0.78	± 7.94
n	6	5	6	5	5	5

Table III : Biliary excretion of radioactivity after an 500 µg/kg oral dose of Drotaverine-1-^{14}C HCl expressed in D% (4).

	0 - 30 min	30 - 70 min	70 - 110 min	110 - 150 min	150 - 190 min
$\bar{x}$	7.81	6.5	9.5	6.0	3.2
S.D.	± 4.26	± 6.2	± 6.8	± 5.1	± 1.6
n	8	8	8	8	8

Table IV : Elimination of radioactivity in urine, feces and expired air after oral administration of 15 mg/kg Drotaverine-^{14}C-Acephyllinate expressed in D% (total values were calculated for each animal as the sum of excretion values of different routes).

	0 - 24 h	24 - 48 h	48 - 72 h	0 - 72 h
urine	6.1 ± 3.2 n = 6	2.0 ± 0.9 n = 5	1.2 ± 1.4 n = 5	9.1 ± 2.7 n = 5
faeces	51.3 ± 18.6 n = 4	12.3 ± 9.5 n = 4	3.2 ± 2.0 n = 4	65.3 ± 5.7 n = 4
expired air	0.20 ± 0.01 n = 4	0.12 ± 0.00 n = 4	0.17 ± 0.09 n = 4	0.36 ± 0.09 n = 4
carcass	—	—	—	25.9 ± 13.0 n = 4
total	58.5 ± 15.8 n = 4	14.8 ± 10.2 n = 4	4.2 ± 3.5 n = 4	96.1 ± 9.5 n = 4

Table V : Elimination of radioactivity in urine, feces and expired air after oral administration of 15 mg/kg Drotaverine--Acephylline-^{14}C-ate expressed in D% (n = 4) (total values were calculated for each animal as the sum of excretion values of different routes).

	0 - 24 h	24 - 48 h	48 - 72 h	0 - 72 h
urine	7.9 ± 6.2	2.9 ± 3.3	0.4 ± 0.1	11.1 ± 9.5
faeces	63.1 ± 10.2	19.8 ± 3.0	6.4 ± 1.8	89.3 ± 5.3
expired air	0.06 ± 0.01	0.02 ± 0.02	0.00 ± 0.00	0.08 ± 0.04
total	71.1 ± 4.0	22.6 ± 6.4	6.8 ± 1.8	101.6 ± 3.7

DISCUSSION

The aim of our studies was to collect information on the metabolic fate and pharmacokinetics of the two main components of Drotaverine-Acephyllinate in rat. For this reason two labelled forms were used (i.e. in the Drotaverine and Acephylline moieties respectively).

In case of Drotaverine-^{14}C-Acephyllinate administration the fate of the Drotaverine part was examined supposedly influenced by the presence of Acephylline. As indicated in Tables II and III biliary excretion of radioactivity is two times higher after Drotaverine-^{14}C HCl treatment than after Drotaverine-^{14}C-Acephyllinate administration.

In vivo loop studies showed that Drotaverine-^{14}C-Acephyllinate was quickly absorbed from both duodenum and ileum, there was no significant difference between the two segments (because of the quick absorption the scattering was greater than the difference).

After oral administration, the major part of both labelled forms was found in the gastrointestinal tract. This can be explained in the case of Drotaverine-^{14}C-Acephyllinate, by biliary excretion, in the case of Drotaverine-Acephylline-^{14}C-ate, by poor absorption.

Following intravenous administration of Drotaverine-^{14}C-Acephyllinate the compound passed the blood-brain barrier but 2 hours after treatment there was no radioactivity in the central nervous system. Radioactivity of stomach and intestines indicated mucous and biliary excretion respectively.

There was rapid urinary elimination in the case of Drotaverine Acephylline-^{14}C-ate treatment but after 2 hours the fecal excretion became the dominant route.

Both compound given orally were excreted mainly in the feces, the expired air contained practically no radioactivity.

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