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Disposition and Tissue Levels of [^{3}H]Vindesine in Rats

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SUMMARY

The excretion, blood levels, and tissue distribution of [^{3}H]vindesine have been studied in the rat. After an i.v. administration of 500 μg/kg, [^{3}H]vindesine was found to be distributed very rapidly to tissues. After the distribution phase, blood levels declined with a half-life near 10 hr. Excretion was mainly via the bile, and [^{3}H]vindesine and its metabolites in bile were poorly reabsorbed in the gastrointestinal tract. Levels were relatively high in most tissues studied but appeared to be efficiently cleared from all tissues except thymus and testes. Uptake into peripheral nerves was considerably higher than into the central nervous system.

INTRODUCTION

Vindesine is a vinblastine modification product with an antitumor spectrum similar to that of vincristine (4, 9). Preliminary clinical studies have been presented recently (5, 6, 8), as have the results of toxicology studies in laboratory animals (10). A comprehensive report on the chemistry of vindesine will be forthcoming shortly.[2]

Although radioimmunoassay has made it possible to investigate pharmacokinetics in man (6), little is known about the disposition of vindesine in test animals. Radiovindesine has now been prepared, and its disposition in rats is described below.

MATERIALS AND METHODS

Labeled Drug. Radioactive vindesine sulfate was prepared by mild acid-catalyzed exchange with [^{3}H]trifluoroacetic acid. This preparation was performed by the Amersham/Searle Corp., Skokie, Ill. Although the position of tritiation is unknown, a similar exchange reaction with vincristine was reported to result principally in the exchange of aromatic protons (7). Purity was estimated to be 95% on the basis of thin-layer chromatography in several systems. Purity was confirmed at periodic intervals by chromatography on silica gel in a chloroform:diethylamine (40:2) system. The [^{3}H]vindesine had a specific activity of 3.4 mCi/mg and was stored at −20° in a glass container in aqueous methanol (pH 4.3) solution. For animal studies aliquots were taken and diluted to a specific activity of 250 μCi/mg by addition of unlabeled vindesine sulfate. After removal of methanol in a stream of nitrogen, the labeled drug was dissolved in 0.9% NaCl solution for injection.

Animals. Male Purdue-Wistar weighing 200 g were used throughout the study. Food and water were available to the rats at all times. Rats were dosed i.v. via the tail vein. The dose of 500 μg/kg selected corresponds roughly to the usual dose of 3 mg /sq m used in man.

Analysis. Radioactivity measurements were made by counting aliquots of bile and urine directly. Blood samples were put on tared cellulose pads and immediately weighed. These were air dried and then burned in a Model 306 Packard Instrument sample oxidizer in preparation for counting. Tissue samples or complete organs were put into tared cellulose cones and then immediately weighed. After drying, these were also burned in preparation for counting. A sample with a known amount of radioactivity was burned with each group to measure recovery efficiency, which was 97 to 99% in most cases.

Thin-Layer Chromatography of Bile. No completely satisfactory thin-layer chromatographic system could be developed for the chromatography of bile containing [^{3}H]vindesine. When small volumes (5 to 10 μl) of bile containing carrier vindesine were run on silica gel plates with a solvent system of chloroform:methanol:diethylamine (95:5:5), some streaming occurred but a reasonably well-defined radiovindesine spot did occur. This sytem was thus used to estimate the amount of unchanged vindesine in bile samples. Since only the radioactivity in the defined spot was counted as vindesine, the value obtained is probably quite low.

RESULTS

Levels of [^{3}H]Vindesine in Whole Blood. Three 200-g male rats received a 500 μg/kg dose of [^{3}H]vindesine i.v. Injection was made 4 cm from the tail tip, and the tail was well massaged to insure proper uptake into the general circulation. Blood samples (50 μl) were taken periodically from the tip of the tail for determination of total radioactivity content. Results were calculated in terms of [^{3}H]vindesine equivalents and are the average values for the 3 rats, which are plotted in Chart 1. It is not known how much of the tritium present in blood represents unmetabolized drug and how much represents metabolites.

After the injection, levels of drug fell very rapidly during the distribution phase.

After a distribution phase during which tissue uptake was rapid, drug levels fell exponentially during the period of 1 to 7 hr. The half-time of this decline was estimated to be about

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[2] K. Gerzon, C. J. Barnett, and G. J. Cullinan, submitted for publication to *The Journal of Medicinal Chemistry*.

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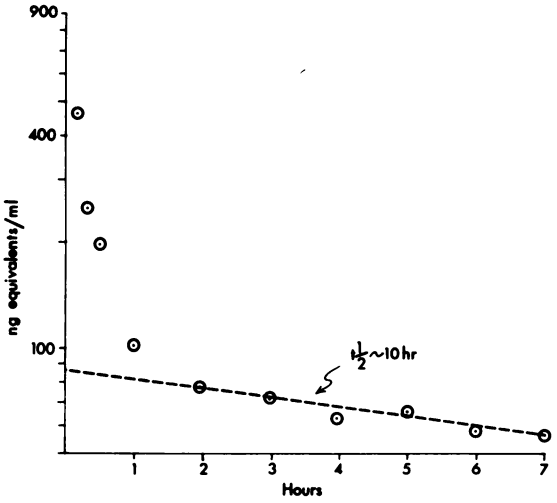

Chart 1. Rate of disappearance of tritium from rat whole blood after [³H]vindesine, 500 μg/kg. i.v. Each *point* is an average value from 3 rats.

10 hr (Chart 1). The apparent value of distribution was about 5.8 liters/kg body weight.

Elimination Studies. The rats received injections of the drug and were placed in restraining cages the 1st 24 hr. Urine was collected soon after voiding, and the feces were collected at 24-hr intervals. Aliquots of urine were counted, and the entire fecal sample was burned in preparation for counting. After the 1st 24 hr the rats were kept in metabolism cages for 3 additional days. The results, which are summarized in Table 1, show that, in the rat, elimination via the bile duct into the gastrointestinal tract is of much greater significance than elimination in urine.

Excretion into Bile. The bile ducts of 2 rats were exteriorized under secobarbital anesthesia (40 mg/kg i.p.). After completion of the surgery each rat was given radiovindesine at 500 μg/kg i.v. Bile was collected at timed intervals, and aliquots were counted. The results are given in Table 2. It is obvious that the primary route of elimination of vindesine is by way of the bile. The rate of appearance of tritium in bile is very rapid during the distribution phase (0 to 0.5 hr) and much slower during the 0.5- to 7-hr phase. In this respect the results are consistent with the blood level results in Chart 1.

In order to determine the extent of reabsorption the following experiment was performed. Two cannulas were put into the common bile duct of a rat. One was directed toward the liver to collect bile, and the 2nd one was directed toward the gut for the infusion of bile. Bile collections from a rat given [³H]vindesine were pooled, and the amount of radioactive content was determined. Five ml of this pooled bile were infused at the rate of 1 ml/hr. Bile and urine were collected during the infusion and then continuously for 2 days. The total amount of radioactivity infused was equivalent to 44.4 μg of vindesine. Aliquots of bile and urine were counted directly, and the feces were burned for counting. The results are given in Table 3. The amount of tritium that appeared in bile and urine suggested that only limited reabsorption of [³H]vindesine occurs.

Tissue Distribution of [³H]Vindesine in Rats. Three 200-g male Purdue-Wistar rats were given [³H]vindesine at 500 μg/kg i.v. At selected times, each rat was asphyxiated with CO_2, and a blood sample (6 to 8 ml) was obtained by heart puncture. The rats were then dissected, and organ samples up to 1 g were weighed immediately after removal. Weighed fractions of liver, lung, brain, erythrocytes, plasma, and muscle were sampled, and 1 each of the kidneys and testes were used in making the radiohydrogen determination. The peripheral lymph nodes were collected outside the thoracic and abdominal cavities. The femur did not include the joints, therefore making the marrow a larger fraction of the whole sample. The hair was closely clipped from the belly of the rats, and about a 4-cm-square sample was collected. The muscle sample was collected from the hind leg. At the time that the muscle sample was taken, a segment of sciatic nerve was also secured. All the samples were dried and then

Table 1
Disposition of [³H]vindesine in rats

Time (hr)	Accumulated % dose in urine				Accumulated % dose in feces			
	Rat A	Rat B	Rat C	Av.	Rat A	Rat B	Rat C	Av.
1	2.4	4.3	3.3	3.3				
2	3.1	5.5	3.5	4.0				
3	3.8	6.0	3.8	4.5				
5	4.4	6.4	4.6	5.1				
24	7.4	9.8	7.5	8.2	55.4	47.8	50.0	51.1
48	8.0	10.6	8.2	8.9	66.4	67.2	67.8	67.1
72	8.4	10.8	8.4	9.2	71.1	71.8	74.4	72.4
96	8.8	11.3	8.8	9.6	73.6	73.1	76.0	74.2

Table 2
Excretion of [³H]vindesine into bile

Time	% of dose (accumulated) in bile from:	
	Rat A	Rat B
15 min	19.5	22.3
30 min	26.7	28.5
60 min	30.5	33.2
75 min	32.7	34.8
120 min	35.1	38.1
3 hr	37.5	40.4
4 hr	39.0	43.0
9 hr	43.3	49.1
15 hr	48.3	55.7
21 hr	52.6	61.0
25 hr	55.2	
25–48 hr	58.7	
48–74 hr	61.3	

Table 3
Reabsorption of [³H]vindesine and metabolites from bile

Time (hr)	% of radioactivity from infused bile appearing in:		
	Bile	Urine	Feces
1	0.02		
2	0.23	0.40	
3	0.32		
4	0.50		
5	0.71		
6	0.94		
24	2.19	0.87	
48	3.01	1.14	62.8

burned in preparation for counting. Total tritium was converted to [^{3}H]vindesine ng equivalents. The results, expressed in ng per g of wet tissue, are given in Table 4. The half-lives of decline were estimated by using the values through 48 hr.

Absorption p.o. of [^{3}H]Vindesine in the Rat. Results of the experiments on reabsorption (see above) suggest that [^{3}H]vindesine is probably poorly absorbed from the gastrointestinal tract. To confirm this, 2 rats were dosed p.o. at 500 μg/kg. Urine was collected for 24 hr at which time selected tissues were removed for tissue level determination. Only 0.4% of the dose appeared in urine in 24 hr. The 24-hr tissue levels of [^{3}H]vindesine are presented in Table 5. These levels are all very low, about 1 to 3% of the 24-hr levels after i.v. administration (see Table 4). It seems clear from this work that [^{3}H]vindesine was poorly absorbed p.o. in the rat.

Chromatography of Bile. Three rats with indwelling bile cannula received i.v. doses of radiovindesine of 500 μg/kg, and bile was collected for 5 hr. Samples of bile were chromatographed on thin-layer plates in a system that allowed estimation of the amount of vindesine present (see "Materials and Methods"). At 1 hr an average of 44% of the dose appeared in bile of which a minimum of 40% was unchanged vindesine. At 5 hr 54.8% of the radioactivity was eliminated, 41% of which was vindesine. The only other spots on the plates were 2 spots near the origin, each of which contained less than 5% of the radioactivity. The remaining radioactivity was streaked along the plate from the origin to the vindesine spot ($R_F \sim 0.8$). Since samples of bile containing added, purified [^{3}H]vindesine behaved in a similar fashion, it is probable that much of this streaked radioactivity is also unmetabolized vindesine.

DISCUSSION

The whole-blood level curve (Chart 1) appears to be biphasic during the 7-hr study, with $t_{1/2}$ values of 15 min for the initial phase and 10 hr for the 2nd phase. The rapid distribution phase and the biphasic decline curve, as well as the large volume of distribution found in the rat are all consistent with the results of pharmacokinetic studies in man (6).

Vindesine is avidly taken up and bound to almost all tissues studied. In several tissues levels are 10 to 20 times those seen in blood. It is interesting that spleen has the highest levels at all times studied. Spleen levels have also been reported to be high for vinblastine (1) and vincristine (3). In the case of these alkaloids, however, spleen levels were not found to be markedly higher than those in organs of excretion (liver, kidney) or of lung.

The comparative results with sciatic nerve, spinal cord, and brain tissue suggest that, although vindesine penetrates the central nervous system to only a very slight degree, it is taken up in significant amounts by peripheral

Table 4

Distribution of vindesine in rat tissues (ng/g wet tissue)

Male rats were dosed i.v. at 500 μg/ml. Tissues were analyzed for total radioactivity as described in "Materials and Methods."

	2 hr		4 hr		8 hr		16 hr		24 hr		48 hr		168 hr		$t_{1/2}$	Significance level by
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	(2–48 hr.)	*F* test
Spleen	910	195	865	66	925	81	725	113	689	82	378	116	19	11	36	0.005
Salivary gland	832	216	599	136	590	97	283	38	181	40	57	9	11	6	12	0.005
Heart	540	98	391[b]	-	180	23	73	4	51	14	17	2	3	0	10	0.005
Kidneys	537	75	410	54	315	10	179	11	160	24	77	3	19	2	17	0.005
Lungs	518	93	499	33	314	65	187	16	113	23	53	8	10	4	14	0.005
Liver	512	122	475	48	562	51	301	64	355	116	183	81	36	1	31	0.01
Adrenals	500	117	531	51	440	31	241	52	175	23	75	6	10	2	16	0.005
Mesenteric lymph nodes	415	62	346	33	402	15	264	25	236	18	135	30	17	3	29	0.005
Peripheral lymph nodes	317	48	284	29	305	14	307	49	293	65	154	7	14	6	49	0.025
Femur	258	38	282	28	220	11	202	43	148	13	79	3	10	1	26	0.005
Muscle	248	31	250	43	169	43	122	17	60	16	18	0	2	0	12	0.005
Thymus	221	41	176[b]	-	217	25	227	7	251	32	286	80	101	52	-[c]	-
Skin	169	6	128	16	148	20	88	3	65	11	-[d]		-[d]		17[e]	0.025
Abdominal fat	144	31	62	2	136	47	85	1	39	9	11	4	2	1	11	0.01
Sciatic nerve	91	15	61	18	47	12	50	13	21	7	-[d]		-[d]		13[e]	0.05
Whole blood	52	11	54	5	74	38	25	4	15	2	4	1	-[d]		11	0.005
RBC	38	24	40	8	8	2	5	1	11	9	4	2	1	0	16	0.1
Plasma	32	10	23	3	16	2	12	2	9	2	4	1	2	1	16	0.001
Testes	26	4	36	5	38	2	22	2	34	3	36	9	24	2	-[c]	-
Spinal cord	17	6	19	3	10	2	8	1	7	3	6	2	3	0	29	0.05
Brain	11	3	7	1	9[b]	-	4	0	4	1	5	2	2	1	45	0.1
Lenses of eyes	7	1	16	0	6	2	4	2	6	4	5	1	1	1	50	0.1

[a] Average of 3 rats/group.
[b] One sample was lost.
[c] Regression lines had positive slope. No $t_{1/2}$ was calculated.
[d] Not determined.
[e] Based on 2- to 24-hr values.

Table 5
Twenty-four-hr tissue levels of [³H]vindesine after p.o. administration

Tissue	ng/g	
	Rat 1	Rat 2
Liver	6.0	13.0
Lung	1.0	3.0
Kidney	5.0	8.0
Thymus	2.0	5.0
Spleen	3.0	9.0
Testes	1.0	1.0
Brain	1.0	1.0
Muscle	0.3	1.0
Mesenteric lymph nodes	2.0	5.0
Salivary gland	1.0	4.0
Whole blood	0.2	0.4

nerve. In spite of the high tissue levels attained, the drug is well cleared from tissues as indicated by the $t_{1/2}$ values which were estimated for each tissue (Table 4). Two exceptions are thymus and testes. Thymus levels are relatively high at 2 hr and do not appear to decline over the 1st 48-hr study period. This apparent lack of decline in the thymus is due at least in part to the fact that a dose of 500 μg/kg caused a degeneration of thymus such that the gross weight declined by 50% in 48 hr. Although testes had very low levels of drug, these levels did not appear to decrease with time up to 168 hr. Data on these 2 tissues have not as yet been reported for vinblastine or vincristine. However, unpublished work in our laboratory indicates that [³H]vindesine levels in thymus and testes are similar to those seen with [³H]vinblastine and [³H]vincristine.

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