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Whole-body Autoradiography and Quantitative Organ-level Distribution Study of Deramciclane in Rats

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Abstract

The distribution of ^{3}H-labelled deramciclane (EGIS-3886), a new 5-HT_2 antagonist with anxiolytic activity, has been investigated by whole-body autoradiography and quantitative organ-level determination after intravenous and oral administration to male and female rats at a dose of 3 mg kg^{-1}. Pregnant dams were also studied, but by autoradiography only. In the autoradiographic study 32 organs were investigated, while in the quantitative organ-level study the radioactivity in 15 organs were determined.

There are no sex differences in the distribution of deramciclane, absorption is rapid, elimination is comparatively fast, no specific organ is targeted, and the accumulation of the compound is very unlikely. Penetration of the blood-brain barrier was complete and extremely fast, a very important feature of a potential anxiolytic drug. There is no penetration of the foetus in pregnant dams.

The study demonstrated that deramciclane has advantageous pharmacokinetic properties in rats.

Deramciclane fumarate (EGIS-3886; 1*R*,2*S*,4*R*-(−)–*N*,*N*-dimethyl-2-(1,7,7-trimethyl-2-phenylbicyclo-[2,2,1]hept- 2- yl)oxyethanamine- 2-*E*-butenedioate (1:1); Figure 1) is a drug specifically developed as a new type of anxiolytic (Gachályi et al 1988, 1996). It was synthesized by Budai et al (1980) at EGIS Pharmaceuticals (Budapest, Hungary).

Deramciclane is pharmacologically and chemically different from benzodiazepine-type anxiolytics and from buspirone. Like buspirone, deramciclane acts via the 5-HT-ergic system, but at different sites. A striking property of deramciclane is its high affinity for 5-HT_{2A} and 5-HT_{2C} receptors (Gachályi et al 1997). Deramcilane has no sedative or muscle-relaxant side-effects (Gachályi et al 1988, 1996; Kovács et al 1989; Berényi et al 1990).

A comparative pharmacokinetic study of 3 mg kg^{-1} deramciclane administered orally to rat, dog, rabbit and man revealed significant species-differences for the main pharmacokinetic parameters (Klebovich et al 1998). Absorbed deramciclane (Lengyel et al 1998) is subject to intense and rapid metabolism in different species, as has been shown in in-vitro (Monostory et al 1994; Klebovich et al 1995) and pilot in-vivo (Hazai et al 1995) studies of metabolism.

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The goal of the work described in this paper was to investigate the distribution of deramciclane in rats by means of whole-body autoradiography and quantitative organ level determination after single oral and intravenous administration of 3 mg kg^{-1} and repeated oral administration of 3 mg kg^{-1} day^{-1} on seven consecutive days.

Materials and Methods

Materials

Deramciclane (EGIS-3886, purity 99·7%) was from EGIS Pharmaceuticals (Budapest, Hungary). Radiolabelled test material deramciclane-camphor-3-^{3}H was from the Central Institute for Chemistry, Hungarian Academy of Sciences (Budapest, Hungary). Its specific activity was 126 Mbq mg^{-1} and its radiochemical purity >98·5% (Szammer et al 1996). Hydrogen peroxide (30% (w/w) solution in water), *n*-hexane (pure), 1-butanol (pure), ethyl alcohol (95%, spectrophotometric grade), and propylene glycol (analytical grade), were from Reanal (Budapest, Hungary). Carmellose sodium (purum)

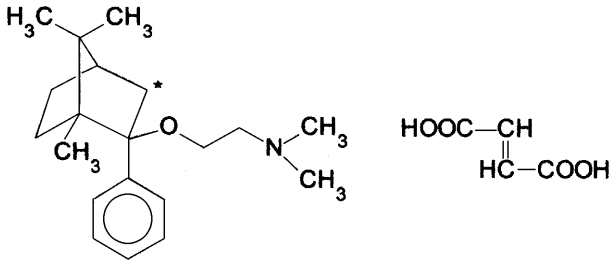

Figure 1. The chemical structure of deramciclane fumarate; the position of ^{3}H-labelling is indicated by asterisks.

was from Fluka (Buchs, Switzerland), aether ad narcosim (Ph. Hg. VI.) from Chinoin (Budapest, Hungary), acetic acid from Erdökémia (Budapest, Hungary), Pico-fluor 40 liquid scintillation solution from Packard (Groningen, The Netherlands), tetraethylammonium hydroxide, 20% aqueous solution, and silica gel 60 F_{254} pre-coated TLC plates from Merck (Darmstadt, Germany), and Hyperfilm-^{3}H high-performance autoradiography film from Amersham (Sweden).

Preparation of dose form for administration

The labelled and unlabelled compounds, weighed separately, were dissolved in ethyl alcohol–propylene glycol–distilled water, 5 : 40 : 55 (%w/w) to give 1·5 mg g^{-1} solutions. These solutions were mixed in the ratios 1 : 9 and 1 : 99 for whole-body autoradiography and for organ-level studies, respectively, to give the specific activity required. The specific activities of solutions used in whole-body autoradiography experiments and organ-level studies were 558 175 disintegrations min^{-1} mg^{-1} (i.e. 9·30 MBq μg^{-1}) and 56 810 disintegrations min^{-1} μg^{-1} (i.e. 0·95 MBq mg^{-1}), respectively.

Checking the stability of the test substance

The stability of the test substance in solution was checked by thin-layer chromatography on silica gel 60 F_{254} plates with 1-butanol–acetic acid–water, 4 : 1 : 1, as mobile phase. This system was suitable provided no spot other than the compound under investigation was observed on the chromatogram.

Administration

Drugs were administered to experimental animals, 180–260 g, orally, or by gastric gavage, or intravenously, via a tail vein.

Whole-body autoradiography

In each group two male and two female rats were treated and samples were taken 5 min and 2, 8 and 24 h after intravenous administration and 30 min and 2, 8 and 24 h after oral administration. After repeated oral administration on seven consecutive days samples were taken 24 h after the final treatment.

Two pregnant dams were treated orally on the 18th day of pregnancy and sampling was performed 2 and 24 h after treatment. Sections were obtained, by standard procedures, by use of a PMV Cryomicrotome MP 450; (LKB, Sweden). Autoradiograms were prepared using Hyperfilm ^{3}H (Amersham); after exposure for 32 days the films were developed by use of Fibro X-ray film-developing equipment (Frings, Bonn, Germany).

All autoradiographic film was inspected visually and the relative densities for 32 organs and tissues (adipose tissue, adrenal glands, bladder, blood, bone, bone-marrow, cerebellum, cerebrum, eye, lachrymal gland, heart, hypophysis, large intestine, liver, lung, lymphatic gland, muscle, nasal mucosa, oesophagus, ovaries, pancreas, renal cortex, renal pelvis, salivary gland, skin, small intestine, spinal cord, spleen, stomach, testicles, thymus and thyroid gland) were evaluated by scoring.

Quantitative organ-level studies

In each group five males and five females were treated according to the time schedules: after intravenous administration, after 15 min and 2 and 24 h; after oral administration, after 30 min and 2, 8 and 24 h; after repeated oral administration, 24 h after the final treatment. At scheduled time points after treatment the animals were killed by decapitation; dissection and sample processing were started immediately.

Selected organs (adrenal glands, bone marrow, brain, brown fat, heart, kidneys, liver, lung, muscle, ovaries, pancreas, spleen, testicles, thymus and thyroid gland) obtained by dissection were weighed and processed according to standard procedures. In experiments with repeated oral administration (subacute study) samples of blood (0·05 mL) were drawn both before treatment and 30 min after treatment.

Measurement of radioactivity. Tetraethylammonium hydroxide (0·5 mL), hydrogen peroxide (0·2 mL) and Pico-fluor 40 universal liquid scintillation solution (10 mL) were added to samples of organs and tissues and counting was performed for 5 min with a Packard Tri-Carb 2000 CA liquid-scintillation spectrometer (Packard, Groningen, The Netherlands) using automatic quench correction by an external standard ratio method and chemiluminescence correction. Representative blank sample values were subtracted from sample

values obtained to calculate net values of the disintegrations min^{-1}/sample. The detection limit was taken as twice the background noise (for blood samples 25 ng equiv. mL^{-1} whereas for organs and tissues it was between 30 and 40 ng equiv. g^{-1} wet sample).

Quantitative evaluation of organ-level data. From the net disintegrations min^{-1} values obtained by measurement of the radioactivity of the organs sampled, the microgram-equivalent of deramciclane (g/organ) (μg equiv. g^{-1}) value for each organ was calculated; for blood samples the value was expressed as microgram-equivalents of deramciclane mL^{-1} blood (μg equiv. mL^{-1}).

Organs were examined for differences between levels of radioactivity in samples obtained from the male and female experimental animals (the genitals were not considered). Because no differences were apparent (see below), the organs obtained from different sexes were not differentiated.

Initial results were then calculated—average values, sample standard deviations and the relative standard deviations were computed for each organ studied at each sampling time. Final results were then calculated—individual data differing from the corresponding average by an amount twice the standard deviation were excluded from the data set. Also, if an anomalous tendency in organ-level values was apparent for any animal, i.e. at least five (out of 14) organs had to be excluded for the rat under scrutiny, then all data from this animal were excluded from further evaluation. The organ-level values and the blood-level data obtained at different sampling times were subjected to principal-component analysis (Massart et al 1988).

All calculations for determination of quantitative organ levels were performed with Quattro Pro V.5.0 (Borland International, USA) and SAS V. 6.08 (The SAS Institute, Hedelberg, Germany) software resident in an IBM-compatible computer.

Results and Discussion

Whole-body autoradiography

Digital autoradiography (DAR), which has the unique feature that slides can be investigated after a comparatively short time, was used for preliminary examinations to obtain rapid results (not presented here) for sections attracting special interest. Final results were obtained, however, by contact autoradiography because the resolution of this technique is greater. Representative autoradiograms are shown in Figures 2–4. No specific sex-dependence could be detected in distribution of radioactivity after administration (intravenous and oral) of [^{3}H]deramciclane.

Single intravenous administration

Five-min group. The autoradiogram is indicative of rapid penetration of radioactivity into the tissues

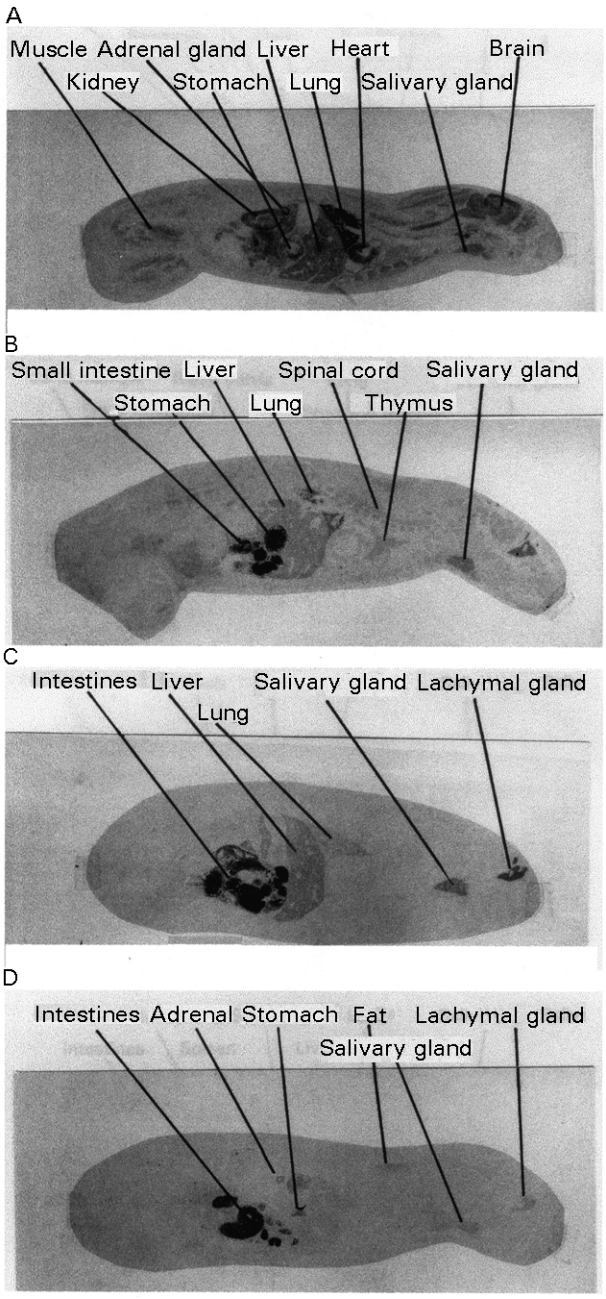

Figure 2. Whole-body autoradiograms of rats obtained after single 3 mg kg^{-1} intravenous administration of deramciclane. A. Female rat in the 5th minute after treatment, B. male rat in the 2nd hour after treatment, C. male rat in the 8th hour after treatment, D. female rat in the 24th hour after treatment.

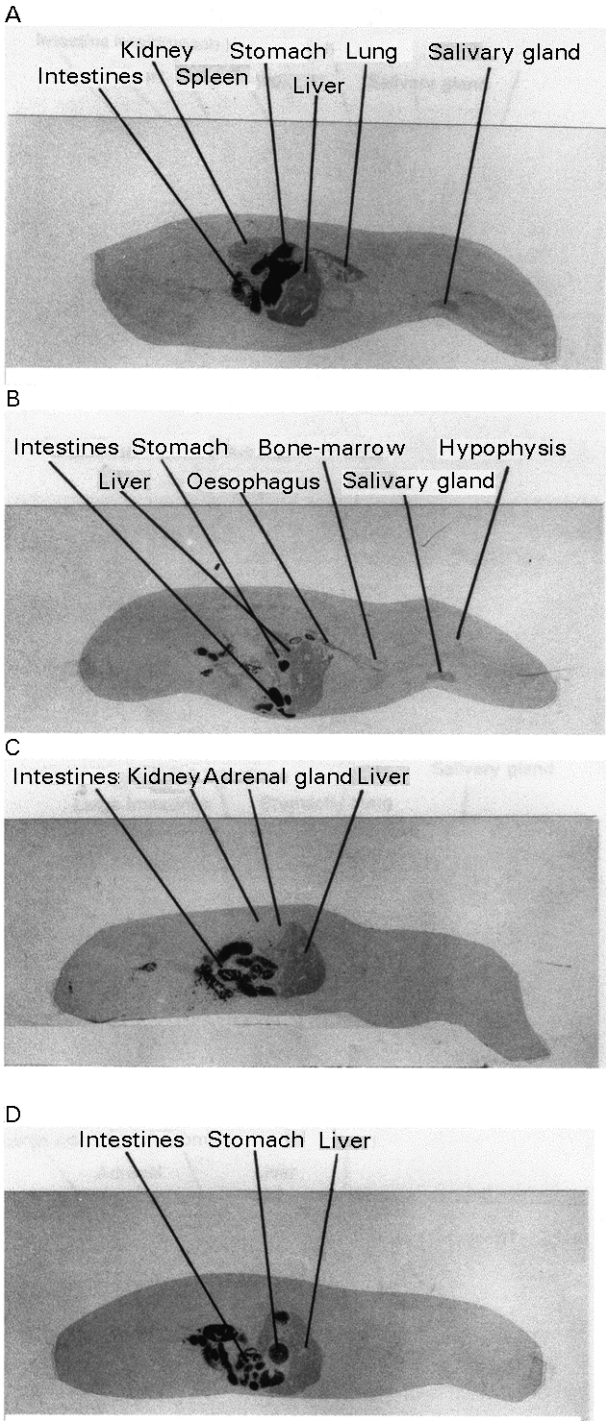

Figure 3. Whole-body autoradiograms of rats obtained after single 3 mg kg^{-1} oral administration of deramciclane. A. Female rat in the 30th minute after treatment, B. female rat in the 2nd hour after treatment, C. male rat in the 8th hour after treatment, D. male rat in the 24th hour after treatment.

and organs. Five minutes after treatment tissue levels were usually higher than blood levels. The highest concentration was observed in the lung and substantial levels were detected in other well-perfused organs (liver, adrenal glands, spleen, kidneys,

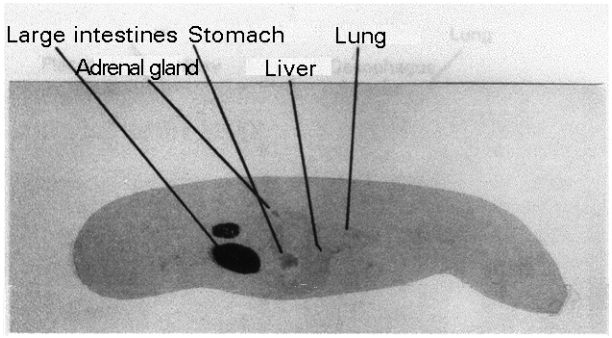

Figure 4. Whole-body autoradiogram of a rat, in the 24th hour after the final dose, after repeated oral administration of deramciclane (3 mg kg^{-1} day^{-1}) for seven consecutive days.

heart), in muscles and in the salivary and lachrymal glands. The high density in brain and the spinal cord is indicative of substantial penetration of radioactivity through the blood-brain barrier. The accumulation of radioactivity in the stomach wall after such a short time deserves attention. The high activity level in the small intestine indicates that excretion starts with the bile.

Two-hour group. It is apparent from the autoradiogram that the radioactivity decreased significantly in the brain and heart. Medium levels could be observed in the lung, liver, spleen, renal cortex, uterus, salivary gland and lachrymal gland. Enrichment could be detected in the thymus, in bone-marrow and in the stomach. High concentrations of radioactivity were accumulated by the intestines and in the renal pelvis, indicating continued excretion in the urine and bile.

Eight-hour group. Radioactivity had decreased in most organs and tissues; weak radioactivity was detected in the lung, liver, adrenal glands, salivary gland, and lachrymal gland, whereas high levels of radioactivity were observed in the stomach and the intestines.

Twenty-four-hour group. By 24 h after dosing the level of radioactivity remained above the detection limit in the liver, adrenal glands, stomach, salivary gland, and lachrymal gland. Accumulation of radioactivity was high in the intestines. The presence of radioactivity in the gastrointestinal tract and liver 24 h after a single intravenous application indicated the incomplete elimination of the dose administered.

Single oral administration

Thirty-min group. Tissue levels were, in general, lower than after intravenous application. Because

of the oral dosing the highest concentrations were found in the oesophagus, stomach and small intestines. As with intravenous application, a moderate density of radioactivity was detected in the lung, liver, spleen, adrenal glands, kidneys and salivary gland. It is worthy of note that the radioactivity in the brain was low.

Two-hour group. There was no major change in tissue distribution during this period (i.e. it was very similar to that observed for the 30 min group). Levels of radioactivity decreased slightly in the lung and heart and increased in the bone-marrow and thymus.

Eight-hour group. In the 8th hour after oral administration a decrease of radioactivity was observed in most organs and tissues. Radioactivity levels remained high in the small intestines only, and increased in the large intestine, indicating excretion in the faeces. The level in the kidneys was comparatively low, suggesting that urinary excretion did not make a significant contribution to elimination.

Twenty-four hour group. By 24 h after single oral administration (Figure 3D) levels of radioactivity in most organs and tissues had diminished to a value below the detection limit. The active components could be detected in the gastrointestinal and urinary tracts and in the liver, indicating an incomplete excretion of the dose applied. A moderate level of radioactivity was also found in the adrenal glands.

Repeated oral administration

Repeated oral administration did not result in immense changes in distribution. It is very unlikely that there is intense accumulation and retention of radioactivity in rats. After the final treatment significant radioactivity could be detected in the stomach and intestines, moderate levels in the kidneys and liver, and weak accumulation in the lung and adrenal glands, results similar to those obtained 24 h after single administration. Other tissues showed very weak (or background) radioactivity.

Pregnant dams

The distribution of radioactivity in pregnant and non-pregnant dams was very similar. According to the relatively low blood level at the time-point studied, 2 h after treatment weak radioactivity could be observed in the placenta and there was no detectable uptake of radioactivity by the foetus. Neither placenta nor foetus could be seen in digital autoradiograms 24 h after application.

Quantitative organ-level study

Table 1 summarizes the average radioactivity levels (with the corresponding standard deviations) in the organs in each group investigated, according to final results. Of the complete data set of individual values (not presented here) only 68 items of data were not considered in the final evaluation. Of the 80 experimental animals investigated three were considered as deviant.

For some organs in the 24-h post-treatment data sets, after both intravenous and oral administration, levels of radioactivity were below the detection limits. In these instances the value 0·00 is given in the table and, although an average value was calculated, no standard deviation is given. (It should be remembered that an average value of 0·00 does not indicate lack of radioactivity in the sample, only that it was below the detection limit.)

Investigation of sex dependence of tissues studied

It was decided that a difference would be declared for an organ if, in each set of experiments, a difference with a significance level of 5% were obtained. The Wilcoxon test was used and the probability values computed by the SAS program (not presented here) showed that no organ met this requirement—i.e. there was no sex-dependence.

Intravenous administration

Fifteen-min group. After excluding one animal the relative standard deviations of the data obtained for individual organ levels were in the range 10–50%.

For most organs the level of radioactivity was highest at this time after intravenous administration. (The exceptions were bone marrow, adrenal glands, testicles and ovaries.) For each organ studied the radioactivity was higher than for the blood samples. It is apparent from Table 1 that a relatively very high level of radioactivity was measured in lung tissue—the level (36·27 μg equiv. g^{-1}) was approximately 50 times higher than that detected in the blood. The concentration of radioactivity found in the lung is more than four times that in the liver, which had the highest radioactivity level of the other tissues. It should be mentioned that the high level of radioactivity present in the brain is a clear indication that the investigated

Table 1. Concentrations of radioactivity (μg equiv g^{-1}; μg equiv mL^{-1}) in tissues after administration of [^{3}H]deramciclane to rats.

Organ	Intravenous			Oral				Repeated oral
	15 min	2 h	24 h	30 min	2 h	8 h	24 h*	24 h
Adrenal glands	7·72 ± 2·71	7·90 ± 2·22	1·21 ± 0·71	1·00 ± 0·83	2·34 ± 1·74	1·10 ± 0·60	0·70	3·28 ± 0·87
Blood	0·66 ± 0·10	0·32 ± 0·07	0·08 ± 0·01	0·26 ± 0·11	0·23 ± 0·05	0·18 ± 0·06	0·07	0·31 ± 0·03
Bone marrow	2·17 ± 0·32	3·35 ± 0·43	0·15 ± 0·18	0·76 ± 0·98	0·98 ± 0·61	0·25 ± 0·06	0·05	0·27 ± 0·11
Brain	7·78 ± 1·29	2·41 ± 0·24	0·02 ± 0·03	0·10 ± 0·08	0·44 ± 0·57	0·07 ± 0·02	0·02	0·17 ± 0·01
Brown fat	4·66 ± 2·32	3·35 ± 1·23	0·13 ± 0·05	0·44 ± 0·47	0·39 ± 0·20	0·17 ± 0·09	0·01	0·22 ± 0·08
Heart	4·57 ± 0·64	1·31 ± 0·19	0·06 ± 0·01	0·34 ± 0·26	0·43 ± 0·28	0·16 ± 0·07	0·05	0·24 ± 0·05
Kidneys	7·19 ± 0·94	2·74 ± 0·40	0·19 ± 0·02	1·22 ± 0·60	1·59 ± 0·75	0·60 ± 0·14	0·13	0·79 ± 0·08
Liver	7·92 ± 1·14	5·01 ± 0·51	1·31 ± 0·49	10·82 ± 5·30	5·51 ± 1·18	4·57 ± 1·22	1·73	5·92 ± 2·73
Lungs	36·27 ± 7·46	12·80 ± 1·97	0·42 ± 0·17	1·53 ± 0·83	3·46 ± 2·75	0·69 ± 0·31	0·16	1·07 ± 0·59
Muscle	1·56 ± 0·50	1·45 ± 0·60	0·04 ± 0·01	0·20 ± 0·13	0·26 ± 0·18	0·12 ± 0·04	0·01	0·19 ± 0·03
Ovaries	5·52 ± 1·39	5·99 ± 0·53	0·26 ± 0·06	0·59 ± 0·40	0·97 ± 0·68	0·59 ± 0·11	0·07	0·68 ± 0·22
Pancreas	6·28 ± 2·09	2·92 ± 0·46	0·15 ± 0·08	0·44 ± 0·32	0·66 ± 0·61	0·35 ± 0·19	0·03	0·31 ± 0·06
Spleen	5·48 ± 0·63	3·97 ± 0·46	0·14 ± 0·03	0·70 ± 0·51	1·32 ± 1·17	0·29 ± 0·12	0·05	0·34 ± 0·07
Testicles	1·02 ± 0·10	1·64 ± 0·08	0·24 ± 0·09	0·05 ± 0·02	0·50 ± 0·49	0·34 ± 0·47	0·12	0·30 ± 0·04
Thymus	2·91 ± 0·29	2·74 ± 0·34	0·05 ± 0·01	0·30 ± 0·20	0·78 ± 0·54	0·17 ± 0·08	0·02	0·29 ± 0·10
Thyroid gland	6·64 ± 2·23	4·77 ± 2·25	0·00	0·58 ± 0·54	0·86 ± 0·73	0·15 ± 0·09	0·01	0·33 ± 0·15

*Because of the low level of radioactivity no standard deviation is given. Data are mean ± s.d.

compound penetrates its target tissue through the blood-brain barrier.

Two-hour group. In the 2nd hour after treatment the concentration of radioactivity in the blood decreased from 0·66 μg equiv mL^{-1} measured in the 15th minute after treatment to 0·32 μg equiv mL^{-1} (Table 1). Simultaneously, radioactivity levels in the organs studied also decreased by factors of 1–3 times. As for the 15-min group, the concentration of radiolabelled compounds was higher in each of organs studied than in the blood. Again, the highest level of radioactivity (13 μg equiv. mL^{-1}) was measured in the lung; the second highest level was in the adrenal glands.

Twenty-four-hour group. In the 24th hour after intravenous treatment the radioactivity level of blood decreased to 0·08 μg equiv. mL^{-1}, approximately one tenth the concentration measured in 15 min after treatment. The decrease in radioactivity levels in the organs was also substantial. The highest radioactivity levels were measured in the liver and adrenal glands (1·31 and 1·21 μg equiv. g^{-1} respectively) but the concentration of labelled compounds in the lung decreased to 0·42 μg equiv. g^{-1}. Radioactivity in the brain and thyroid gland was present at levels near to or below the detection limit.

Comparison of groups. Radioactivity levels at different times after treatment are compared in Figure 5A; the normalized values (i.e. with the highest value considered as 100%) are shown in Figure 5B. Although it is evident that no comparison can be made on the basis of these abundance

figures, use of principal-component analysis enabled some remarks to be made. (It is worth remarking that to the best of the authors' knowledge this statistical approach has not yet been used for evaluation of quantitative organ-level studies.) The method is based on the concept of reducing data such that an n-dimensional data structure is represented in two dimensions. By this method each organ at each time after treatment is represented by two numbers, enabling simple visual representation as shown in Figures 6 and 7. By use of this type of representation it is possible to group the organs under investigation. From Figure 6A it is apparent that most of the organs can be combined in one group, but the lung (g) is far away from this group, which is indicative of completely dissimilar behaviour. The extremely high concentration of radioactivity in the lung decreases to the average value after 24 h; in other words the rate of change is much higher. The opposite tendency is apparent for the other two atypical organs (adrenal glands and liver)—the rate of the change is lower than the average, and again results in these organs occupying a different group. These groupings are in complete agreement with the observations discussed above.

Figure 6B shows how normalized values of the radioactivity measured in the organs changes with time. Normalization of the data emphasizes the tendencies in the changes. The data are, however, more disperse—adrenal glands (m) are again atypical organs, as are ovaries (o), bone marrow (j) and testicles (c). The radioactivity in these organs increased during the period from 15 min to 2 h. It is

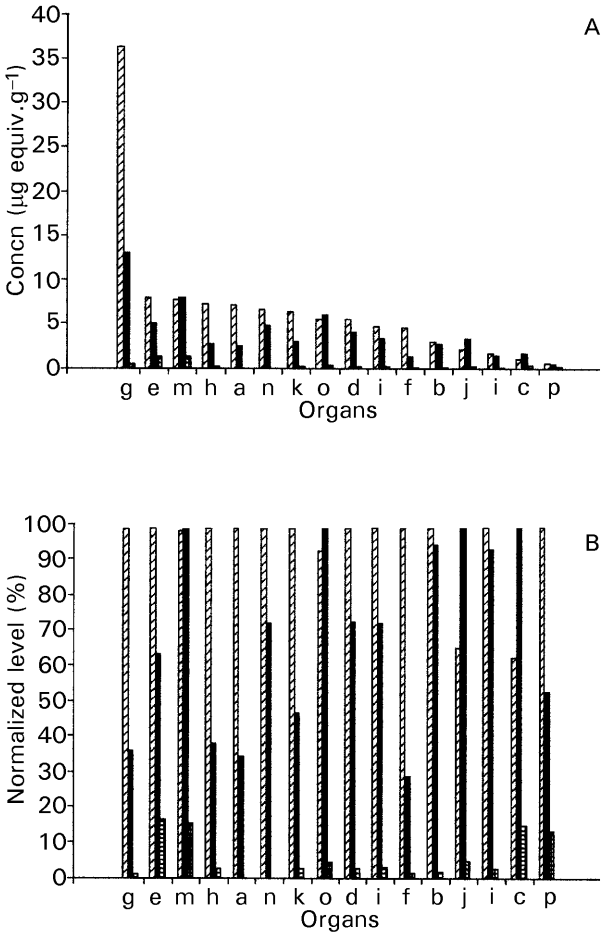

Figure 5. Changes with time of the levels of radioactivity in the organs studied in experiments with intravenous administration. A. radioactivity levels, B. normalized values of radioactivity levels; ▨ 15 min, ■ 2 h, ⊞ 24 h, a brain, b thymus, c testicles, d spleen, e liver, f heart, g lung, h kidneys, i brown fat, j bone-marrow, k pancreas, l muscle, m adrenal, n thyroid gland, o ovaries, p blood.

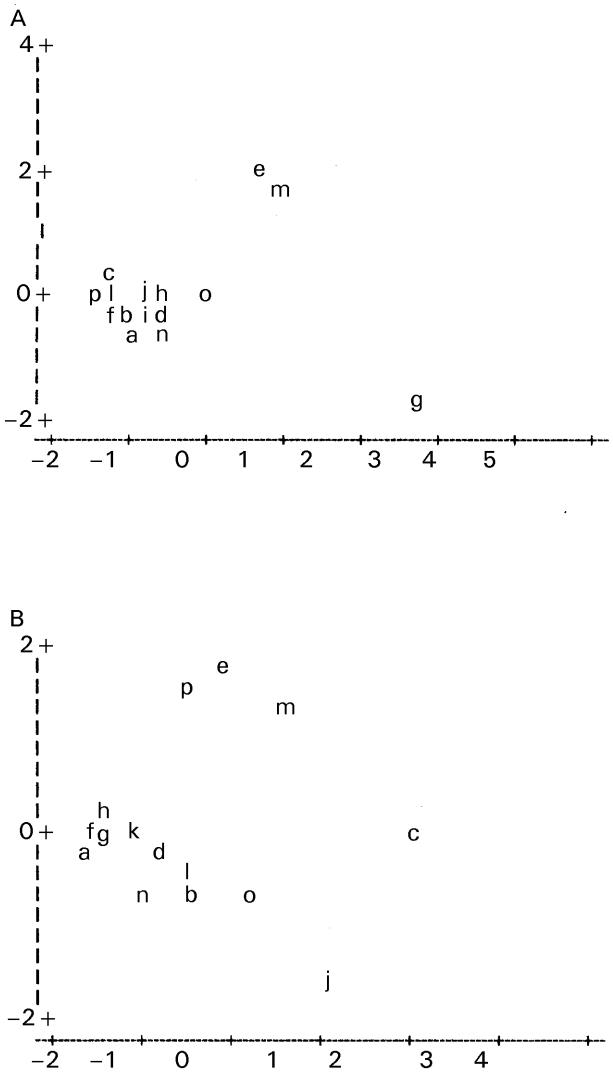

Figure 6. Principal-component analysis of organ level data obtained in intravenous experiments. A. radioactivity levels, B. normalized values of radioactivity levels; a brain, b thymus, c testicles, d spleen, e liver, f heart, g lung, h kidneys, i brown fat, j bone-marrow, k pancreas, l muscle, m adrenal, n thyroid gland, o ovaries, p blood.

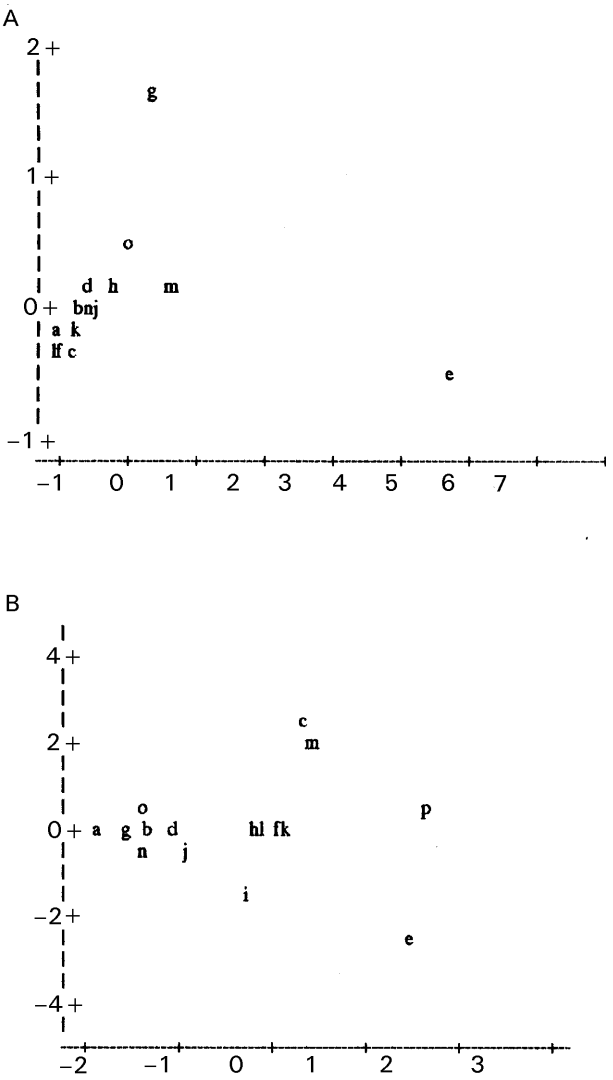

Figure 7. Principal-component analysis of organ-level data obtained after oral administration. A. radioactivity levels, B. normalized values of radioactivity levels; a brain, b thymus, c testicles, d spleen, e liver, f heart, g lung, h kidneys, i brown fat, j bone-marrow, k pancreas, l muscle, m adrenal, n thyroid gland, o ovaries, p blood.

interesting to note that the pattern for lung tissue (g) proved similar to that for most other organs.

Single oral administration

Thirty-min group. The radioactivity levels show that the relative standard deviations calculated are definitely higher than for the data set obtained after intravenous administration. The diversity might be attributable to comparatively large individual variability in the absorption process. One animal had to be excluded from the data set because the levels detected were beyond the acceptance limit.

It is apparent that (with the exception of the liver) the radioactivity levels were substantially lower than those measured at early time points after intravenous administration. In contrast with the intravenous treatment, the level of radioactivity in the lung was not as high, and did not differ very much from levels in the other organs investigated. The very high concentration ($10{\cdot}82\,\mu g$ equiv. g^{-1}) of radiolabelled compounds detected in the liver was approximately 40 times that detected in the blood ($0{\cdot}26\,\mu g$ equiv. g^{-1}). Although the second highest levels were measured in the lung, the levels were lower than after intravenous treatment; the concentration of radioactivity was approximately six times that detected in blood. The radioactivity in other organs varied between 0·05 (testicles) and 1·22 (kidneys) μg equiv. g^{-1}; because of the high diversity of data there is no sense in arranging the data in sequence.

Two-hour group. These data are as diverse as those recorded 30 min after treatment. Again the radioactivity levels in one animal were so high that it had to be omitted during evaluation. With the exception of the liver, levels of radioactivity did not reach the values obtained after intravenous treatment. Whereas the level of radioactivity in blood ($0{\cdot}23\,\mu g$ equiv. mL^{-1}) was almost the same as it was 30 min after treatment, it decreased to $5{\cdot}51\,\mu g$ equiv. mL^{-1} in liver. The second highest level of radioactivity was again measured in the lung, followed by the adrenal glands. It should be noted, however, that the order might also be the reverse, because of the high standard deviations of the results. Levels of radioactivity in each organ were higher than that in the blood (with the possible exception of muscle, in which the concentration was $0{\cdot}26 \pm 0{\cdot}18$ compared with a blood concentration of $0{\cdot}23 \pm 0{\cdot}05\,\mu$equiv. mL^{-1}).

Eight-hour group. The radioactivity in blood decreased to a value of $0{\cdot}18\,\mu g$ equiv. L^{-1}. Liver contained $4{\cdot}57\,\mu$equiv. g^{-1} radiolabelled compounds, this organ again being the most abundant in radioactivity. The next greatest abundance was found in the adrenal glands and lung ($1{\cdot}1 \pm 0{\cdot}6$ and $0{\cdot}69 \pm 0{\cdot}31\,\mu$equiv. g^{-1}, respectively). Levels of radioactivity were lower in the brain, thymus, heart, brown fat, muscle and thyroid gland than in the blood. Radioactivity levels were lower than after 2 h and were similar to the 30-min values.

Twenty-four hour group. The concentration of radioactivity in the blood dropped to a value of $0{\cdot}07\,\mu g$ equiv. mL^{-1}. In some organs (brain, thymus, heart, brown fat, pancreas, muscle, and thyroid gland) the radioactivity was below (or near to)

the detection limit; this is indicated by the value 0·000 in the data. Because of this, although average values are given in Table 1, no statistical treatment of these data was conducted. As for the earlier sampling times the organ most abundant in radioactivity was the liver (1·73 μg equiv. g^{-1}) followed by the adrenal glands (0·70 μg equiv. g^{-1}).

Comparison of groups. As was found for the intravenous data, principal-component analysis enabled separation of the organs into groups (Figures 7A, B). It is apparent from Figure 7A that the behaviour of the liver (e) and the lung (g) was completely different from that of the other organs and tissues studied. During the time-period investigated the changes in the level of radioactivity in these organs were more pronounced than in the others. As was stated above the normalized values show how the concentrations change with time. In Figure 7B it is apparent that the atypical organs were liver (e) and blood (p); this might be because the radioactivity levels in these organs decreased more than those of the other organs between 30 min and 2 h, even though radioactivity levels could still be measured in the 24th hour after treatment.

Repeated oral administration

The samples contained more radioactivity than samples taken 24-h after single oral treatment; levels of radioactivity in all organs were above the detection limit. With the exception of the adrenal glands, in none of the organs were radioactivity levels higher than the corresponding peak concentration data found in experiments with single oral treatment.

Blood was drawn both before (24-h sample) and after (30-min sample) treatment on seven consecutive days. Levels of radioactivity in the blood are given in Table 2. It is evident that changes in radioactivity levels were not significant during the 7-day period of treatment. The initial concentration values (24-h sample) were similar to that for the 30-min sample indicating the achievement of a steady state.

Table 2. Blood level data (μg equiv. mL^{-1}) found in studies of repeated oral administration.

Day	Before treatment	After treatment
1st	–	0·33 ± 0·27
2nd	0·05 ± 0·01	0·23 ± 0·13
3rd	0·13 ± 0·02	0·23 ± 0·14
4th	0·15 ± 0·03	0·21 ± 0·09
5th	0·16 ± 0·01	0·23 ± 0·11
6th	0·18 ± 0·02	0·39 ± 0·20
7th	0·27 ± 0·03	0·35 ± 0·09

Data are means ± standard deviations.

In most of the organs the level was similar to that in the blood—a significantly higher concentration was measured in the liver only, and moderately higher levels were found in lung and kidneys, indicating continuous elimination. These results again suggest the achievement of a steady state.

Attention should be paid to the adrenal glands, because the abundance of radioactivity in this organ proved higher than the corresponding peak concentration (2-h sample) obtained after single oral administration and the level of radioactivity in the adrenal glands was second highest after the liver.

Conclusions

Results from both whole-body autoradiography and quantitative organ-level study show there are no sex differences in the distribution of deramciclane. Both absorption and elimination were shown to be rapid. On the basis of the results from the quantitative organ-level study no specific organ was targeted by deramciclane and accumulation of the compound studied seems very unlikely. Penetration of the blood-brain barrier is complete and extremely fast. There is no penetration of the foetus in pregnant dams.

This study demonstrated that deramciclane is a potential anxiolytic compound with advantageous pharmacokinetic properties.

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