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Pharmacokinetic properties of the antimuscarinic drug [^{3}H]-hexahydro-sila-difenidol in the rat

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Summary. The pharmacokinetics of tritiated hexahydro-sila-difenidol ([^{3}H]-HHSiD) were examined in rats. Furthermore, the distribution of radioactivity was studied by means of whole body autoradiography.

After i.v. administration of 2.9 mg/kg HHSiD plus [^{3}H]-HHSiD to anaesthetized rats bearing a catheter implanted in the ductus choledochus and receiving a mannitol infusion, HHSiD was rapidly distributed and metabolized. Only 5% of the radioactivity was recovered in blood after 23 s and 0.4% after 2.5 h. 64% of the plasma radioactivity could be extracted with hexane from the samples taken 23 s after administration. 52% of the radioactivity was eliminated within 2.5 h, 13% by urinary and 39% by biliary excretion.

Following oral administration of 8.6 mg/kg HHSiD plus [^{3}H]-HHSiD there was an absorption of approximately one fourth of the administered radioactivity within 4 h. By means of whole body autoradiography (i.v. injection) as well as by tissue distribution measurement the highest levels of radioactivity were found in bile, urine, lung, kidney, adrenals, liver and pancreas. Thus, after i.v. administration to rats HHSiD is rather quickly distributed, metabolized and excreted. This explains its low antimuscarinic potency in vivo.

Key words: Pharmacokinetics – [^{3}H]-Hexahydro-sila-difenidol – Sila-drug – Rat – Autoradiography

Introduction

The antimuscarinic agent hexahydro-sila-difenidol (HHSiD, Fig. 1) is used in experimental pharmacology to differentiate subtypes of muscarinic receptors (m-cholinoceptors) due to its selectivity pattern M3 ≥ M1 > M2 (Mutschler and Lambrecht 1984; Fuder et al. 1985; Lambrecht et al. 1988, 1989; Waelbroeck et al. 1989). In in-vitro functional experiments as well as in binding studies HHSiD and pirenzepine showed a similar affinity for muscarinic M1 receptors (Lambrecht et al. 1988, 1989; Lazareno and Roberts 1989; Waelbroeck et al. 1988, 1989). On the other hand, the M1-antimuscarinic potency of HHSiD observed in in-vivo studies with pithed rats (antagonism of McN-A-343-induced increase in mean arterial pressure) after i.v. administration (Lambrecht et al. 1984; Lambrecht and Mutschler 1985) was 1.5 orders of magnitude lower than that of pirenzepine. A different pharmacokinetic behaviour of the two drugs may be responsible for this discrepancy.

Besides this, very little is known about the pharmacokinetics of organosilicon compounds and basic information referring to this is lacking (Tacke and Linoh 1989).

It was the aim of this study to investigate the pharmacokinetics of HHSiD in rats. Our interest concentrated on a determination of plasma levels, tissue distribution and elimination pathways of [^{3}H]-HHSiD and its metabolites after i.v. administration.

Although it was not possible to differentiate between HHSiD and its metabolites because total radioactivity was measured, pharmacokinetic parameters were calculated from these data. They might therefore be regarded as purely experimental quantities.

An abstract of this work has been presented previously to the Mainz spring meeting of the German Society of Pharmacology and Toxicology, March 1988 (Rettenmayr et al. 1988).

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Materials and methods

Materials. [^{3}H]-Hexahydro-sila-difenidol ([^{3}H]-HHSiD; 12.8 $\times 10^{14}$ Bq/mol, 98% radiochemical purity) was synthesized in cooperation with NEN (Boston, USA). The drug was labelled with tritium in the piperidino ring. HHSiD hydrochloride (all quantities refer to the hydrochloride) was synthesized according to the literature (Tacke et al. 1985). The other chemicals were of reagent grade and were used as purchased.

Autoradiographic studies. The distribution of radioactivity in male Wistar rats (body weight approximately 200 g) was studied by means of the whole body autoradiography methods of Ullberg (1954, 1958) and van der Kleijn (1969).

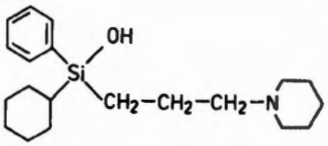

Fig. 1. Chemical structure of hexahydro-sila-difenidol (HHSiD)

Rat 1: 26 min after injection of about 7.4×10^6 Bq [^{3}H]-HHSiD (corresponding to 1.9 µg) in 0.5 ml of Tyrode solution into the tail vein, the rat was anaesthetized with 60 mg/kg of pentobarbitone sodium i.p., shaved and fixed on a specially developed device in stretched position. Thirty-five minutes after i.v. injection, the rat was sacrificed and simultaneously stiffened by submersion in isopentane cooled with solid carbon dioxide (−80°C). Tail and legs were removed and the body was embedded in carboxymethyl cellulose gel (5%). Sagittal sections of 30 µm thickness were made with a microtome (LKB 2250-PMV, MV, Stockholm, Sweden) in a refrigerated room (temperature approximately −15°C). After fixing the sections on scotch tape (Permanent Mending Tape No. 810, 3M Co., USA), the tissue was freeze-dried, pressed onto Kodak X-OMAT AR films and exposed for 15 weeks at a temperature below −10°C.

Based on the results of this pilot experiment (rat 1) a few changes of the experimental design were made to determine the tissue distribution in more detail. For example, in rat 1 most of the radioactivity was found in the urinary bladder and in the small intestine (excretion with the bile). Thus, the time between application of radioactivity and death of the animal was shortened in rat 2.

Rat 2: A mixture of 1.8×10^7 Bq [^{3}H]-HHSiD (corresponding to 4.8 µg) and 0.55 mg of unlabelled HHSiD in 0.5 ml of physiological salt solution (0.9% NaCl, w/v) was injected i.v. Three minutes later the rat was anaesthetized with halothane, fixed in the stretched position and killed 4.5 min after administration as described above. In order to reduce electrostatic charge when removing the film after the exposure time of 6 weeks, rat 2 was embedded in a more concentrated carboxymethyl cellulose gel (8%). All other conditions were the same as described by Ullberg (1954, 1958) and van der Kleijn (1969).

Pharmacokinetic studies, i.v. administration. Male Wistar rats of about 300 g ($n = 4$) were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.). The trachea was intubated and the animals were artificially respirated ($N_2O/O_2 = 1:1$). The jugular vein was cannulated with a polyethylene cannula and a 5% mannitol solution (4 ml/h) was administered in order to obtain a constant urine flow. A small amount of pentobarbitone sodium (22 mg/kg/h) was added to the infusion solution for maintenance of anaesthesia. Blood pressure was measured via a cannula placed in the carotid artery. The abdomen was opened and the bile duct and the urinary bladder were cannulated (for details see Mulder et al. 1981). Finally, the femoral artery was catheterized for rapid blood sampling. During the experiment normal body temperature (37.5–38.5°C) was maintained by placing the animal on a heating pad (the temperature was monitored rectally). [^{3}H]-HHSiD was given as an i.v. bolus injection via the femoral vein 30 min after finishing the operation. In order to obtain a therapeutically relevant dose, tritiated HHSiD was diluted with unlabelled HHSiD [approximately 2.2×10^6 Bq, corresponding to 0.6 µg plus 0.9 mg unlabelled HHSiD in 0.5 ml of physiological salt solution (0.9% NaCl, w/v)] and administered to each of four anaesthetized rats.

Immediately after injection of HHSiD together with ^{3}H-HHSiD blood, bile and urine samples were collected in polypropylene tubes at varying intervals. The samples from the first two rats had been collected in untreated vials. Because the results were found to be influenced by adsorption of HHSiD to the walls of the blood vials all vials used for rat 3 and 4 were siliconized (Silicon, Roth, Karlsruhe, FRG). There was no drug adsorption onto vial walls from bile and urine samples. Therefore, under Results plasma concentrations only of rat 3 and 4 are presented. The blood vials were heparinized (each vial contained 2 globules impregnated with Li-heparin from a Monovette, Sarstedt, Eindhoven, The Netherlands). Plasma samples were prepared immediately after every blood collection by centrifugation ($2000 \times g$, 10 min). After drawing the last sample 2.5 h after administration the rats were killed by an overdose of pentobarbitone sodium and samples of different tissues (25–200 mg) were taken. The skin was removed and the rest of the body homogenized. Samples were taken from the homogenized rat and from different sections of the skin.

Processing of samples. To 10 or 25 µl aliquots of plasma, urine or bile 10 ml of scintillation fluid (Aqualuma, Lumac, Schaesberg, The Netherlands) were added and radioactivity was determined.

In order to separate the parent drug and lipophilic metabolites from more hydrophilic metabolites, aliquots of the various samples were extracted with n-hexane and radioactivity was determined in the n-hexane extract. For this purpose 10 or 25 µl aliquots of plasma, urine or bile of two rats were diluted with water to a volume of 100 µl in siliconized Eppendorf vials. The samples were alkalized by addition of 20 µl of 0.1 mol/l NaOH and extracted three times with aliquots of 1 ml of n-hexane (centrifugation for 10 min at $18000 \times g$). The three n-hexane extracts of each sample were pooled and the organic solvent was allowed to evaporate. Radioactivity was measured in 10 ml Aqualuma. In some preliminary experiments it was found that at least 97% of HHSiD, along with hexane soluble metabolites, is extracted with this method.

The skin samples and the other homogenized tissue samples were dissolved in Soluene 100 (Packard, Groningen, The Netherlands) at 60°C and radioactivity was determined after addition of 15 ml of a mixture of Insta-Gel (Packard) and 0.5 mol/l HCl (9:1. v/v).

The radioactivity was measured in a liquid scintillation counter (Packard). The quenching was corrected by external standardization.

Oral administration. In order to determine the absorbed fraction of HHSiD after oral administration, a dose of [^{3}H]-HHSiD was given to one rat (body-weight: 282 g) after withdrawal of food for 24 h. A mixture of 6.3×10^6 Bq [^{3}H]-HHSiD (corresponding to 1.7 µg) plus 2.42 mg of unlabelled HHSiD (8.6 mg/kg) in 1.2 ml of water was injected into the stomach. The experimental design was the same as in the i.v. experiments except the duration of sampling (4 h).

At the end of the experiment the skin was removed. Stomach and intestine were taken from the body and homogenized. The rest of the body was also homogenized and samples were taken for measurement of radioactivity.

Pharmacokinetic calculations. Plasma concentration and excretion rate data were fitted to the usual multiexponential equations using the NONLIN computer program (Metzler et al. 1974). Data were weighted reciprocally. Using standard methods basic pharmacokinetic parameters such as distribution half-life ($t_{1/2\ Dis}$), elimination half-life ($t_{1/2\ El}$), half-lives of urinary and biliary excretion rate ($t_{1/2\ U}$, $t_{1/2\ Bil}$), plasma clearances (CL) and mean residences time (MRT) were calculated from these fits (Gibaldi and Perrier 1982). The biliary and renal clearances (CL_{Bil}, CL_R) were determined by $D_{Bil,\ U} \cdot CL/100$. The percentage of dose excreted with urine and bile extrapolated to infinity (D_U, D_{Bil}) %D was calculated by the area under the excretion rate curve $AUC_{0-\infty} \cdot 100/D$. Time of maximum excretion rate of bile and urine (t_{maxB}, t_{maxU}) were obtained from the curves.

Results

Whole body autoradiography

In the animal that was sacrificed after 4.5 min (rat 2; Fig. 2), the radioactivity had already spread over the whole body. The highest concentration of radioactivity

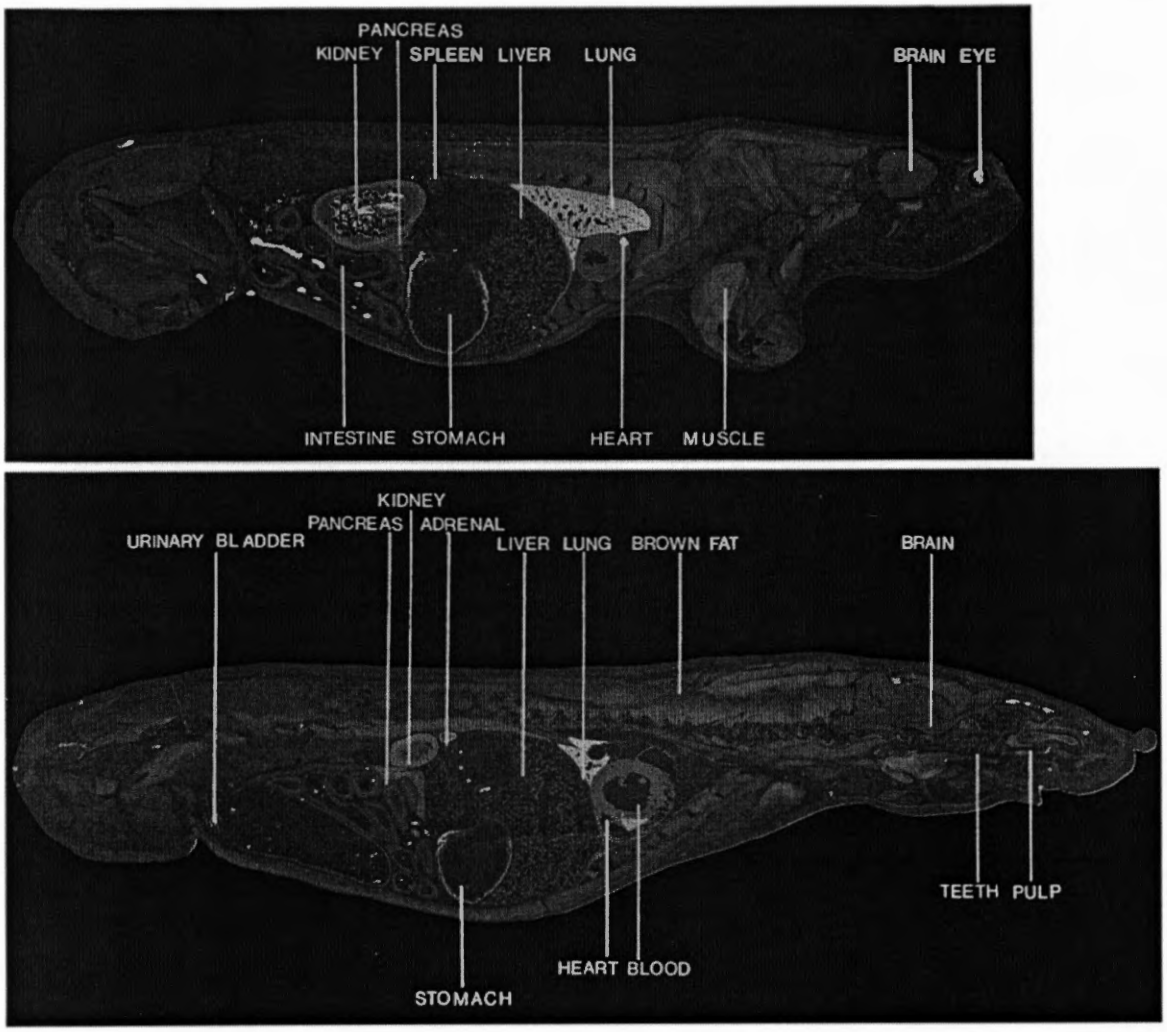

Fig. 2. Autoradiograms of two different sagittal sections of one rat, showing the distribution of radioactivity 4.5 min after i.v. injection of 1.8×10^7 Bq [^{3}H]-HHSiD plus 0.55 mg HHSiD. White areas in the autoradiograms indicate the presence of radioactivity

was found in the lung. High concentrations could also be observed in the kidney, urine and gastric mucosa. A less pronounced accumulation of radioactivity was found in the adrenals, liver (the liver showed a spotted pattern), brain, muscle, intestinal walls, heart and in the glandular tissues, such as pancreas and salivary gland. On the other hand, the autoradiograms showed no radioactivity in the blood.

After 35 min (rat 1; Fig. 3) extremely high concentrations of radioactivity could be observed in the urinary bladder, the duodenum and on the skin. The liver still, and more clearly, showed a spotted pattern, whereas the radioactivity of the stomach had shifted slightly further away from the mucosa. Only low concentrations were detectable in kidney, pancreas and lung. The rest of the body showed hardly any radioactivity.

I. v. administration to anaesthetized rats

Plasma radioactivity. The plasma concentrations presented are the mean values of data obtained from two rats (No. 3 and 4). Figure 4 shows the data from a single experiment.

The radioactivity remaining in the plasma 23 s after i.v. administration of HHSiD together with [^{3}H]-HHSiD represented only 5% of the theoretical value at time zero (plasma volume was assumed to be 7.5 ml). The radioactivity decreased to 1% of the calculated initial value within the first 10 min and to 0.4% within 2.5 h. The estimated terminal half-life was 3.3 h. 64% of the plasma radioactivity could be extracted with hexane from the samples drawn 23 s after administration. This percentage decreased to 11% after 2.5 h. Half-life of the plasma radioactivity was 1.4 h in case of the extracts.

Urinary excretion. A measurable urinary excretion (Fig. 5) of radioactivity started within 3 min after the i.v. dose ($n = 4$). The maximum excretion rate was reached after 12.5 min with a 95-fold higher concentration in urine than in plasma at the same time. The radioactivity extracted with hexane ranged from 46% in the beginning (total radioactivity of the respective sample expressed as

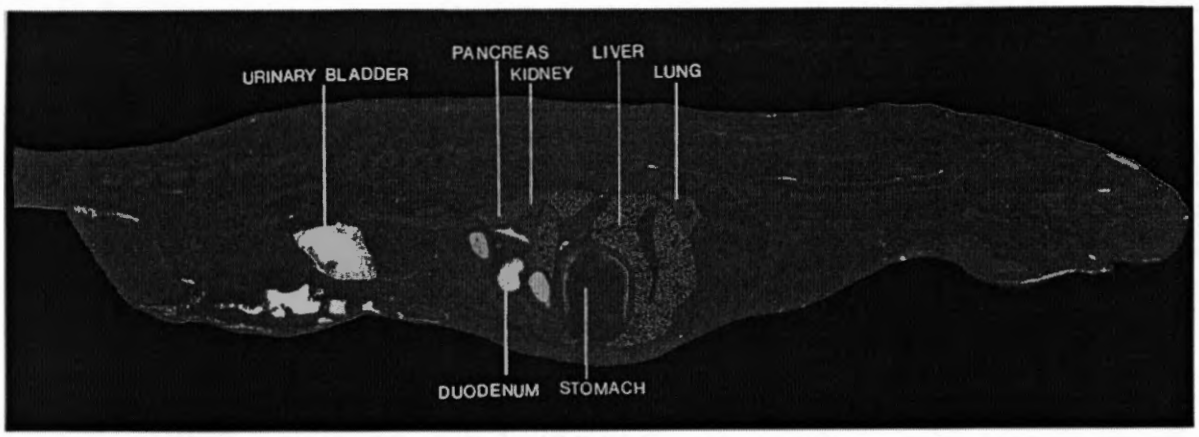

Fig. 3. Autoradiogram showing the distribution of radioactivity 35 min after i.v. administration of 7.5×10^6 Bq [^{3}H]-HHSiD to a rat

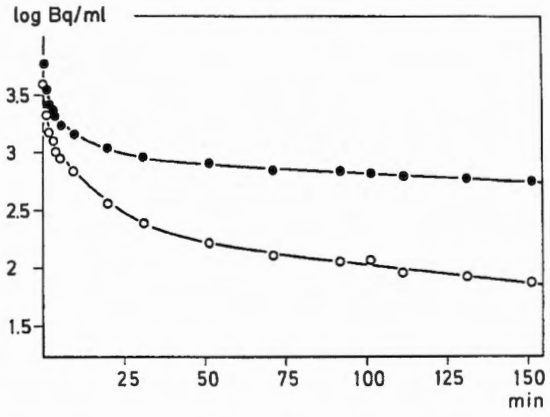

Fig. 4. Plasma radioactivity after i.v. injection of 2.2×10^6 Bq [^{3}H]-HHSiD plus 3 mg/kg HHSiD in rat No. 3 [total activity (●) and radioactivity (HHSiD and its lipophilic metabolites) extracted with hexane (○)]

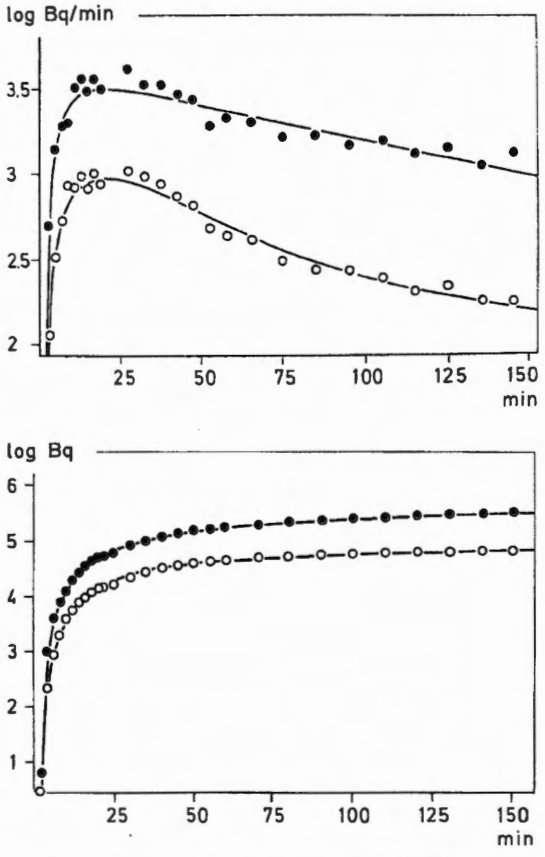

Fig. 5. Urinary excretion rates (*upper panel*) and cumulative urinary excretion (*lower panel*) of radioactivity after i.v. injection of 2.2×10^6 Bq [^{3}H]-HHSiD plus 3 mg/kg HHSiD in rat No. 3 [total activity (●) and radioactivity (HHSiD and its lipophilic metabolites) extracted with hexane (○)]

100%) to 13% at the end. The maximum excretion rate of hexane-soluble radioactivity was observed 20 min after administration. The calculated half-lives were 1.4 h (total activity) and 1.7 h (radioactivity extracted with hexane).

Biliary excretion. Radioactivity was detectable in bile (Fig. 6) already in the first minute after i.v. administration ($n = 4$). The maximum excretion rate was reached after 6 min with a 360-fold higher concentration in bile than in plasma at the same time. The concentration of radioactivity in bile was higher than in any other tissue or body fluid. During the period of 9 min to 2.5 h after i.v. administration of the drug, the radioactivity extracted from bile with hexane accounted for about 1 – 2% of the total activity of the respective sample. Half-lives from excretion rates were 1.6 h (total activity) and 0.8 h (radioactivity extracted with hexane).

Total excretion. 52% of the i.v. administered radioactivity was excreted within 2.5 h, excretion in urine and bile amounting to 13% and 39%, respectively. Extrapolation to infinity resulted in a total recovery of 17% in urine and 52% in bile. The radioactivity extracted with hexane was 2.9% of the dose (4.2% after extrapolation) in case of urine and 0.7% (0.7 after extrapolation) in case of bile.

Distribution of radioactivity. At the end of the experiments (2.5 h after i.v. administration) 47.3% of the dose could

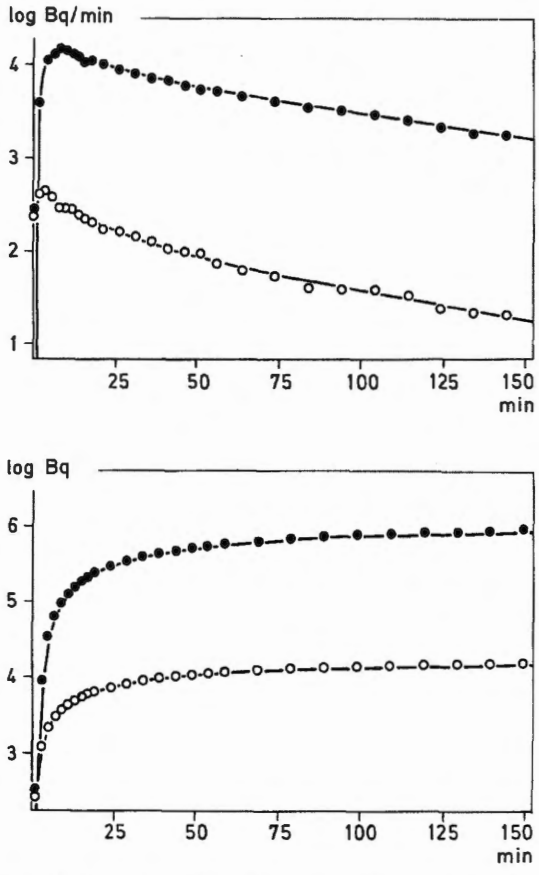

Fig. 6. Biliary excretion rate (*upper panel*) and cumulative biliary excretion (*lower panel*) of radioactivity after i.v injection of 2.2×10^6 Bq [^{3}H]-HHSiD plus 3 mg/kg HHSiD in rat No. 3 [total activity (●) and radioactivity (HHSiD and its lipophilic metabolites) extracted with hexane (○)]

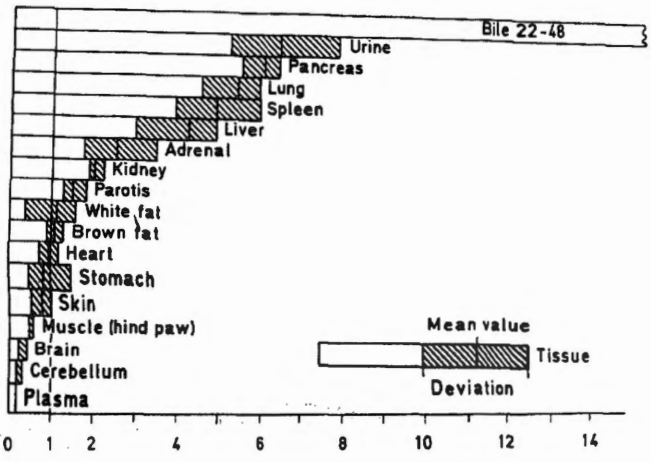

Fig. 7. Tissue distribution of radioactivity 2.5 h after i.v. administration of [^{3}H]-HHSiD plus 3 mg/kg HHSiD [values of three rats (Bq/g) relative to the average total radioactivity of the respective homogenized rat (Bq/g) which was arbitrarily set as 1]. *Shaded parts* of columns are maximum deviations from sample mean

still be detected in the animals. At that time drug levels in all tissues were higher than the corresponding plasma concentrations. High levels of radioactivity were found in bile, urine, pancreas, lung, spleen, liver, adrenal and kidney (Fig. 7).

Pharmacokinetic parameters. From the plasma concentration time curves and the urine and bile excretion curves pharmacokinetic parameters were calculated. These data are summarized in Table 1.

Oral administration

Plasma concentration. Thirty minutes after oral administration plasma radioactivity reached a plateau until the end of the experiment (4 h, Fig. 8). The highest yield of radioactivity extracted with hexane was observed after 12 min. The extracted fraction ranged from 15% of the total activity in the beginning to 1.5% at the end.

Distribution of radioactivity. At the end of the experiment (4 h after oral administration) the radioactivity was distributed as follows: Skin 1.1%, stomach and intestine 73.5%, rest of the body 9.3%, excreted via urine 2.9%, excreted via bile 13.1% (sum = 99.9%).

Total excretion. Four hours after oral administration 16% of the administered radioactivity had been excreted (2.9% via urine and 13.1% via bile). Extrapolation to infinity resulted in a recovery of 2.9% in urine and 15% in bile. The fraction of radioactivity extracted with hexane was 0.11% of the dose (0.11% after extrapolation) in case of urine and 0.16% (0.17% after extrapolation) in case of bile.

Discussion

After i.v. injection of [^{3}H]-HHSiD together with unlabelled HHSiD, the rate of drug disappearance from plasma is particularly striking: 95% of the administered radioactivity disappeared from plasma within 23 s and 99% within 10 min. Due to this rapid distribution no radioactivity could be seen in the blood of the autoradiograms taken 4.5 min after the injection (Fig. 2), although radioactivity was detectable in most tissues. The presence of tritium in the brain shows that HHSiD can pass the blood brain barrier. An extremely high tritium concentration was detected in the lung after 4.5 min.

In the autoradiograms taken 35 min after i.v. administration (Fig. 3), very high concentrations of tritium in the urinary bladder and the duodenum indicate a rather quick and effective urinary and biliary elimination.

The radioactivity noticed on the skin is probably the result of a contamination with urine. The spotted pattern of activity distribution in the liver is caused by the intralobular concentration of radioactivity in the bile.

Twenty-three seconds after i.v. administration only 64% of the total radioactivity in plasma could be extrac-

Table 1. Pharmacokinetic parameters obtained after i.v. administration of [^{3}H]-HHSiD plus unlabelled HHSiD with total and hexane-extractable (in brackets) radioactivity. For explanation of terms see Methods

Rat No.		1	2	3	4
Weight (g)		315	305	300	304
Dose [^{3}H]-HHSiD (Bq × 10^6)		1.87	2.26	2.17	2.37
Dose HHSiD (mg)		0.73	0.88	0.90	0.98
CL	(ml/min)			7.7 (47.6)	5.7 (46.5)
CL_{Bil}	(ml/min)			3.4 (0.4)	3.0 (0.3)
CL_R	(ml/min)			1.4 (2.2)	1.0 (1.7)
D_U	(%)	19.6	13.6	17.9 (4.6)	17.9 (3.7)
D_{Bil}	(%)	62.5	49.6	44.7 (0.8)	52.6 (0.6)
$t_{1/2El}$	(h)			3.2 (1.5)	3.4 (1.3)
$t_{1/2Dis}$	(s)			38.8 (37.0)	17.3 (20.5)
$t_{1/2Bil}$	(h)	2.2	1.6	1.0 (0.8)	1.5 (0.9)
$t_{1/2U}$	(h)	1.6	1.6	1.2 (2.0)	1.1 (1.4)
t_{maxB}	(min)	7	8	8 (2)	6* (2)
t_{maxU}	(min)	22	27*	21 (20)	20 (18)
MRT	(min)			262 (90)	279 (72)

* Measured maximum was taken

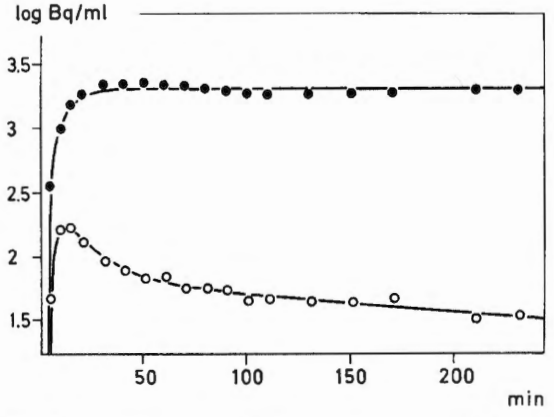

Fig. 8. Plama concentration-time curve after oral administration of 6.3×10^6 Bq [^{3}H]-HHSiD plus 8.6 mg/kg HHSiD [total activity (●) and radioactivity (HHSiD and its lipophilic metabolites) extracted with hexane (○)]

ted with hexane, thus indicating that already at that time 36% of the radioactivity in plasma is due to hydrophilic metabolites no longer extractable with hexane. By analogy to the metabolism of the carbon compound hexahydro-difenidol (Tacke and Linoh 1989), the main metabolic pathway of the silicon analogue HHSiD is the introduction of one, two or three hydroxy groups preferably located at the cyclohexyl ring but also at the phenyl moiety (Rettenmayr et al. 1988; Strohmann et al. 1988). After i.v. administration the radioactivity extracted from plasma with hexane is ranging from 64% in the beginning to 11% at the end, and in case of oral administration from 12% to 2%, respectively. These data can best be explained by a first pass metabolism.

Incubation of blood with [^{3}H]-HHSiD and determination of radioactivity in plasma and centrifuged blood cells showed that the amount of radioactivity was about the same in both fractions (46% in plasma and 54% in centrifuged blood cells) (unpublished observations).

It appears from Table 1, where biliary and renal clearances together (hexane extract) are much lower than plasma clearance, that there must be another elimination route, probably the metabolic clearance.

It is interesting to note that there is a good correlation between the pharmacokinetic results (course of the concentration-time curve of HHSiD extracted from plasma, Fig. 4) and the course of the pharmacodynamic effects on the eye after oral administration of 5 mg/kg HHSiD to mice. The dilatating effect on the pupil was reported to be quite strong in the beginning and to decrease rapidly during the first hour (W. Kromer and U. Brand, Byk Gulden, Konstanz, FRG, unpublished results). Compared to HHSiD the carbon analogue hexahydro-difenidol showed a longer duration of mydriatic effect. A similar experiment using procyclidine and sila-procyclidine showed that the silicon analogue exhibited a longer duration of pupillary dilatation (Tacke et al. 1987).

The main fraction of [^{3}H]-HHSiD radioactivity is concentrated in liver and excreted via bile as can be seen in the total excretion of radioactivity (see Results), which was 52% of the administered dose in case of the bile.

The high amount of tritium found in the stomach and intestine (the bile was collected) after oral administration (74% of the administered dose; 4 h after administration; see Results) indicates a poor absorption of HHSiD. A direct excretion into the intestine cannot play an important role, as faeces and the contents of the small intestine contain only a small amount of radioactivity after i.v. administration to anaesthetized rats with a cannulated bile duct (data not shown).

In conclusion, the results of this study provide strong evidence that pharmacokinetic reasons (the quick metabolization and the fast excretion) are responsible for the reported differences between the in vivo M1-antimuscarinic activities (lower potency of HHSiD compared to pirenzepine in the pithed rat, see Introduction) and the in vitro M1-binding affinities (HHSiD similar to pirenzepine).

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