Cytofluorescence Localization of Adriamycin and Daunorubicin

Merrill J. Egorin, Robert C. Hildebrand, Eugene F. Cimino, and Nicholas R. Bachur¹

Baltimore Cancer Research Center, National Cancer Institute, NIH, Baltimore, Maryland 21211

INTRODUCTION

The pharmacokinetics and tissue distribution of the cancer chemotherapeutic agents adriamycin and daunorubicin have been studied (1, 3, 7, 8, 13), but these studies reveal little of the intracellular localization of these drugs. Both drugs are absorbed by cells (9) and bind avidly to cellular components, particularly to nucleic acids (6, 12). Using an in vitro system, Silvestrini et al. (10) demonstrated that cultured HeLa tumor cells take up both daunorubicin and adriamycin into nuclear structures; but the nuclear localization of adriamycin was delayed compared to that of daunorubicin, becoming apparent only after 1 hr of incubation. In order to investigate the subcellular localization of these agents in vivo, we have used fluorescence microscopy to examine tissues from hamsters treated with these drugs.

MATERIALS AND METHODS

Adriamycin-HCl and daunorubicin-HCl in clinical dosage form prepared by Farmitalia Co., Milan, Italy, were obtained from the Drug Development Branch, DCT, National Cancer Institute, NIH, Bethesda, Md. Other reagents were of analytical grade.

Male golden Syrian hamsters weighing 75 to 100 g were anesthetized with diethyl ether and their femoral veins were isolated. Adriamycin or daunorubicin (5 mg/ml in 0.9% NaCl solution) was injected i.v. over a 10-sec period with a 32-gauge needle to a dose of 5 mg/kg. Control animals received similar volumes of i.v. 0.9% NaCl solution without drug. Duplicate animals were killed by cervical dislocation at 0.5, 15, and 60 min postinjection. The hearts, lungs, livers, and kidneys were rapidly excised and sliced, and the slices were rapidly processed.

Drug Extraction. Tissue samples (0.1 to 0.8 g) were frozen under powdered CO₂ immediately after excision and subsequently extracted with 0.3 N HCl in 50% ethanol as previously reported (5). Fluorescence of the extracts (excitation, 470 nm; emission, 585 nm) was measured on an Aminco SPF 125 spectrophotofluorometer and compared to a series of adriamycin and daunorubicin standards.

RESULTS AND DISCUSSION

Adriamycin and daunorubicin fluoresced orange-red when excited with UV (2). Tissue sections from adriamycin or daunorubicin-treated animals also fluoresced a striking orange-red (Figs. 1 to 6). This drug-specific fluorescence emanated from all the tissues, with the greatest fluorescence intensity localized in the nuclei of cells. The nuclear pattern was verified in the toluidine blue-treated sections examined under white light. Control tissue sections viewed in UV contained no orange-red fluorescence; but the walls of large blood vessels, the walls of pulmonary airways, and outlines of myocardial bundles, alveolar cell masses, and kidney tubules contained areas of blue-green fluorescence. In lung alveoli, discrete punctate green-yellow fluorescence was seen in both drug-
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Table 1

Tissue drug content

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Drug&lt;sup&gt;a&lt;/sup&gt;</th>
<th>0.5 min</th>
<th>15 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>A</td>
<td>5.46</td>
<td>6.26</td>
<td>6.97</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>9.47</td>
<td>5.92</td>
<td>2.39</td>
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<tr>
<td>Lung</td>
<td>A</td>
<td>3.11</td>
<td>3.40</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>13.32</td>
<td>7.72</td>
<td>2.09</td>
</tr>
<tr>
<td>Liver</td>
<td>A</td>
<td>1.59</td>
<td>5.35</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>10.31</td>
<td>8.32</td>
<td>4.91</td>
</tr>
<tr>
<td>Kidney</td>
<td>A</td>
<td>12.36</td>
<td>9.46</td>
<td>3.80</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>14.06</td>
<td>13.51</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> A, adriamycin-treated; D, daunorubicin-treated.

treated and control tissues and was clearly different from the orange-red nuclear fluorescence associated with drug treatment (Fig. 5).

The nuclear, drug-specific fluorescence was seen at every time sampling, even in samples obtained at 0.5 min postinjection. Despite the findings from <em>in vitro</em>-cultured cell experiments (9, 10), our observations indicated that adriamycin entered normal hamster tissues readily and was rapidly localized in the nucleus. Although no quantification of the microscopic fluorescence was possible, nuclear fluorescence intensity decreased in the 60-min samples compared to the 0.5- and 15-min samples in all of the tissues examined. These observations parallel our measurements of the total drug fluorescence (Table 1) of the tissues.

Although drug-specific fluorescence filled kidney and liver cytoplasm in the earliest samples (Fig. 6), this cytoplasmic fluorescence disappeared from 60-min specimens. The early cytoplasmic drug loading of kidney and liver cells may reflect their role in drug metabolism and excretion. Perhaps the cytoplasmic fluorescence of the kidney tubular cells may be related to tubular secretion of the anthracycline antibiotics. Throughout the experiment, all nuclei remained fluorescent, and no redistribution of the fluorescence was observed.

In heart muscle specimens (Fig. 1), the nuclei contained the drug fluorescence. We observed no other subcellular organellar fluorescence as might be seen if mitochondria were stained.

Since a substantial amount (10 to 50%) of daunorubicin fluorescence in the hamster tissues at 60 min is daunorubicin (3), and since almost all drug fluorescence is located in the nuclei, it would appear that the metabolite daunorubcinol is also in the nucleus. Similarly, adriamycinol, a metabolite of adriamycin that has been identified in human urine (11) and in rabbits (4), may be localized to the cell nucleus also.

Subcellular localization of these drugs could possibly be determined by cell fractionation and drug assay. However, cell fractionation may lead to redistribution of the drug during processing. For this reason, we feel the frozen tissue fluorescence microscopy technique inherently has less uncertainty.

REFERENCES

Figs. 1 to 6. Fluorescent photomicrographs of frozen tissue sections. All tissues are from adriamycin (5 mg/kg, i.v.)-treated hamsters removed 0.5 min after administration.

Fig. 1. Heart. X 250.
Fig. 2. Kidney. X 100. Note glomerulus in right upper corner.
Fig. 3. Lung. X 100. Punctate fluorescence is intrinsic.
Fig. 4. Liver. X 100.
Fig. 5. Lung. X 400. Blue-green color is intrinsic fluorescence.
Fig. 6. Kidney tubules. X 400. Faint red cytoplasmic fluorescence is most prominent at outer margin of kidney tubule cells.
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