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EXCRETION AND DISTRIBUTION OF 16,17-ACETONIDE OF 21-ACETOXY-3-(2′-CHLOROETHOXY)-9α-FLUORO-6-FORMYL-11β,16α,17α-TRIHYDROXY-3,5-PREGNADIEN-20-ONE

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SUMMARY

The excretion of a tritium-labelled synthetic anti-inflammatory steroid, active by local application (16,17-acetonide of 21-acetoxy-3-((2′-chloroethoxy)-9α fluoro-6-formyl-11β,16α,17α-trihydroxy-3,5-pregnadien-20-one) was studied in four animal species (rat, guinea-pig, rabbit and dog). The distribution of the compound was also investigated in the rat.

The steroid, intradermically applied, was removed from the dermis in a consistent amount during the first 48 h and then more slowly in the following 120 h.

In all the species examined the radioactivity, due to the administered compound or to its metabolites, was prominently excreted by the faeces. The highest fecal/urinary ratios were found in the guinea-pig and in the dog.

No accumulation of the compound was detected in the various organs examined in the rat; the relative highest concentration of radioactivity was detected in the liver.

The results are discussed in relation to a peculiar pharmacodynamic aspect of the compound, which is much more active by local application than by systemic administration.

INTRODUCTION

THE PHARMACOLOGICAL experiments performed to define the biological profile of 16,17-acetonide of 21-acetoxy-3-(2′-chloroethoxy)-9α-fluoro-6-formyl-11β,16α,17α-trihydroxy-3,5-pregnadien-20-one (Formocortal) have always shown evidence of a dissociation between its topical anti-inflammatory effect and its systemic anti-inflammatory, corticoid activity as a whole [1].

This behaviour may be due, in the case of topical (percutaneous) application, to lack of penetration through the skin of the compound, thereby preventing its entry into the blood stream. In the case of Formocortal this does not apply. After percutaneous applications to the mini-pig of a cream containing tritiated Formocortal, significant amounts of radioactivity were recovered in the deep dermal layers as well as in the underlying adipose tissue [2]. The dermal penetration of the compound was also demonstrated in humans [3, 4] with some difference in the results, depending on the condition of the epidermis, the concentration of the steroid in the applied cream and the constituents of the excipients.

Once the absorption of the compound has been ascertained, the next step is represented by the identification of its metabolic pattern.

For this purpose the first experiments were carried out to clarify the distribution of Formocortal in the various tissues and the routes of its elimination after intradermal application to various animal species.

METHODS

1. Radioactive compound. The Formocortal used was tritium labelled at C-7. Its specific activity was 0·221 Ci/mmol. The chemical and radiochemical purities of the steroid were assessed spectrophotometrically and by thin layer chromatography on silicagel (benzene : petroleum ether : ethylacetate, 70 : 10 : 40 by vol) respectively. The compound was found to be at least 98% pure.

2. Biological materials. The following animals were used: 30 rats (female, SPF, Sprague-Dawley CRF strain, 120–130 g body weight); 5 guinea pigs (both sexes, 300 g body weight); 5 rabbits (both sexes, albino New Zealand, 2–3 kg body weight); 2 dogs (female Beagles, 10 kg body weight).

The biological samples examined were: urine and faeces from all the animal species used; blood, thymus, kidneys, liver, brain, pituitary gland, adipose tissue, skeletal muscle from rats; the area of the skin where the compound had been injected from rats and rabbits.

3. Biological procedures. The radioactive compound was administered intradermally as an aqueous suspension in the following amount:

Rat : 20·37 μCi (92 n mol)
Guinea-pig: 10·19 μCi (46 n mol)
Rabbit : 43 μCi (190 n mol)
Dog : 40·74 μCi (184 n mol).

Immediately after the injection, the animals were housed in metabolic cages with free access to food and water. Urine and faeces were collected at various intervals during the following 168 h. Groups of 5 rats were sacrificed at various times after treatment and the above mentioned organs and tissues were collected. The treated skin was excised from rats and rabbits at the end of the experiment.

4. Determination of the radioactivity. The tissues were freeze-dried and each dry residue weighted. No appreciable radioactivity was detected in the water after lyophilization of the tissues. The freeze-dried organs as a whole or homogeneous aliquots of them were then burnt in a TRI-CARB oxidizer (Packard mod. 305); the tritiated water was collected directly in the counting vials together with the scintillation fluid.

Lyophilized tissues containing appreciable amounts of fat (adipose tissue, skin) were extracted with ethyl ether/methanol (3 : 1 v/v) and the radioactivity was determined both in the extracted material and in the burnt residue.

The radioactivity in the faeces was determined by burning aliquots of the lyophilized material, thoroughly ground in a Mulinex grinder. Urines were properly diluted with distilled water and counted directly.

All processed samples were counted in a liquid scintillation spectrometer TRI-CARB (Packard mod. 3375); the counting efficiency was determined by the channel ratio method.

RESULTS

All the data refer to the radioactivity found, regardless if due to the injected compound or to its metabolites not yet identified.

(1) *Elimination*

The results are summarized in Table 1 which shows the amounts and the corresponding ratios of the radioactive material excreted in urines and faeces at

Table 1. Urinary and faecal excretion of [7-^{3}H]-formocortal (% of the administered radioactivity ± S.E.)

Species	Hours after administration 0–8	8–24	24–48	48–72	72–96	96–120	120–144	144–168	Total
	(a) *Urine*								
Rat	7·4 ± 0·54 (30)	4·9 ± 0·37 (25)	2·8 ± 0·22 (20)	1·7 ± 0·17 (15)	1·2 ± 0·13 (10)	1·3 ± 0·27 (5)		0·5 ± 0·7 (5)	19·8
Guinea-pig (5)	3·52 ± 0·43	2·91 ± 0·12	1·45 ± 0·18	1·15 ± 0·17	0·74 ± 0·10	0·78 ± 0·18	0·48 ± 0·08	0·29 ± 0·06	10·42
Rabbit (5)	3·81 ± 2·91	15·91 ± 3·15	4·31 ± 0·89	1·37 ± 0·52	0·74 ± 0·43	0·59 ± 0·12	0·35 ± 0·035	0·36 ± 0·064	27·45
Dog (2)	2·49	2·74	2·67	1·85	0·90	0·63	0·40	0·20	11·89
	(b) *Faeces*								
Rat	7·6 ± 0·79 (30)	13·6 ± 0·91 (25)	17·8 ± 1·61 (20)	7·6 ± 0·70 (15)	3·8 ± 0·57 (10)	5·10 ± 0·77 (5)		1·6 ± 0·16 (5)	57·1
Guinea-pig (5)	7·98 ± 2·23	17·98 ± 4·26	15·44 ± 1·17	12·19 ± 2·93	5·37 ± 1·38	3·56 ± 0·81	2·73 ± 0·82	1·64 ± 1·73	66·99
Rabbit (5)	4·27 ± 0·85	30·47 ± 4·63	13·71 ± 1·64	5·24 ± 1·08	2·29 ± 0·42	0·98 ± 0·19	0·56 ± 0·09	0·39 ± 0·07	57·92
Dog (2)	2·08	20·35	13·91	11·14	6·48	3·62	2·31	1·88	61·80
	(c) *Faecal/Urinary ratio*								
Rat	1·03	4·33	6·36	4·47	3·17	3·92		3·20	2·88
Guinea-pig	2·27	8·94	10·65	10·60	7·26	4·56	5·69	5·65	6·42
Rabbit	1·12	1·91	3·18	3·82	3·09	1·66	1·60	1·08	2·11
Dog	0·83	8·24	5·21	6·02	7·20	5·75	5·78	9·40	5·19

In parenthesis the number of animals.

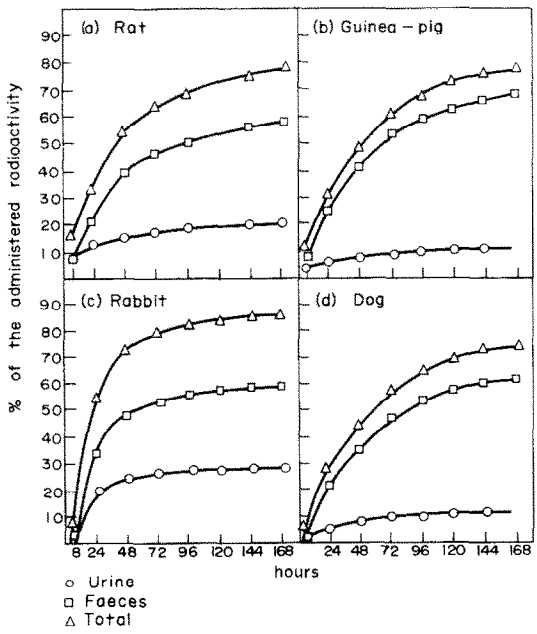

Fig. 1. Excretion of [7-^{3}H]-formocortal in various animal species.

various time intervals. Figure 1(a–d) represents the overall course of elimination of the radioactivity in the four species examined. From these data it appears that in all the species some radioactivity is still detected in the excreta 168 h after the intradermal injection, whereas its maximal excretion appears within the first 24 h.

Throughout the period of the experiment all species excreted much more radioactivity in the faeces than in the urines. The total radioactivity eliminated by the faeces after 7 days ranged from 60–75% whereas only 10–25% were eliminated in the urine.

The highest fecal/urinary radioactivity ratios were found in the guinea-pig and in the dog, the lowest in the rat and rabbit, where the radioactivity in the faeces was nevertheless more than twice that found in the urine.

(2) *Removal from the injection sites*

Table 2 summarizes the data concerning the amount of radioactivity found at various times in the injection sites in the rat. In the first 8 h after treatment more

Table 2. Removal of [7-^{3}H]-formocortal from the intradermal injection site (5 rats per group)

Hours after administration	% of the administered radioactivity found ± S.E.
8	42·0 ± 2·31
24	37·9 ± 3·92
48	20·8 ± 2·40
72	14·4 ± 2·07
96	17·9 ± 1·94
168	5·8 ± 1·07

Table 3. Distribution of [7-^{3}H]-formocortal in various organs and tissues of rat (5 animals per group)

Hours after administration	Blood nCi/ml ± S.E.	Liver	Skeletal muscle	Kidney	Brain	Thymus	Fat	Pituitary	Adrenals
				nCi/g ± S.E.					
8	4·85 ± 0·92	30·82 ± 0·98	4·82 ± 1·06	15·11 ± 0·21	12·33 ± 1·96	11·84 ± 1·00	11·88 ± 3·35	7·88*	15·46 ± 2·08
24	4·09 ± 0·46	22·28 ± 3·24	3·37 ± 0·58	10·50 ± 1·40	11·67 ± 1·32	14·28 ± 1·93	5·49 ∓ ·86	8·47*	14·47 ± 2·05
48	2·21 ± 0·40	9·04 ∓ ·68	1·28 ± 0·12	4·75 ± 0·30	8·54 ± 0·84	7·71 ± 0·37	2·02 ± 0·12		
72	1·64 ± 0·15	6·25 ± 0·70	0·96 ± 0·077	3·57 ± 0·13	6·21 ± 0·29	6·23 ± 0·27	1·54 ± 0·12		
96	1·73 ± 0·15	6·40 ± 0·89	1·06 ± 0·068	3·87 ± 0·34	7·80 ± 1·65	7·50 ± 1·46	1·58 ± 0·14		
168	0·92 ± 0·16	3·34 ± 0·69	0·61 ± 0·05	2·18 ± 0·30	3·07 ± 0·56	2·95 ± 0·55	0·98 ± 0·13		

*5 pituitaries pooled.

than 50% of the steroid is removed from the injection site. In the following period the removal of the compound occurs at such a slow rate that 5% of the radioactivity is still present in the skin 7 days after the injection. In five rabbits treated in the same way, only 0·21 ± 0·026% of the radioactivity was present in the injection site 7 days after treatment.

(3) *Distribution*

Table 3 shows the data concerning the concentration of radioactive material in the organs and tissues. In all cases the concentration of radioactivity is maximal during the first 8–24 h after treatment. During the following days no accumulation of the material was detected. Assuming that the values found in the blood could be taken as a background, one can observe that the skeletal muscle shows the same radioactivity as the blood, while the other organs and tissues contain more radioactivity, particularly in the earliest samples. The highest amount of radioactivity was found in the liver. In any case, the total radioactivity present in the organs is very low when compared with the amount found in the injection site or with that already excreted (Fig. 2). In an additional group of rats sacrificed 8 h after treatment and not included in Fig. 2, the radioactivity detected in the intestinal tract rose, as expected, to high levels: 26·9%. The recovery of the administered material ranged from 75–80%, a satisfactory value if one considers that only the radioactivity quantitatively determined adds up to this figure (urine, faeces, and organs which were dissected out *in toto*).

DISCUSSION

The results concerning the kinetic of Formocortal (disappearance from the injection site, blood levels at the various times, elimination rate) support the

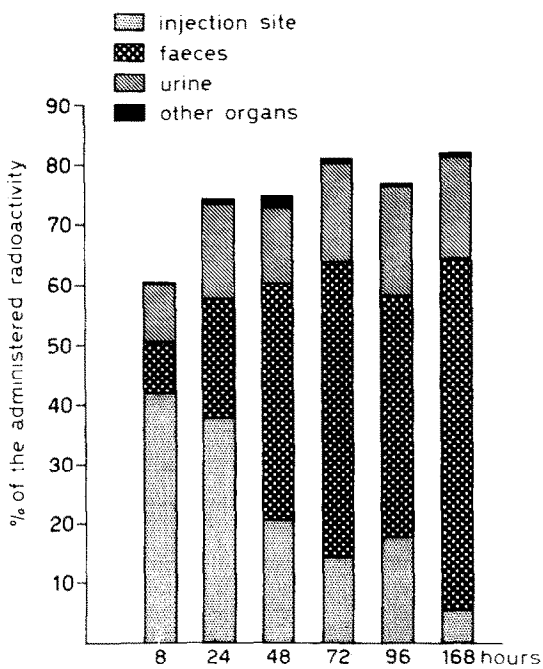

Fig. 2. Recovery of [7-^{3}H]-formocortal in rats.

assumption that this steroid, after an initial prompt and marked release, is then slowly removed from the dermis.

The excretion of the radioactive material and its disappearance from the dermis proceed at the same rate; thus no accumulation occurs in any area of the body.

The prominent elimination via liver-bile–intestine–faeces is evident not only in the rat, but also in other animal species (dog, rabbit, guinea-pig). This pattern of elimination is not obvious for all the species examined. The rabbit and the guinea-pig have been described as "poor biliary excretors" of drugs [5]). The dog excretes injected corticosterone, or its metabolites, in urine: faeces ratio equal to 55:20 [6]. For the rat this ratio has been reported to vary from 15:80–40:50 depending on the sex and the strain [7].

The hypothesis that Formocortal rapidly reaches the liver where it is metabolized into inactive compounds, while supported by some of the results obtained, still remains unanswered. It must be remembered that for Fluocinolone acetonide (16, 17-acetonide of 6α, 9α-difluoro-11β, 16α, 17α, 21-tetrahydroxy-1, 4-pregnadien-3,20-dione), a steroid with high systemic activity, an intense biliary excretion has also been described after intravenous administration [8].

The identification of the biliary metabolites of Formocortal and the determination of the length of time that the compound remains in the blood before it reaches the liver could clarify the above hypothesis.

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