Biodistribution of Intravenously Injected [^{14}C] Doxorubicin and [^{14}C] Daunorubicin in Mice: Concise Communication

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[^{14}C] doxorubicin (adriamycin) and [^{14}C] daunorubicin (daunomycin) are cardiotoxic antibodies used in cancer therapy. These drugs were examined as possible agents for the measurement of regional myocardial blood flow. The antibiotics were injected intravenously into mice, which were then killed after various intervals. At a chemical dosage of 0.5 mg per kilogram, the content of the heart never exceeded 0.60% of the administered dose for doxorubicin and 0.55% for daunorubicin. The cardiotoxic effect of these drugs, therefore, is probably related to a specific sensitivity of the heart, rather than to an avid uptake of the drugs by the cardiac muscle. Further studies seem warranted, using a lower chemical dosage and higher specific activity.

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Extensive use has been made of thallium-201 chloride for the measurement of regional myocardial blood flow. This cyclotron-produced radionuclide is expensive and the dose is usually limited to 2 mCi per study. The antineoplastic drugs, doxorubicin (adriamycin) and daunorubicin (daunomycin), have recently been used in cancer therapy. Both may produce cardiotoxicity that is severe and irreversible (*1*). Previous work by Bachur (*2*) and Arena (*3*) indicate that doxorubicin and daunorubicin accumulate in the cardiac muscle of mice. In the present experiments, we determined the distribution of the C-14-labeled drugs in mice at various times after i.v. injection. This was done to examine the hypothesis that doxorubicin and daunorubicin might be useful for measuring regional myocardial perfusion.

METHODS

Doxorubicin hydrochloride and daunorubicin hydrochloride* were obtained at specific activities of 2.3 and 6.9 mCi/mM, respectively. Each drug was diluted in normal saline (pH 5.5) to a concentration of 50 μg per cc. Laboratory white mice with an average weight of 27 g were injected intravenously with a dose of 0.5 mg per kilogram of each drug. The mice were killed at 15 min, 30 min, 60 min, and 4 hr after injection. Blood samples (100 μl) were taken, and the heart, lungs, liver, and a skeletal

TABLE 1. [^{14}C] DOXORUBICIN DISTRIBUTION IN MICE*

Time after injection	% dose per gram				
	Blood	Heart	Lungs	Liver	Muscle
15 min	1.30	5.3	7.8	19.8	1.6
30 min	0.71	5.2	6.9	13.3	1.5
60 min	†	3.7	5.7	13.4	1.3
4 hr	†	2.5	5.0	9.6	1.8
	% dose per organ‡				
15 min	2.5	0.60	1.5	29.5	
30 min	1.3	0.57	1.6	22.9	
60 min	†	0.46	1.5	22.6	
4 hr	†	0.32	1.0	15.7	

* Each value represents a mean of three animals.
† Counts not significantly above background.
‡ Blood volume was calculated as 7% of body weight.

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TABLE 2. [^{14}C] DAUNORUBICIN DISTRIBUTION IN MICE*

Time after injection	% dose per gram				
	Blood	Heart	Lungs	Liver	Muscle
15 min	0.69	4.0	9.5	13.2	1.3
30 min	0.54	4.0	9.6	12.8	1.3
60 min	0.39	3.9	8.2	13.5	1.2
4 hr	0.25	1.7	3.3	6.4	1.1
	% dose per organ†				
15 min	1.3	0.55	2.2	21.3	
30 min	1.0	0.51	2.0	21.9	
60 min	0.73	0.48	1.9	19.9	
4 hr	0.45	0.22	0.93	9.9	

* Each value represents a mean of three animals.
† Blood volume was calculated as 7% of body weight.

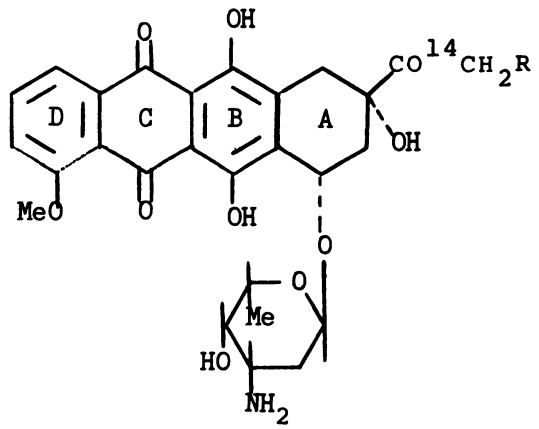

FIG. 1. Molecular configurations of doxorubicin (R = OH) and daunorubicin (R = H).

muscle sample, were excised and weighed. Tissue samples of 100–150 mg were obtained and dissolved overnight in 1 cc of Unisol solubilizer. The next day 0.5 cc of methanol and 10 cc of Unisol Complement were added to the samples. Four drops of 30% hydrogen peroxide were used to reduce native coloration in each vial, and the samples were counted in a scintillation spectrometer. All samples were corrected for quenching using the channels' ratio method. The corrected counts were then used to calculate percentage of dose for each organ and percentage dose per gram.

RESULTS AND DISCUSSION

The results are shown in Tables 1 and 2. The distributions of doxorubicin and daunorubicin were similar. The accumulated dose of both antibiotics in the liver was 20–30%. The heart-to-lung ratio per gram of tissue was 0.75 for doxorubicin and 0.5 for daunorubicin.

Doxorubicin and daunorubicin are closely related in structure, the difference being the hydroxy substitution at the C-14 position (Fig. 1). The large accumulation of doxorubicin and daunorubicin found in the liver confirms previous reports that the liver and bile are major sites for the metabolism of these drugs (*5*). Although these antibiotics accumulate in the heart muscle, the heart-to-lung ratio was only one quarter as great as that obtained with ^{201}Tl Cl, where the heart-to-lung ratio in mice was 2:1 at 10 min after injection (*4*). Our 60-min cardiac concentration of [^{14}C] doxorubicin is in independent agreement with the 60-min cardiac value obtained by Bachur, who used a fluorescent method to detect doxorubicin metabolites in mice (*2*). These two independent methods—ours, using the C-14-labeled antibiotic, and Bachur's, using a fluorescent measurement of metabolites—gave comparable cardiac concentration levels at the 60-min time interval. When Bachur's data are recalculated, the percentage of the administered dose in the heart was comparable to that of ^{201}Tl Cl at the same time period (*4*). Our previous experience with ^{201}Tl Cl indicated that measurement of heart activity at 60 min only is inadequate to assess the usefulness of a myocardial imaging agent. We therefore examined the concentrations at 15, 30, 60 min, and 4 hr after injection.

Previous studies of doxorubicin and daunorubicin distribution in animals have been performed using doses of 1–15 mg per kilogram. Although it was our objective to obtain adequate cardiac radioactivity while decreasing toxicity using a lower chemical dose of the antibiotics, the low specific activities of the labeled compounds limited the injected dose to 0.5 mg/kg. If cardiac accumulation sites are saturated at this dosage level, myocardial radioactivity might be enhanced by administering doses of higher specific activity and lower chemical quantity.

The mechanism of the cardiotoxicity of doxorubicin and daunorubicin is not known. Previous animal studies indicate that these drugs do not concentrate in the heart in large amounts (*5*). Our results with the C-14-labeled antibiotics suggest that cardiac toxicity is due to a selective sensitivity of the heart to these drugs, rather than to the accumulation of large chemical quantities.

CONCLUSIONS

1. When injected at a chemical dosage of 0.5 mg/kg body weight, doxorubicin and daunorubicin do not seem to be suitable for measurement of regional myocardial blood flow.

2. Further studies of these compounds at higher specific activities and lower chemical dosage seem warranted.

3. Cardiac toxicity is probably related to a specific sensitivity of the heart, rather than to avid accumulation of the drugs in the cardiac muscle.

FOOTNOTE

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REFERENCES

1. ADAMSON RH: Daunomycin (NSC-82151) and adriamycin (NCS-123127): A hypothesis concerning antitumor activity and cardiotoxicity. *Cancer Chemother Rep* 58: 293–294, 1974

2. BACHUR NR, REITER W, ARENA E: Cardiac uptake of adriamycin (NSC-123127) not affected by strophanthin G (NSC-25485). *Cancer Chemother Rep* 59: 765–766, 1975

3. ARENA E, D'ALESSANDRO ND, DUSONCHET L, et al: Influence of α-6-deoxy-5-oxytetracycline on some pharmacological characteristics of daunomycin. *J Antibiotics* 26: 339–342, 1973

4. STRAUSS HW, HARRISON K, LANGAN JK, et al: Thallium 201 for Myocardial Imaging: Relation of Thallium-201 to Regional Myocardial Perfusion. *Circulation* 51: 641–645, 1975

5. BACHUR NR, HILDEBRAND RC, JAENKE RS: Adriamycin and Daunorubicin Disposition in the Rabbit. *J Pharmacol Exp Ther* 191: 331–340, 1974