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Pharmacokinetic Investigation of 17α-Desoxymethasone (A 41 304) in Rats

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^{3}H-A 41 304 ointment in the concentration of 0.27% was applied to the back skin of rats, and its absorption and distribution in the blood and organs and its excretion in the urine and feces were examined in the course of time.

1. Most of the total amount absorbed was excreted in the urine of male and female rats within 1—2 days after the beginning of the application and the excretion in the feces was slightly slower than in the urine. The ratio of the excreted amount in the feces to that in the urine was approximately 1 : 4.

2. There was a sex difference in the total amount of the excreted radioactivity for 7 days after the beginning of the application; the amount in female rats was only about 60% of that in male ones. The excreted amount was decreased significantly by castration in male rats. The investigation with thin-layer chromatography. revealed that approximately half the absorbed amount of the compound was excreted unchanged in the urine and that the percentage was higher in female rats than in male ones.

3. The radioactivity began to appear in the blood from about 1 hr after the beginning of the application. The maximum radioactivity level was noted in the blood at 24 hr and in main organs at 31 hr. The radioactivity was little detected 7 days after the beginning of the application.

4. The liver and adrenal had higher radioactivity levels than other organs. In each organ the radioactivity reached its maximum 31 hr after the beginning of the application. No sex difference was noted in the distribution of this compound in the organs.

Synthetic corticoids have been developed as anti-inflammatory agents for the therapy of inflammation such as rheumatoid arthritis. Fates of all synthetic corticoids administered to a living body, however, have not been clarified yet.

It is generally known that steroid hormones are mainly metabolized in the liver, though partly in other organs such as the kidney, intestines, spleen, and muscles and in other tissues. This manner of metabolism has been proved by the experimental result that steroid hormones are metabolized to a smaller extent in animals hepatectomized or with hepatic disease than in normal animals. Moreover, Nambara[2] has also made it clear that steroid hormones are metabolized mainly in the liver by *in vitro* experiments with the perfused liver and slices of the liver and kidney. Synthetic corticoids are also mainly metabolized in the liver, though partly in the kidney, and then excreted in urine as conjugate.

1) Location: *a*) *3-2, 1-Chome, Minamidai, Kawagoe-shi*; *b*) *Aobayama, Sendai-shi.*
2) T. Nambara, *The Journal of Practical Pharmacy*, 21, 287 (1970).

Synthetic corticoids have both prominent anti-inflammatory and anti-pruritic actions, and clinically they are often used in the form of ointment. Therefore, many pharmaceutical investigations have been made on their parenteral absorption and metabolism in the skin. Since Baker, *et al.* (1950)[3] observed the atrophy of epidermis due to the dermal application of an alcoholic solution of hydrocortisone in rats, Livingood (1958),[4] Malkinson (1955—1963)[5] and Feldmann, *et al.* (1967)[6] have reported on the dermal absorption of hydrocortisone and other corticosteroids mainly from a clinical viewpoint. From their investigations it has become clear that corticosteroids are absorbed through both transepidermal and transfollicular routes,[7] and it is now known that about 2—3% of the applied amount is absorbed through the normal skin. In comparison with these many clinical examinations there have been few close pharmacokinetic investigations with animals on percutaneous absorption of synthetic corticosteroids and their fates in a living body. Therefore, with rats we investigated the percutaneous absorption, the distribution, and the excretion of ^{3}H-17α-desoxymethasone (A 41 304-[2-^{3}H]), a synthetic corticoid developed by Hoechst AG, prior to its clinical use. In this paper the results are reported.

Experimental

Compound——A 41 304-[2-^{3}H] (9α-fluoro-11β,21-dihydroxy-16α-methyl pregna-1,4-diene-3,20-dione-[2-^{3}H], 1.18 mCi/mg) in the concentration of 0.27% was used in the form of ointment (3.19 mCi/g). To make 1 g of A 41 304 ointment, 2.7 mg of A 41 304, 100 mg of isopropyl myristate, 530 mg of wool wax alcohol ointment, and 20 mg of wool wax alcohol were added to distilled water.

The purity of A 41 304-[2-^{3}H] was examined chromatographically using Kieselgel GF 254 and carbon tetrachloride–acetone (1: 1) and chloroform–acetone–carbon tetrachloride (7: 2: 1) as developing solvents. The test compound was proved to be pure, the *Rf* values obtained with the former and the latter solvents being 0.61 and 0.41 respectively. As authentic samples, nonradioactive A 41 304 and dexamethasone, dissolved in 1 N methyl alcohol, were used.

Experimental Animals——Wistar strain of male and female rats weighing 180—200 g were used. Each group consisted of 3 rats. The ointment containing ^{3}H-A 41 304 on the center of polyethylene paper (2 cm $\times$2 cm) was applied in the amount of 50 mg/100 g body weight to depilated backs of the rats, which were covered with Micropore® (Sumitomo 3M Ltd.), sticky tape for operation, and fixed with commercially available gumtape, as shown in Fig. 1. After the beginning of the application of the compound, the animals were individually kept in metabolic cages, and given commercial laboratory solid feed (CLEA CA-1) and tap water *ad lib*. Except in the groups killed within 24 hr, the fixed tape was stripped off and the compound was completely washed off with methanol 24 hr after the beginning of the application. After intravenous injection of 1 ml of heparin, the rats were killed by decapitation. Blood was collected and main organs (the liver, adrenal, kidney, spleen, heart, brain, seminal vesicle, testicle, ovary and uterus) were removed. Urine and feces were collected from the rats in individual metabolic cages every day for 7 days from the beginning of the application. The term "24-hr urine" in the present paper indicates the urine pooled for 24 hr from the beginning of the application, and "4-day urine" the urine pooled for 24 hr from 72 to 96 hr after the beginning of the application.

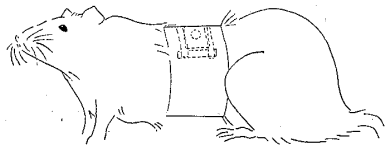

Fig. 1. Bandage for the Dermal Application of the Ointment (^{3}H-A 41 304) in Rats

Determination of Radioactivity (^{3}H-A 41 304)——The organs removed were rinsed with physiological saline solution and weighed. After weighed, a part of each organ was homogenized by a teflon homogenizer in distilled water three times the volume of the part of each organ. An aliquot of the homogenate (0.1 ml) was solubilized by heating at 60° for 3 hr in 1 ml of hyamine, and then 10 ml of dioxane scintillator (6 g of PPO, 0.2 g of dimethyl POPOP, 90 g of naphthalene, and 100 ml of toluene were added to dioxane to make 1 liter) was added.

3) B. L. Baker and C.W. Caster, *Endocrinol.*, **47**, 234 (1950).
4) C.S. Livingood, *J. Invest. Dermat.*, **31**, 19 (1958).
5) F.D. Malkinson and E.H. Furgnson, *J. Invest. Dermat.*, **25**, 281 (1955); F.D. Malkinson, *J. Soc. Cosm. Chem.*, **7**, 109 (1956); F.D. Malkinson, E.H. Furgnson, and M.C. Wang, *J. Invest. Dermat.*, **28**, 211 (1957); F.D. Malkinson, *J. Soc. Cosm. Chem.*, **11**, 146 (1960); F.D. Malkinson, *Arch. Dermat.*, **88**, 427 (1963).
6) R.J. Feldmann and H.I. Maibach, *J. Invest. Dermat.*, **48**, 181 (1967).
7) A. Scott and F. Kalz, *J. Invest. Dermat.*, **26**, 149 (1956).

An aliquot of blood (0.1 ml) was added to 1 ml of 0.1 N NaOH, decolorized by 0.1 ml of 30% hydrogen peroxide, and then 10 ml of the dioxane scintillator was added. The urine diluted in distilled water was directly added in the volume of 0.1 ml to 10 ml of the dioxane scintillator. Feces was dried at 110° for 1 hr, and 1 g of the feces measured accurately was homogenized in distilled water three times the amount of the feces. An aliquot of the homogenate (1 ml) was washed twice with 10 ml of petroleum ether and extracted three times with 7 ml of methylene chloride. After the extract was concentrated under reduced pressure and dissolved in methanol to make a certain volume, 0.1 ml of the solution was added to 10 ml of the dioxane scintillator. The recovery of the radioactivity in the feces was 92%. The radioactivity of the samples was measured by a liquid scintillation spectrometer (Packard Co., Model 3380) and quenching was corrected by the internal standard method.

Thin-Layer Chromatography——Twenty-four-hr urine and 4-days urine were extracted with 7 ml of methylene chloride. After the extracts were concentrated under reduced pressure and dissolved in 1 ml of methanol, 0.01 ml of the solution was spotted on Kieselgel GF 254 (Merck Co.) activated for 1 hr at 110°, and was developed using benzene–96% ethanol (85: 15) as a developing solvent. The silica gel was zonally scraped (0.5 cm wide) and suspended in 10 ml of toluene scintillator (4 g of PPO, 0.1 g of dimethyl POPOP, and 1000 ml of toluene) and radioactivity was determined.

Results

Excretion of ^{3}H-A 41 304

Table I shows changes with time in excretion of radioactivity in the urine and feces. Within 2 days after the beginning of the dermal application of ^{3}H-A 41 304, 8.1 and 3.9% of the applied amount of radioactivity were excreted in male and female rats respectively, which amounted to 89 and 70% respectively of the total radioactivity excreted. Most of the radioactivity in the feces was excreted within 4 days after the beginning of the application. The radioactivity was excreted in the feces more slowly than in the urine, and the excretion in the urine was slower in female rats than in male ones though such a sex difference was not seen in the feces. The total radioactivity excreted in the urine and feces of female rats was 61% of that of male ones, that is, a sex difference was apparently noted in the excreted amount of radioactivity.

Only a trace amount of radioactivity was observed in the urine of male and female rats after 7 days as shown in Table I. In both sexes, the total amount of the excreted radioactivity in the feces was about 20% of that in the urine.

Table I. Excretion of the Radioactivity in Urine and Feces after Dermal Application of ^{3}H-A 41 304 to Rats

Days after application	Radioactivity excreted (%)			
	Male		Female	
	Urine (u)	Feces (f)	Urine (u)	Feces (f)
1	3.12 ±0.47 (18)	0.038±0.013 (3)	0.59 ±0.22 (18)	0.015±0.007 (3)
2	4.38 ±1.17 (12)	0.53 ±0.23 (3)	2.99 ±0.82 (12)	0.30 ±0.12 (3)
3	0.21 ±0.07 (6)	0.53 ±0.14 (3)	0.70 ±0.19 (6)	0.34 ±0.10 (3)
4	0.065±0.021 (6)	0.14 ±0.06 (3)	0.25 ±0.11 (6)	0.23 ±0.06 (3)
5	0.023±0.010 (3)	0.015±0.004 (3)	0.055±0.010 (3)	0.033±0.012 (3)
6	0.015±0.008 (3)	0.007±0.002 (3)	0.015±0.010 (3)	0.010±0.003 (3)
7	0.004±0.001 (3)	0.002±0.001 (3)	0.003±0.001 (3)	0.003±0.002 (3)
Total	7.82	1.26	4.60	0.94
u+f	male 9.08%		female 5.54%	

All the figures represent the mean ±standard deviation expressed as % of the total radioactivity administered. Figures in parentheses indicate the number of the experimental animals.

Excretion of ^{3}H-A 41 304 in the Urine of Castrated Animals

Because there was a sex difference in the excreted amount of ^{3}H-A 41 304 as shown in Table I, male rats were castrated 45 hr before the application of the compound in order to investigate the influence of castration on the excretion of radioactivity in the urine. As shown in Table II, the total radioactivity excreted in the urine for 4 days in the castrated rats was 78% of that in the normal male rats, closing to the value in the normal female rats.

Table II. Effect of Castration on the Excretion of the Radioactivity in Urine after the Dermal Application of ^{3}H-A 41 304 to Rats

Days after application	Radioactivity excreted (%)		
	Male	Castrated male	Female
1	2.78 ±0.32	1.96 ±0.32	0.70±0.24
2	4.03 ±0.86	3.08 ±1.06	3.12±0.68
3	0.30 ±0.08	0.50 ±0.13	0.56±0.20
4	0.078±0.025	0.069±0.010	0.12±0.04
Total	7.19	5.61	4.50

Three groups each consisted of 3 rats. Male Wistar rats were castrated 45 hr before dermal application of ^{3}H-A 41 304.
All the figures represent the mean ±standard deviation expressed as % of the total radioactivity administered.

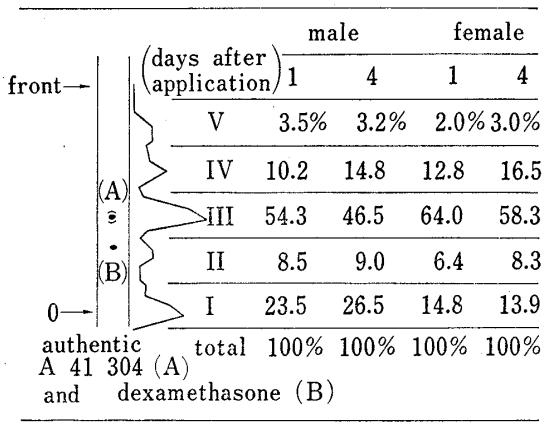

Fig. 2. Thin-Layer Chromatogram of Methylene Chloride Extracts of Urine after the Dermal Application of ^{3}H-A 41 304 to Rats

Data are shown as percent of the total radioactivity on thin-layer chromatogram.
adsorbent: Kiesergel GF 254 (Merck)
solvent: benzene–96% ethanol (85: 15)

Metabolites of ^{3}H-A 41 304 in the Urine

Twenty-four-hr urine and 4-day urine were extracted with methylene chloride and examined chromatographically for metabolites. After the extracts were developed with benzene–ethanol (85: 15), the radioactivity at each spot, I—V, was determined. As shown in Fig. 2, in the 24 hr urine 54.3 and 64.0% of the total radioactivity were recovered in males and females respectively from the part which had the same *Rf* value as that of authentic A 41 304. In the 4 day urine the radioactivity detected at the same part was 46.5% in males and 58% in females. And about 6—9% of the total radioactivity was detected at the part of the same *Rf* value as that of dexamethasone in the animals of both sexes.

Absorption of ^{3}H-A 41 304 and Its Disappearance from Blood

In males the radioactivity of ^{3}H-A 41 304 was demonstrated at 1 hr after the beginning of the application and increased with time, reaching a maximum level at 24 hr. It decreased to 1/8 and 1/135 of the maximum radioactivity level at 2 days and 3 days after the beginning of the application respectively. In females, the appearance of the radioactivity in the blood was a little later than in males, but the maximum radioactivity level was also attained after 24 hr. And it decreased to 1/3 and 1/35 of the maximum level 2 days and 3 days after the beginning of the application respectively. Its disappearance from the blood was also slightly slower in female rats than in male ones. After 7 days the radioactivity was not detected in the blood of either sex. The experimental results are shown in Table III.

Table III. Absorption of the Radioactivity and Its Disappearance from the Blood after Dermal Application of ^{3}H-A 41 304 to Rats

Time after application	Male		Female	
	$\times 10^3$ dpm/ml	Radioactivity excreted (%/ml)	$\times 10^3$ dpm/ml	Radioactivity excreted (%/ml)
1 hr	186	(0.030)	58	(0.009)
4	1056	(0.170)	325	(0.051)
8	1294	(0.198)	1006	(0.159)
24	1846	(0.268)	1430	(0.214)
31	1405	(0.227)	1251	(0.188)
2 days	232	(0.033)	468	(0.073)
4	11	(0.002)	36	(0.006)
7	3.4	(<0.001)	1.6	(<0.001)

All figures show the average of values of 3 animals. Figures in parentheses indicate the values expressed as % of the total radioactivity administered.

Distribution of ^{3}H-A 41 304 in the Organs

Table IV shows the time-course of the radioactivity in main organs when ^{3}H-A 41 304 was absorbed through the rat skin. The organ distribution of the radioactivity was represented as the ratio of the radioactivity distributed in the whole organ to the total amount applied and as the ratio of the radioactivity distributed per gram of tissues to the total amount applied. The radioactivity began to appear in each organ from 4 hr after the beginning of the application and reached its maximum level at 31 hr, and then gradually decreased with time. After 7 days only a trace amount of radioactivity was observed. In the liver and adrenal, a greater concentration of radioactivity was detected than in the other organs. The maximum radioactivity level was attained in the organs such as the liver, adrenal, kidney, spleen, heart and genital organs a little later than in the blood, and its duration was longer. In the brain the radioactivity was only a little detected.

Table IV. Distribution of ^{3}H-A 41 304 in the Organs

Time after application		Liver		Adrenal		Kidney		Spleen		Heart		Brain		Seminal vesicle		Testicle	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Male	1 hr	3	25	18	<1	3	5										
	4	58	514	13	<1	5	8	1	<1	1	1	2	3	1	1	2	3
	8	67	537	39	1	6	10	5	3	2	2	1	2	3	3	2	4
	24	74	681	68	1	15	27	8	6	6	4	4	7	6	5	4	10
	31	100	782	99	2	21	33	10	7	12	8	2	4	22	19	5	11
	2 days	48	397	52	1	20	33	8	5	7	5	3	5	19	13	4	9
	4	5	38	36	1	8	14	4	2	3	2	1	2	3	2	4	10
	7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Famale	1 hr	2	15	15	<1	3	5							ovary		uterus	
	4	30	222	19	<1	5	7	1	<1	2	1	2	3	5	<1	2	1
	8	41	273	28	1	5	8	3	2	6	4	3	5	10	1	2	1
	24	46	310	52	1	28	37	7	3	5	3	2	4	22	2	4	1
	31	136	879	146	3	31	42	16	7	14	8	9	14	52	5	20	10
	2 days	97	695	87	2	21	29	7	3	5	4	3	4	15	1	5	2
	4	20	141	34	1	9	13	3	1	2	2	1	1	7	<1	2	1
	7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1	<1	<1	<1	<1

A: Radioactivity in 1 g of organ expressed as $\times 10^{-3}$ % of the total radioactivity administered.
B: Radioactivity in whole organ expressed as $\times 10^{-3}$ % of the total radioactivity administered.
All figures show the average of values of 3 animals.

Discussion

When ^{3}H-A 41 304 was applied to the skin of rats, the excretion of the radioactivity was observed not only in the urine but in the feces, suggesting that a part of the compound was metabolized in the liver and was then excreted into the alimentary tract. It was demonstrated that there was a sex difference in the amount of the excreted radioactivity and that the excretion in castrated male rats approximated to that in female ones. These findings suggest that the difference in the excretion of the radioactivity resulted from influences of hormonal factors. On the other hand, no sex differences were demonstrated in (1) the distribution of the radioactivity in the organs, (2) its disappearance from the blood and the organs, (3) the pattern of the excretion with time, and (4) metabolites in the urine as examined on thin-layer chromatogram. It can therefore be presumed that the absorption of the compound is sex-dependent or that there is a difference in the metabolic turnover rate and excretion after the absorption.

Conney, *et al.*[8] have already reported that there was a sex difference in the enzyme activity related to the hydroxylation of steroid hormones in the liver and that 3—4 times the extent of the activity noted in female rats was observed in male ones. It has been also reported that enzyme induction by various steroid hormones was sex-dependent too.[9] Therefore, a possibility cannot be excluded that the sex difference in the excretion of this compound resulted from the sex difference in the metabolic enzyme activity in the liver, or the sex difference in the enzyme induction or potentiation.

Fukushima, *et al.*[10] have reported that when 4-^{14}C-cortisol was administered to normal human subjects about 90% of the absorbed amount was metabolized, and Sandberg, *et al.*[11] have reported that only 10% of the radioactivity absorbed was excreted unchanged in the urine after the administration of 4-^{14}C-prednisolone. On the other hand, in the present experiment approximately 50% of the compound absorbed was found to be excreted unchanged in the urine of rats after the application. From this fact, it is considered that the metabolism of A 41 304 is relatively difficult in rats. Furthermore, the fact that the biological half-life of steroid compounds is prolonged by the halogenation of C-9 or the methylation of C-16 in the steroid structure may be helpful for explaining the result of the present experiment that a relatively large amount of A 41 304 absorbed was excreted unchanged. At the same time, the fact that not only the unchanged A 41 304 but metabolites with the same *Rf* value as that of dexamethasone in thin-layer chromatogram were excreted in the urine suggests that hydroxylation at the position of 17α was one of metabolic processes in a living body. This might be the reason of the fact that the activity corresponding to 1/4 of that of dexamethasone was found in A 41 304 without 17α-OH group in our laboratory, while glucocorticoid activity is generally thought to be dependent on 17α-OH in the structure of corticoid derivatives.[12]

^{3}H-A 41 304 was already observed in the blood 1 hr after the beginning of the dermal application and was excreted in the urine after 4 hr, which suggests that the compound was rather rapidly absorbed percutaneously. It was also found that ^{3}H-A 41 304 was very rapidly metabolized and excreted, since the excretion of the radioactivity was hardly observed and only a trace amount of radioactivity was noted in the blood, organs, *etc.* 7 days after the

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10) K. Fukushima, H.L. Bradlow, L. Hellmann, B. Zumoff, and T.F. Gallagher, *J. Biol. Chem.*, **235**, 2246 (1960).
11) A.A. Sandberg and W.R. Slaunwhite, Jr., *J. Clin. Endocrinol. Met.*, **17**, 1040 (1957).
12) G.W. Liddle and M. Fox, "Information and Diseases of Connective Tissue," W.B. Saunders Comp., Philadelphia & Lond., 1961, p. 302.

beginning of the application. Although the compound was applied in the form of ointment with covering for 24 hr, the total amount of its excretion for 7 days was less than 10% of the total amount applied even in male rats. Therefore it is considered that there is a threshold, which is not so high, for the absorption per time unit through the skin. And the fact that the compound disappeared from the organs in a short time may show that it hardly accumulated in the organs after the application to the skin.

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