Biochemical Pharmacology, 1960, Vol. 3, pp. 277–282. Pergamon Press Ltd., Printed in Great Britain.

THE HYPERCALCEMIC ACTIVITY OF DIHYDROTACHYSTEROL$_2$ AND DIHYDROTACHYSTEROL$_3$ AND OF THE VITAMINS D_2 AND D_3 AFTER INTRAVENOUS INJECTION OF THE AQUEOUS PREPARATIONS—II

COMPARATIVE EXPERIMENTS ON RATS

J. R. ROBORGH and TH. J. DE MAN

Biological Department, N.V. Philips-Duphar, Weesp, Holland

(*Received* 12 *January* 1960)

Abstract—In experiments on rats, the hypercalcemic activities of dihydrotachysterol$_2$, dihydrotachysterol$_3$, vitamin D_2 and vitamin D_3 have been compared at different time intervals (1, 2, 4 and 7 days) after the administration of one intravenous injection of the crystalline compounds dispersed in an aqueous medium.

It appears that dihydrotachysterol$_3$ is the most active hypercalcemic agent, followed, in decreasing order, by dihydrotachysterol$_2$, vitamin D_3 and vitamin D_2. This is exactly the same order that was found in a previous study of the effects of an oral administration of oily solutions of the same substances. The maximal serum calcium levels appear to be obtained 2–4 days after the intravenous injection.

The activity ratios prove to be highly dependent on the time interval again and show a fair agreement with the activity ratios found in the previous investigation.

INTRODUCTION

IN A previous report[3] the hypercalcemic activities of dihydrotachysterol$_2$, dihydrotachysterol$_3$, vitamins D_2 and D_3 have been compared at different time intervals after a single oral administration to rats of the crystalline compounds in pure peanut oil. Dihydrotachysterol$_3$ appeared to be the most active agent, followed in decreasing order by dihydrotachysterol$_2$, vitamin D_3 and vitamin D_2. The activity ratios proved to be highly dependent on the time interval, while the maximal serum calcium levels were reached 2 to 4 days after the administration.

Since the absorption by the intestinal wall played an important role in those experiments and this factor could have been determinant with regard to the ultimate effect in serum calcium increase, we decided to compare also the activity ratios of the four crystalline compounds at different time intervals after a single dose given by the parenteral route, notably the intravenous mode of administration.

EXPERIMENTAL

For intravenous injection, it was necessary to use aqueous dispersions of the substances under investigation. Therefore a 25%-aqueous solution of a solubilizer, belonging to the group of polyoxyethylene derivatives of fatty acid esters, was used for composing the preparations. For the determination of the hypercalcemic activity the

same method[1] was followed as used in our previous communication. For each series of experiments (dihydrotachysterol$_3$/dihydrotachysterol$_2$, dihydrotachysterol$_2$/vitamin D_3 or vitamin D_3/vitamin D_2), 180 male rats (average body weight $\pm$ 200 g) of our own inbred stock were prepared by feeding a stock diet, to which 1% calcium carbonate was added during at least 10 days. These animals were divided into five uniform groups four for the two substances to be compared (each compound at two dosage levels) and one control group.

On the day of dosing, all the rats received an intravenous injection of 0·25 ml of the respective aqueous preparations or the blank dilution medium (control group). The site of injection was the vena femoralis, made accessible by cutting off a little skin flap of the thigh (inner side). This manipulation as well as the injection was done under a slight either narcosis.

The quantities administered per rat (of about 200 g) were:

dihydrotachysterol$_2$	0·04 and 0·16 mg
dihydrotachysterol$_3$	0·01 and 0·04 mg
vitamin D_2	0·25 and 1·0 mg
vitamin D_3	0·25 and 1·0 mg

After 1, 2 and 4 days, respectively, twelve rats of each group were anaesthetized and their blood collected individually. The blood samples were centrifuged and the sera mixed in pairs (equal volumes) within the groups. In these mixed sera the calcium determinations were performed according to the method of Clark and Collip.[2]

With the choice of the parenteral route combined with the use of an aqueous dispersion, a rapid serum calcium increase could be expected and we thought the maximal time interval of 4 days would suffice at the onset.

Nevertheless an elongation of the time of observation appeared to be necessary in the course of the experiments, for which reason analogous experiments (60 rats) with a time interval of 1 week were added thereafter.

Each series of experiments was executed twice (experiments I and II), at different moments, several months apart. The obtained data were worked up statistically in pairs as in our foregoing study.

RESULTS AND DISCUSSION

In Tables 1, 2 and 3, the data of the series of experiments (1, 2 and 4 days) and of the separate experiments (1 week) are collected respectively for the following comparisons: dihydrotachysterol$_3$/dihydrotachysterol$_2$, dihydrotachysterol$_2$/vitamin D_3 and vitamin D_3/vitamin D_2. In all cases, the serum calcium values are expressed as increases over the corresponding control values (in mg %). Also the standard deviations of the mean values are given and, in parentheses, the numbers of the determinations made.

It is seen from the tables that maximal serum calcium increases occur at about 2–4 days after the intravenous injections. According to expectation, there is a clear trend of a somewhat more rapid serum calcium increase than in the previous experiments, when the compounds, dissolved in peanut oil, were administered orally.

On the basis of the data from the tables, the activity ratios have been calculated according to the method of *U.S. Pharmacopoeia*.[4] The results are summarized in Table 4. In the last column the weighted mean values of the duplicate determinations are given with their P 0·95 intervals.

TABLE 1. COMPARISON DIHYDROTACHYSTEROL$_3$ WITH DIHYDROTACHYSTEROL$_2$; MEAN INCREASE SERUM CALCIUM OVER CONTROLS (mg %)

Days after administering	Dihydrotachysterol$_3$				Dihydrotachysterol$_2$			
	0·01 mg		0·04 mg		0·04 mg		0·16 mg	
1 (expt. I)	0·15±0·20	(6)	0·48±0·17	(6)	0·40±0·12	(6)	1·27±0·29	(6)
1 (expt. II)	0·38±0·20	(6)	0·37±0·13	(6)	0·11±0·15	(6)	0·67±0·15	(6)
2 (expt. I)	1·47±0·13	(6)	2·72±0·16	(6)	1·29±0·14	(6)	2·74±0·24	(6)
2 (expt. II)	1·08±0·05	(6)	2·37±0·08	(6)	1·07±0·12	(6)	2·77±0·33	(6)
4 (expt. I)	−0·16±0·26	(6)	2·49±0·29	(6)	0·15±0·18	(6)	2·08±0·09	(6)
4 (expt. II)	1·13±0·10	(6)	2·93±0·14	(6)	1·06±0·13	(6)	2·74±0·15	(6)
7 (expt. I)	0·48±0·09	(6)	1·68±0·12	(6)	−0·10±0·10	(6)	0·81±0·08	(6)
7 (expt. II)	−0·09±0·06	(6)	1·07±0·17	(6)	−0·15±0·09	(6)	0·50±0·03	(6)

TABLE 2. COMPARISON DIHYDROTACHYSTEROL$_2$ WITH VITAMIN D$_3$; MEAN INCREASE SERUM CALCIUM OVER CONTROLS (mg %)

Days after administering	Dihydrotachysterol$_2$				Vitamin D$_3$			
	0·04 mg		0·16 mg		0·25 mg		1·0 mg	
1 (expt. I)	1·37±0·13	(6)	1·88±0·28	(6)	1·12±0·19	(6)	1·20±0·36	(6)
1 (expt. II)	0·47±0·29	(6)	0·75±0·21	(6)	0·56±0·19	(6)	0·47±0·08	(6)
2 (expt. I)	0·98±0·19	(6)	3·44±0·53	(6)	0·88±0·19	(6)	3·56±0·28	(6)
2 (expt. II)	1·08±0·25	(6)	2·35±0·28	(6)	1·39±0·17	(6)	3·14±0·48	(6)
4 (expt. I)	0·33±0·11	(5)	1·46±0·11	(6)	0·76±0·11	(6)	2·47±0·11	(6)
4 (expt. II)	0·07±0·26	(6)	2·00±0·19	(6)	0·55±0·17	(7)*	2·14±0·18	(6)
7 (expt. I)	0·08±0·20	(5)	0·64±0·11	(6)	0·18±0·11	(6)	1·44±0·31	(6)
7 (expt. II)	−0·02±0·09	(6)	0·13±0·17	(6)	0·02±0·11	(6)	1·25±0·30	(6)

* As two of the twelve serum samples have not been pooled, seven observations resulted.

TABLE 3. COMPARISON VITAMIN D$_3$ WITH VITAMIN D$_2$; MEAN INCREASE SERUM CALCIUM OVER CONTROLS (mg %)

Days after administering	Vitamin D$_3$				Vitamin D$_2$			
	0·25 mg		1·0 mg		0·25 mg		1·0 mg	
1 (expt. I)	1·22±0·14	(6)	1·13±0·17	(6)	0·53±0·10	(6)	0·94±0·10	(6)
1 (expt. II)	0·35±0·25	(6)	1·52±0·20	(6)	−0·12±0·09	(6)	0·65±0·14	(6)
2 (expt. I)	1·46±0·16	(6)	3·26±0·27	(6)	1·07±0·17	(6)	2·50±0·41	(5)
2 (expt. II)	1·61±0·11	(6)	3·19±0·24	(6)	1·03±0·09	(6)	3·19±0·45	(6)
4 (expt. I)	0·95±0·18	(6)	2·50±0·24	(6)	0·60±0·18	(6)	3·09±0·21	(5)
4 (expt. II)	0·97±0·04	(6)	2·58±0·19	(6)	0·62±0·11	(6)	2·15±0·21	(6)
7 (expt. I)	−0·60±0·09	(6)	0·61±0·26	(6)	−0·89±0·08	(6)	0·33±0·13	(6)
7 (expt. II)	0·00±0·09	(6)	2·09±0·22	(6)	0·04±0·07	(6)	1·79±0·32	(6)

As can be seen from Tables 1, 2 and 3, the increases in serum calcium fail to show a sufficient dose–response relation in some cases, resulting in the absence of a reliable slope of the curve. The calculated activity ratios for those cases (1 and 7 days) are only approximate therefore and provided with a plus and minus sign in Table 4.

TABLE 4. ACTIVITY RATIOS AT DIFFERENT TIMES AFTER ADMINISTERING

	Days after administ.	Expt. I	Expt. II	Weighted mean value and P 0·95 interval	
Dihydrotach. 3/ Dihydrotach. 2	1	± 1·2	± 3·6	± 2·4	—
	2	4·35	3·36	3·80	77–130%
	4	4·12	4·43	4·31	84–119%
	7	10·40	± 6·50	± 8·40	—
Dihydrotach. 2/ vit. D_3	1	±56	±24	±40	—
	2	6·2	3·8	5·6	71–142%
	4	± 3·1*	4·9	± 3·8	(63–159)%
	7	± 3·1	± 1·9	± 2·4	—
Vit. D_3/vit. D_2	1	±11·0	2·60	± 6·8	—
	2	1·62	1·43	1·50	71–140%
	4	0·93	1·41	1·12	79–126%
	7	1·38	1·10	1·20	78–128%

* The two lines fail to be statistically parallel.

The hypercalcemic activity of vitamin D_2 appears to be the weakest. It is followed, in increasing order, by vitamin D_3, dihydrotachysterol$_2$ and dihydrotachysterol$_3$, which is exactly the same order as was found in our previous experiments with oral application of the oily solutions.

The mutual activity ratios find expression in Table 5, in which we have put the hypercalcemic activity of dihydrotachysterol$_2$ equal to one for each time interval (2, 4 and 7 days), and recalculated the activity relations. For convenience we repeat in this table the data of our previous study ("oily oral").

TABLE 5. ACTIVITY RATIOS (DIHYDROTACHYSTEROL$_2$ EQUAL TO ONE)

Application:	"Aqueous intravenous"			"Oily oral"		
Days after dosing:	2	4	7	2	4	7
vitamin D_2	0·12	0·23	±0·35	0·15	0·20	0·20
vitamin D_3	0·18	0·26	±0·42	0·24	0·39	0·28
dihydrotachysterol$_2$	1·0	1·0	1·0	1·0	1·0	1·0
dihydrotachysterol$_3$	3·8	4·3	±8·4	3·6	6·0	3·4

In general a fair agreement seems to exist between the two series of values.

As we now dispose of the results of the dihydrotachysterols and of the vitamins D in different media and with different modes of application, viz. "oily oral" and "aqueous intravenous", it appears interesting to demonstrate the influence thereof.

This can be done by calculating the average serum Calcium increase for each compound and at each level from this paper and from our previous publication from all the experiments given in the Tables 1, 2, 3 and 1a, 1b, 1c, respectively. The results

are given in Tables 6, 7, 8 and 9. They clearly show a better utilization of the four compounds on a weight basis when intravenously given as aqueous dispersions. These obvious differences will be caused by losses and a limitation of the rate of absorption

TABLE 6. COMPARISON VITAMIN D_2 "OILY ORAL" WITH "AQUEOUS INTRAVENOUS": MEAN INCREASE SERUM CALCIUM OVER CONTROLS (mg %)

Days after dosing	"Oily oral"			"Aqueous intravenous"	
	1·0 mg	4·0 mg	16·0 mg	0·25 mg	1·0 mg
1	0·34	1·12	0·80	0·20	0·80
2	0·95	2·71	4·64	1·05	2·88
4	0·52	2·61	4·14	0·61	2·58
7	−0·09	1·57	2·79	−0·42	1·06

TABLE 7. COMPARISON VITAMIN D_3 "OILY ORAL" WITH "AQUEOUS INTRAVENOUS": MEAN INCREASE SERUM CALCIUM OVER CONTROLS (mg %)

Days after dosing	"Oily oral"				"Aqueous intravenous"	
	0·25 mg	1·0 mg	4·0 mg	16·0 mg	0·25 mg	1·0 mg
1	—	0·90	0·80	0·90	0·81	1·08
2	0·79	1·48	3·54	4·79	1·34	3·29
4	0·60	1·57	3·52	5·29	0·80	2·42
7	0·49	0·81	2·38	3·16	−0·10	1·35

TABLE 8. COMPARISON DIHYDROTACHYSTEROL$_2$ "OILY ORAL" WITH "AQUEOUS INTRAVENOUS"; MEAN INCREASE SERUM CALCIUM OVER CONTROLS (mg %)

Days after dosing	"Oily oral"			"Aqueous intravenous"	
	0·25 mg	1·0 mg	4·0 mg	0·04 mg	0·16 mg
1	0·88	1·36	1·64	0·59	1·14
2	1·66	3·56	4·70	1·10	2·82
4	1·63	3·61	4·47	0·41	2·07
7	0·96	2·49	3·67	−0·05	0·52

TABLE 9. COMPARISON DIHYDROTACHYSTEROL$_3$ "OILY ORAL" WITH "AQUEOUS INTRAVENOUS"; MEAN INCREASE SERUM CALCIUM OVER CONTROLS (mg %)

Days after dosing	"Oily oral"			"Aqueous intravenous"	
	0·1 mg	0·4 mg	1·6 mg	0·01 mg	0·04 mg
1	1·11	1·25	1·06	0·26	0·42
2	2·33	3·74	5·50	1·28	2·54
4	3·22	4·58	5·46	0·48	2·71
7	1·20	2·81	4·07	0·20	1·38

through the intestinal wall in the case of oral administration of the solution in oil. These factors are probably of the same order of magnitude in view of the fair similarity, which appears to exist between the activity ratios of the four compounds investigated by two different modes of administration (see Table 5).

A quantitative evaluation of the data of the Tables 6–9 is not justified on statistical grounds, but two days after the administration an activity ratio: "aqueous intravenous"/"oily oral" in the order of 3 to 4 could be estimated for each of the four compounds.

Acknowledgements—We wish to thank Dr. J. A. Keverling Buisman and Mr. P. Westerhof for preparing crystalline dihydrotachysterol$_2$ and dihydrotachysterol$_3$ and Drs. R. J. E. Esser and Dr. K. J. Keuning for the calcium-determinations.

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