Comparative Pharmacokinetics of Daunomycin and Adriamycin in Several Animal Species

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SUMMARY

The physiological disposition of adriamycin and daunomycin has been studied in several animal species. Both drugs were rapidly cleared from plasma, deposited in tissues, and then excreted slowly. Drug and metabolites were excreted via the bile and urine. Daunomycin was extensively metabolized by mice, rats, dogs, and hamsters, whereas no evidence was obtained for the metabolism of adriamycin in rats and mice. Hamsters metabolized adriamycin to an aglycone. The metabolite of daunomycin in all species appeared identical to daunorubicinol, described by Bachur and Gee. In hamsters, daunorubicinol as well as another metabolite of daunomycin were observed. This unknown metabolite, \( D^* \), appeared to be an aglycone but not the aglycone of daunomycin or daunorubicinol. The aglycone, \( D^* \), was also observed in several tissues of rats and mice that were exposed to hypobaric stress.

INTRODUCTION

Daunomycin (NSC 82151), an antibiotic of the anthracycline group consisting of the aglycone, daunomycinone, and an amino sugar, daunosamine, has shown antitumor activity against several animal and human neoplasms. The cytotoxic effects of daunomycin on mouse leukemic cells are related to the degree of retention of daunomycin by these neoplastic tissues. Therapeutic doses of daunomycin produce toxic effects in several organs of man and animals, including bone marrow, gastrointestinal tract, and heart. The toxicity of daunomycin to the host may be related to the degree of retention of daunomycin by these tissues and persists for several days following a single i.v. dose. Rusconi et al. (16) reported that 10% of the radioactivity is excreted in urine and 40% is excreted in feces. The distribution and urinary excretion of daunomycin equivalents in man are similar.

Adriamycin, a new antitumor antibiotic, differs from daunomycin only by substituting a hydroxyl group for a hydrogen atom in the acetyl radical. Interest has been focused on adriamycin because its therapeutic index is higher than that of daunomycin in several experimental tumor systems.

In this study, we have compared the physiological disposition of adriamycin and daunomycin in several animal species and have discussed these data with respect to comparative toxicity and chemotherapeutic activity of daunomycin and adriamycin.

MATERIALS AND METHODS

Adult, male BDF\(_1\) mice (10 to 30 g) and adult, male Sprague-Dawley rats (130 to 200 g) were maintained on a standard Purina chow diet with food and water ad libitum. Adriamycin hydrochloride or adriamycin hydrochloride was dissolved in 0.9% NaCl solution and administered via a caudal vein at a dose of 10 mg/kg to rats and mice. Adult, male Syrian golden hamsters were given i.p. injections of either daunomycin or adriamycin, at both 10 mg/kg and 20 mg/kg. Mice were housed in conventional cages, and rats and hamsters were housed in individual No. 110 metabolism chambers (Maryland Plastic, Inc., New York, N. Y.) during the drug studies. The technique for preventing coprophagy by the rats has been described by Barnes et al. (6) and modified by Yesair et al. (21).

In the physiological disposition studies, blood was obtained by heart puncture from rats that were anesthetized with Pentothal sodium (approximately 20 mg/kg i.v.) approximately 1 min earlier. After blood was collected by heart puncture, tissues were taken immediately from the animals and frozen in liquid nitrogen. These tissues could be stored at \(-4^\circ\) to \(-20^\circ\) for extended periods of time without breakdown of daunomycin and its metabolite to their anthracycline moieties.

Male rats (350 to 500 g) selected for bile cannulation were anesthetized with chloral hydrate USP (Fisher Scientific Co., Fairlawn, N. J.) at a dose of 360 mg/kg given i.p. and the bile duct was cannulated with Adams polyethylene tubing, P.E. 10 (E. F. Mahady and Co., Cambridge, Mass.) These surgically prepared rats were restrained but had access to food and water at all times. The daunomycin or adriamycin was injected i.v. into the caudal vein after recovery from anesthesia (about 2 to 3 hr after surgery) and the doses, as clinical dose formulations in 0.9% NaCl solution, were 10 mg/kg.

Female beagle dogs (7 to 10 kg) (Roma Kennels, Dunstable, Mass.) were used in a study to observe the biliary and urinary excretion of daunomycin. Stage 3 anesthesia was induced by i.v. administration of Nembutal (35 mg/kg) and maintained throughout the study period by supplemental doses of that drug. Respiratory rate was maintained at approximately...
20 min with a Harvard apparatus respirator. The common bile duct was cannulated with Adams polyethylene, P.E. 60; the gallbladder and accessory ducts were isolated by ligation. A No. 12 Foley catheter was inserted retrograde through the urethra to remain indwelling in the urinary bladder. The animals were infused initially with 0.9% NaCl solution at an approximate rate of 1 ml/min in the right jugular vein to assure an adequate urinary flow. Inulin-14C (5 mg/ml in 0.9% NaCl solution; specific activity, 4 nCi/mg) was infused into the left jugular vein at approximately 1 ml/min throughout the experiment. Bile and urine samples were collected periodically through the bile cannula and Foley catheter. Blood samples were collected via a 3-way indwelling valve in the right jugular vein; daunomycin (8 mg/ml in 0.9% NaCl solution), 10 mg/kg, was administered via this valve. At autopsy, tissues were taken and frozen immediately in liquid nitrogen, in view of the precaution stated for the rat experiments.

The chamber used for simulating high altitude was a cylindrical Plexiglas vacuum chamber (6 ft long x 3 ft diameter) that contained gloved ports and an air lock. Air was supplied at controlled pressure (12,000 ft) and at flow rate such that air content was completely changed once every 2 hr. Rats and mice were maintained in cages inside the altitude chamber on a standard Purina chow diet with food and water ad libitum.

The method for extracting and quantitating total daunomycin equivalents has been described by Bachur et al. (5). This method does not differentiate between parent drug and fluorescent metabolites. The fluorescence that is associated with the anthracycline moiety of drug and metabolite was determined with a Hitachi Perkin-Elmer MPF 2A fluorescence spectrophotometer. The excitation wavelength was 490 nm, and the excitation slit width was 12 nm. The emission was recorded from 520 to 620 nm, and the excitation slit width was 6 nm. The daunomycin and adriamycin were quantitated at 581 nm, and reference standards were those used in the animal experiments.

A second method for extracting daunomycin and adriamycin equivalents was used for those samples in which drug and fluorescent metabolites were separated by thin-layer chromatography. One g of biological sample was homogenized with 20 volumes of chloroform:methanol (2:1, v/v). After the homogenate was filtered, the filtrate was made biphasic with further additions of water, methanol, and chloroform. The final volume ratio of chloroform:methanol:water was 23.7:19.7:13.5, v/v/v. This extraction procedure was quantitative (> 95%) for all reference standards (daunomycin, its metabolite D2, and adriamycin). These reference standards partitioned 80:20 between the organic and aqueous phases over a wide concentration range (0.1 to 10 μg/g). An aliquot of the organic phase was taken to dryness in a vacuum, and the residue was taken up in 0.2 ml of chloroform:methanol (2:1, v/v).

An aliquot of this concentrated extract was taken for determining total daunomycin and adriamycin equivalents (correcting for the 80:20 distribution noted above), and another aliquot was spotted on activated (1 hr at 110°C), 250-μm, Silica Gel H thin-layer plates under nitrogen and subdued light for characterizing parent drug and metabolite D2. The thin-layer plates were first chromatographed in diethyl ether to separate neutral lipid and subsequently were chromatographed in Solvent System 1, which was chloroform:methanol:acetic acid:water (100:50:14:6, v/v/v/v), to fractionate drug species. After this 2nd chromatography, the fluorescent areas were scraped from the plate into conical test tubes, and fluorescent material was eluted from silica gel by 2 successive washings with 0.5 ml of 0.1 N HCl in 99.5% ethanol. The final volume was diluted with the same solution to 1.0 ml. Fluorescence was determined as described for total drug equivalents.

The conditions for acid hydrolysis of daunomycin, its metabolite D2, and adriamycin and the chromatography of their aglycones were as follows. Aqueous solutions of the drugs were made 3 N in HCl and left overnight at 4°C. After these acidic samples were spotted on activated thin-layer plates of Silica Gel H, separation was accomplished with Solvent System 2, i.e., chloroform:ethanol:acetic acid:water (119:4:5:12, v/v/v/v). The method for calculating \( C \times t \) is described by Coffey et al. (7).

RESULTS

The distribution of tritiated daunomycin has been investigated in normal rats and mice (5, 16). Tritium was taken up rapidly in tissues and was excreted slowly in urine and feces. In our studies, we have followed the physiological distribution of adriamycin and daunomycin in normal rats, mice, dogs, and hamsters and in rats and mice exposed to hypobaric atmospheric conditions.

Chart 1. Plasma levels of daunomycin and adriamycin equivalents in rats. Each rat received daunomycin or adriamycin, 10 mg/kg of body weight, i.v. Plasma was obtained by heart puncture from 5 to 6 anesthetized rats. Adriamycin and daunomycin equivalents were extracted by the acid-ethanol techniques of Bachur et al. (5) and were determined by fluorescence with an excitation wavelength of 490 nm and an emission wavelength of 581 nm.
The concentration of daunomycin and Adriamycin equivalents in plasma of rats decreased rapidly (Chart 1) and remained nearly constant at a low level after 20 min. Similar data for daunomycin have been described for mice (12), rats (16), and humans (1). In the dog, the level of daunomycin equivalents in plasma decreased rapidly and was similar to that seen in the rat (Chart 1). These data indicate a localization of drug, perhaps in tissues and/or excretory products.

The cumulative excretion of both daunomycin and Adriamycin in urine by normal rats (Chart 2) and by rats with cannulated bile ducts (Chart 3) was nearly equivalent. The extent to which Adriamycin and daunomycin were excreted by the biliary route in rats is also shown in Chart 3. The excretion of daunomycin equivalents in urine and bile was essentially the same for 12 hr and possibly until 24 hr. The secretion of Adriamycin into bile was slightly greater than its excretion into urine. In comparison to daunomycin, the excretion of Adriamycin equivalents via the 2 routes was about one-third that of daunomycin equivalents in both normal and bile duct-cannulated rats. However, urinary excretion rates of daunomycin and Adriamycin by normal mice were very similar, as indicated in Chart 2.

Feces of rats dosed with daunomycin contained little (<5% of administered dose) fluorescent material that was not characterized by chromatography. Rusconi et al. (16) showed that 30 to 40% of the administered radioactivity of tritiated daunomycin was excreted in feces within 4 days. The difference between these results suggested that the intestinal flora metabolized daunomycin to nonfluorescent products.

Excretion of daunomycin and its fluorescent metabolites in urine and bile by duct- and bladder-cannulated dogs was also determined. The total quantity of daunomycin equivalents was about 8 to 10% of the i.v. dose in 4-hr urine collection and was less than 1% in 4-hr bile collection. Bile flow was constant at about 0.02 ml/min over the 4 hr, whereas the urine flow rate dropped to zero for 10 to 20 min and then gradually increased to a maximum of 1 to 3 ml/min. The plasma clearance of inulin-14C by kidney stopped initially after administration of daunomycin, 10 mg/kg, but returned to normal within 20 to 90 min later (Chart 4). The plasma clearance of total equivalents of daunomycin reached its peak at the time inulin clearance returned to normal but declined soon thereafter (Chart 4). This decline was probably associated with a decrease in the amount of daunomycin available for clearance and a corresponding increase in Metabolite D2. The drug species in urine were differentiated into drug and metabolite by chromatography on thin layers of Silica Gel H with Solvent System 1 (Chart 5). Initially, daunomycin represented a large percentage of total urinary drug species, but later the major urinary drug species was Metabolite D2. A similar trend in decreased plasma clearance of daunomycin equivalent into bile was also found.

The fluorescent drug equivalents in urine and bile from rats have been fractionated by thin-layer chromatography with...

Chart 2. Urinary excretion of Adriamycin or daunomycin equivalents by rats and mice. Each animal received daunomycin or Adriamycin, 10 mg/kg of body weight, i.v. Adriamycin and daunomycin equivalents were extracted with acid-ethanol and were quantitated by fluorescence as stated in "Materials and Methods."

Chart 3. Biliary excretion of daunomycin and Adriamycin equivalents by bile duct-cannulated rats. Bile duct-cannulated rats received daunomycin or Adriamycin, 10 mg/kg of body weight, i.v. Daunomycin and Adriamycin in urine and bile from 4 of 5 bile duct-cannulated rats were determined by fluorescence.

Chart 4. Plasma clearance of inulin-14C and daunomycin equivalents by kidney of dogs that received daunomycin, 10 mg/kg of body weight, i.v. Bile duct-cannulated dogs, which were under Nembutal anesthesia, were given daunomycin, 10 mg/kg of body weight, i.v. Urine was collected via an indwelling catheter, and blood samples were collected by a 3-way indwelling valve in the right jugular vein. Inulin-14C concentrations were determined as stated in "Materials and Methods."

System 1 (Chart 5). Initially, daunomycin represented a large percentage of total urinary drug species, but later the major urinary drug species was Metabolite D2. A similar trend in decreased plasma clearance of daunomycin equivalent into bile was also found.

The fluorescent drug equivalents in urine and bile from rats have been fractionated by thin-layer chromatography with...
Solvent System 1 and were quantified by fluorescence spectrophotometry. Only 2 fluorescent species were present in urine and bile; i.e., daunomycin at R_F 0.5 and a major metabolite at R_F 0.4. This major metabolite appears to correspond to daunorubicinol, D_2, recently described by Bachur and Gee (4). The rate of excretion of daunomycin and of this major metabolite is represented in Chart 6. Daunomycin excretion into bile (Chart 6) and in urine was initially greater than that of the metabolite. At 2 hr, the metabolite represented more than 50% of daunomycin equivalents. By 24 hr, this metabolite was about two-thirds of the total excreted drug equivalents. A similar trend was seen for urine of both cannulated and normal rats.

Similar studies on the adriamycin equivalents from urine of rats showed no evidence of adriamycin metabolism; only a single fluorescent area was seen with an R_F of 0.4, which is similar to infused drug. After the silica gel was scraped off and eluted with acidic ethanol, 92 to 100% of the fluorescence corresponded to the visible R_F 0.4 spot. However, this area might still have contained an adriamycin metabolite. For evaluation of this possibility, the aglycone of this fluorescence material was prepared and chromatographed. Samples of urine from the adriamycin-treated rats were acid hydrolyzed and chromatographed on thin layers of silica gel with Solvent System 2. This system easily separates the aglycones of daunomycin, its metabolite D_2, and adriamycin (R_F's 0.45, 0.25, and 0.3 respectively). Only 1 fluorescent area at R_F 0.3 was observed, and it accounted for 94 to 100% of the fluorescent material eluted from the silica gel for the individual urine collections (0 to 3, 6 to 12, 12 to 24, and 24 to 48 hr). There was no pattern to suggest that a metabolite of adriamycin was excreted in urine, as was apparent for daunomycin.

Tissues were analyzed in order to determine the general distribution of daunomycin and adriamycin as well as to determine the extent of metabolism of these drugs. The distribution of adriamycin, daunomycin, and Metabolite D_2 in several tissues of rats is shown in Chart 7. Initially, the concentration of daunomycin was greater than metabolite, but the concentration of metabolite became greater than that of daunomycin within 3 to 6 hr. In all the tissues examined, the concentrations of adriamycin equivalents were greater than the concentrations of daunomycin and its metabolites. The efflux of adriamycin equivalents appeared more similar to that of metabolite than to that of daunomycin.

The calculated C X t of adriamycin equivalents in all 4 tissues of rats was several times that for daunomycin, its metabolite, and their combined total (Table 1). Kidney has the highest C X t of daunomycin, its metabolite, and adriamycin equivalents.

Tissue distributions of daunomycin and adriamycin equivalents in mice were similar to those in rats except for the very rapid decrease in daunomycin equivalents in mouse liver at the end of 24 hr (Chart 8). Daunomycin and its metabolite D_2 were the major drug species in all tissues of mice after an i.v. dose of daunomycin; the percentage of daunomycin was greatest at early times, whereas metabolite D_2 was the predominant species after 6 hr. No metabolite of adriamycin was detected in the tissues of any of these animals.

Assay of tissues from dogs revealed high concentrations of daunomycin equivalents in liver, kidney, and lung (Table 2). The daunomycin equivalents that were extracted from liver, kidney, and heart were differentiated into daunomycin and metabolite by Silica Gel H thin-layer chromatography. In all 3 tissues, daunomycin represented a small (< 20) percentage of extractable equivalent, the remainder being metabolite. These findings in dogs corroborate the extensive metabolism of
Table 2
Concentration of total daunomycin equivalents in tissues of dogs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>39</td>
</tr>
<tr>
<td>Liver</td>
<td>32</td>
</tr>
<tr>
<td>Heart</td>
<td>18</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>22</td>
</tr>
<tr>
<td>Lung</td>
<td>37</td>
</tr>
<tr>
<td>Spleen</td>
<td>22</td>
</tr>
<tr>
<td>Muscle</td>
<td>6</td>
</tr>
<tr>
<td>Adipose</td>
<td>5</td>
</tr>
<tr>
<td>Brain</td>
<td>1</td>
</tr>
</tbody>
</table>

* Each value is the mean representing 2 dogs.

Daunomycin and its metabolite D2. In our studies, we found an additional aglycone, D2, which was chromatographically different from the aglycones of daunomycin and its metabolite D2. In Solvent System 2, D2 has an RF of

approximately 0.35, compared to 0.45 and 0.25 for the aglycones of daunomycin and its metabolite D₂, respectively. Further, we have noted that tissues excised from daunomycin-dosed rats and allowed to stand at room temperature converted daunomycin and its metabolite D₂ to the aglycone, D₃, in situ.

Mhatre et al. (15) found an aglycone in tissues of hamster dosed with daunomycin and noted that the chemically prepared aglycone of daunomycin produced cardiac toxicity in hamsters and dogs. We have undertaken a preliminary study of the physiological disposition of daunomycin and adriamycin in hamsters and have observed that aglycone-like compounds of adriamycin and daunomycin, as well as the normal metabolite, D₂, of daunomycin, were present in liver. Four hr after administration of daunomycin, hamster livers contained 20 to 30% D₃ and 30 to 40% D₂. The remainder of the daunomycin equivalent was present as daunomycin D₁. Similar studies with adriamycin revealed that, after 4 hr, the liver contained 20 to 30% of an aglycone of adriamycin plus 70 to 80% adriamycin. Chromatographically, the aglycone-like compound found in tissues from adriamycin-treated animals has an R₉ of 0.15, which is less than that for the aglycone of adriamycin (0.35) in Solvent System 2. Based on our chromatographic data in Solvent System 2, we concluded that the aglycone-like material in tissues from daunomycin-treated hamsters corresponded in R₉ to the new aglycone, which was found to be produced by tissue homogenates under anaerobic conditions.

We also investigated whether this new aglycone metabolite, D₄, of daunomycin could be produced in rats and mice that were exposed to hypobaric atmospheric conditions. We presumed that such exposures in vivo might simulate the hypoxic conditions in vitro under which the D₃ metabolite was produced. Rats and mice were exposed for 24 hr at hypobaric conditions comparable to 12,000 ft altitude. These animals were dosed with daunomycin at atmospheric conditions, and the hypobaric conditions were then restored for 6 hr. At that time, tissues were excised, immediately frozen, and handled in a manner such that we would normally expect to find no drug species other than daunomycin or its metabolite D₂. In extracts of liver and kidney, we found 5 to 10% of the new aglycone metabolite, D₄, of daunomycin, in addition to 50 to 60% D₂ and 30 to 40% D₁.

DISCUSSION

The data presented in this study demonstrate that administered daunomycin was rapidly cleared from plasma, deposited in tissue, extensively metabolized, and slowly excreted in rats, mice, and dogs. Biliary excretion of drug equivalents was extensive and equivalent to urinary excretion in rats but much less than urinary excretion in dogs. The data on physiological distribution of total daunomycin equivalents in rats, mice, and dogs were similar and are in agreement with the data reported by Rusconi et al. (16), Finkel et al. (12), and Bachur et al. (5).

Administered adriamycin, like daunomycin, was rapidly cleared from plasma, deposited in tissue, and slowly excreted into urine and bile in rats and mice. Although daunomycin was extensively metabolized in all animals studied, no evidence was obtained for the metabolism of adriamycin except in hamsters. The calculated C X t for adriamycin equivalents in all rat tissues (liver, kidney, heart, and small intestine) was several times greater than that found for either daunomycin or its metabolite. This longer retention of adriamycin equivalents may correlate with the increased therapeutic index and with the findings that intermittent therapy is as effective as continuous daily treatment with adriamycin.

The rapid deposition of daunomycin, its extensive metabolism by most tissues, and the persistence of both daunomycin and its metabolite, D₂, in tissues may be related to onset of toxicological symptoms. Daunomycin was converted to a single metabolite, D₃, in the tissues of normal rats, mice, and dogs. Chemotherapeutically, the metabolite, D₂, was equivalent to daunomycin against P388 cells growing in mice. Under aerobic conditions, cell-free homogenates of the tissues of these animals metabolized daunomycin to D₂ (3, 4). However, anaerobically, these same tissue homogenates converted daunomycin to an aglycone-like metabolite, D₄, the chromatographic characteristics of which are different from those of the aglycones of daunomycin and D₂. Rats under hypobaric stress metabolized daunomycin to D₄, and hamsters produced small amounts of D₄ under normal conditions. Herman et al. (13) noted that hamsters, in contrast to rats, were very susceptible to daunomycin-induced cardiac toxicity. They further found that hamsters converted daunomycin to an aglycone-like compound. In addition, hamsters, which extensively metabolize adriamycin to its aglycone, may also be susceptible to adriamycin-induced cardiac toxicity. Using isolated dog hearts, Mhatre et al. (15) showed that an increase in coronary perfusion pressure occurred simultaneously with the conversion of daunomycin to an aglycone metabolite.

Having found this aglycone metabolite, D₄, in tissues of hamsters, rats, and mice exposed to hypobaric conditions in vivo, we suggest that production of D₄ in situ may be associated with the cardiac toxicity seen in hamster and reported for man. Further, one might expect to find more of this metabolite in seriously ill patients whose tissues may be poorly oxygenated.

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