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Distribution of tritiated methyl ester of streptonigrin in mice bearing sarcoma 180

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STREPTONIGRIN, an antibiotic with antitumor activity, has been isolated from the broth of *Streptomyces flocculus*.[1] A methyl ester derivative (NSC-45384), which is less toxic than the parent compound, has been synthesized (Fig. 1), and a report of clinical trials with the use of this drug in human cancer patients has been published.[2] Its metabolism in human subjects was studied with the aid of the tritiated compound.[3] Since organ distribution could not be studied in the human, it was of interest to study its distribution in mice bearing sarcoma 180 tumor.

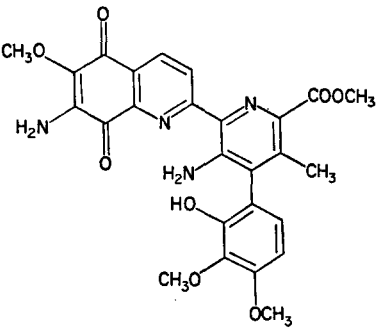

FIG. 1. Methyl ester of streptonigrin, $C_{26}H_{24}O_8N_4$.

METHODS AND RESULTS

Tritiated methyl ester of streptonigrin had a specific activity of 2·1 μc/mg* and was found to be free from contaminants, by paper chromatography.[3] Negligible amounts of exchangeable hydrogen were found by distilling serum to which the compound had been added, and assaying the distillate for tritium. A solution was made of 1·25 mg tritiated drug/ml N,N-dimethylacetamide, and it was given i.p. at a level of 2·5 mg/kg to mice with sarcoma 180. The mice were placed in metabolism cages, and urine, feces, and expired air were collected. They were sacrificed at the end of 24 and 48 hr and the tissues removed for analysis were weighed, dried in vacuum at 50°, and combusted in Thomas Ogg oxygen flasks. The water vapor released from oxidation was frozen,[4] and tritium was assayed in gel-scintillate according to Frenkel *et al*[5] with a Nuclear-Chicago liquid scintillation counter. Corrections were made for quenching by the channels ratio method.[6] Radioautographs were made on sections of liver, kidney, and tumor by applying NTB† liquid emulsion, exposing for 30 days, developing in DK19b, fixing, washing, and staining with hematoxylin and eosin.

Mice were used 5–10 days after tumor transplantation. Tumor was propagated by excising malignant tissue from donor mice (10 to 14 days after transplantation), and injecting with a trocar small pieces (1 to 2 mm)[3] of actively growing tumor into the inguinal region of A-strain mice.

The results in Table 1 show that at 24 hr after drug administration, the highest concentration of tritium was found in the small intestine. The remainder of the tissues could be divided into three groups in the order of descending concentration: group 1: liver, spleen, sarcoma, and large intestine; group 2: lung, kidney, stomach, muscle, skin, and blood cells; and group 3: plasma, heart, and brain. The change in the level in the second 24 hr is expressed by the ratio of the value at 48 hr to that at 24 hr. There was little change of concentration in the stomach. Moderate reduction by about one third occurred in kidney and blood cells. The remainder of the tissues showed considerable decrease. Radioautographs showed generalized distribution of radioactivity throughout the tissue.

A mean of 64·9 per cent of the dose was excreted in 24 hr. An additional 25·9 per cent was excreted during the second 24 hr. The partition between urine and feces varied widely and could not be

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† Nuclear track emulsion, Eastman Kodak Co., Rochester, N.Y.

TABLE 1. DISTRIBUTION OF TRITIUM FROM TRITIATED METHYL ESTER OF STREPTONIGRIN, 2·5 MG/KG

Tissue	DPM/g wet wt*		
	24 hr	48 hr	48/24 hr
Small intestine	11,046 ± 718	926 ± 196	0·08
Spleen	4335 ± 349	1649 ± 216	0·38
Liver	4313 ± 530	819 ± 144	0·19
Large intestine	3428 ± 1,505	597 ± 227	0·17
Sarcoma	3106 ± 588	834 ± 166	0·27
Lung	1785 ± 292	493 ± 103	0·28
Muscle	1675 ± 162	426 ± 162	0·26
Kidney	1482 ± 387	1186 ± 411	0·81
Skin	1462 ± 514	169 ± 36	0·12
Stomach	1051 ± 267	1126 ± 269	1·07
Heart	751 ± 227	388 ± 212	0·52
Brain	255 ± 72	38 ± 6	0·15
Plasma	848 ± 412	609 ± 51	0·72
Blood cells	1727 ± 440	1116 ± 105	0·65
Bile†	966		
Excretion	% of dose		
	24 hr	48 hr	
Urine + feces	64·9 ± 2·7	89·8 ± 3·0	
Expired air‡	0·01		

* Mean disintegrations/min/g of 10 mice (24 hr) and 9 mice (48 hr), ± standard error of the mean.

† One mouse only.

‡ Average of 4 mice.

accurately determined, because gross contamination of feces by the urine occurred even with the use of the feces–urine separator in the metabolism cage. The loss of label via expired air was negligible.

DISCUSSION AND SUMMARY

The results showed that about two-thirds of the label from the drug was excreted during the first 24 hr and a total of about 90 per cent by the end of the second day. Oxidation of the compound to water did not occur to any appreciable extent, since negligible amount of radioactivity was detected in the expired air. The high concentration of the label in the small intestine suggested that this may be an important route of excretion. It was previously reported that in human patients with cancer the administration of the tritiated drug resulted in the excretion of 18·5 per cent of the dose in the urine.[3] Fecal excretion was not determined. With mice, the partition of the label between urine and feces could not be accurately determined, but if results from human studies are applicable, the major route of elimination was in the feces. Organ studies showed no preferential uptake of the label by the sarcoma. Radioautographs did not show particular localization of the label in any cellular compartment.

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