Uptake and Retention of Daunomycin by Mouse Leukemic Cells as Factors in Drug Response¹

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Summary

Survival of mice bearing different transplantable tumors and treated with Daunomycin was compared with the capacity of the tumor cells for uptake of the drug in vitro and for drug uptake and retention in vivo. The data obtained indicate that the ability of these cells to retain Daunomycin in vivo was a determinant of drug response.

Introduction

The antibiotic Daunomycin was shown to have antitumor activity against animal (4, 5, 21) and human (7, 20) neoplasms. Daunomycin is apparently identical to Rubidomycin, which also inhibits growth of certain animal (8) and human (1) malignancies. Daunomycin binds to DNA (13, 14, 19), leading to inhibition of RNA synthesis; this inhibition could be shown both in vivo (3, 6) and in vitro (3, 6, 12-14, 22). Unlike actinomycin D, Daunomycin apparently binds to both DNA strands (3); the two drugs bind to different sites on DNA (18).

In the present study, transport of Daunomycin by animal leukemia cells was studied to seek determinants of drug response. Animals bearing tumors varying widely in sensitivity to Daunomycin were treated with the drug in vivo, and drug levels in the tumor cells were measured at intervals following drug administration. Uptake of the drug in vitro was also studied.

Materials and Methods

Tumor Cells. Animals were inoculated intraperitoneally with \(10^6\) tumor cells \((L1210 = 10^6)\) and were used 2 days before death from the tumor was expected. Sources and methods of propagation of tumor lines have been described (17). P815/VLB is a subline of P815 selected for resistance to Vinblastine. P388/38280 and P388/57155 are sublines of P388 selected for resistance to terephthalanilide derivatives. Survival data were obtained by administration of Daunomycin at 1 mg/kg/day \((1L1210 = 3 \text{ mg/kg/day})\) to animals from Day 1 to Day 9 following inoculation with tumor cells.

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**Factors in Daunomycin Response**

Chart 1. The effect of incubation temperature on the time course of uptake of Daunomycin in vitro by P388 cells from medium containing 70 μg/ml of the drug. The data shown represent the mean of eight experiments together with the standard deviation of the determinations, at specified temperatures using P388 cells.

Chart 2. Uptake of Daunomycin by tumor cells in vivo. Animals were treated with 1 mg/kg of the drug by intraperitoneal injection, and samples of cells were removed at intervals thereafter for determination of cellular drug levels. Ranges are shown for data obtained from triplicate experiments.

**RESULTS**

**Extraction of Daunomycin from Tumor Cells.** After cells had taken up the drug, either in vivo or in vitro, intracellular drug could not be extracted by 0.9% NaCl, 0.25 M sucrose, 0.01 M acetic acid or water, at 0–60°C. Formamide removed Daunomycin from cells quantitatively; the drug was not extracted by ethanol, ethyl acetate, n-butanol, or methanol. The data described here were first obtained with nonlabeled Daunomycin and were later verified with tritiated drug when this became available.

**Uptake of Daunomycin in Vitro.** Daunomycin uptake in vitro was similar in cell lines which differed widely in drug sensitivity in vivo. The data of Chart 1 were obtained using P388 cells, but significant differences were not found when P388/38280, P388/57155, P815/VLB, or L1210 cells were substituted. Uptake of Daunomycin was temperature-sensitive and apparently concentrative. Although the drug level used for the data of Chart 1 was 70 μg/ml, the temperature-sensitivity of uptake and the distribution ratios achieved were not altered by the use of drug concentrations ranging from 10 to 100 μg/ml.

**Uptake and Retention of Daunomycin in Vivo.** The cellular level of Daunomycin reached a maximum within 30 minutes after injection of the drug; after 30 minutes (the shortest time point measured), the cellular drug levels fell (Chart 2). The initial concentration of drug attained in the ascitic fluid was 8–12 μg/ml; this was in part dependent on the volume of ascitic fluid present. Loss of Daunomycin from L1210, P815/ VLB, and P388/38280 cells in vivo was rapid and, within 24 hours, was complete in mice bearing either of the latter two cell lines. Loss of drug from P388 and P388/57155 cells appeared to be much slower; a significant amount of drug remained in the tumor cells after 24 hours.

**Survival Data.** The data obtained, in terms of the percentage increase in mean survival time over untreated controls were the following: P388, 127; P388/57155, 100; L1210, 57; P815/ VLB, 0; P388/38280, 10.
DISCUSSION

The data obtained indicate that uptake of Daunomycin by mouse leukemia cells in vivo or in vitro was rapid, with equilibrium apparently attained within 30 minutes after drug administration. In contrast, uptake of actinomycin D in vivo continued for several hours (16). Daunomycin uptake in vitro was temperature sensitive and, at 37°C, was complete within 10–20 minutes. The uptake process, as previously reported (3), was apparently not saturable. There is no persuasive evidence to suggest barriers to Daunomycin uptake in drug-resistant cell lines. Uptake of the drug in vitro was similar in extent in all cell lines examined, and the amount of drug initially accumulated in vivo was not a predictive index of drug response. Differences in extent of uptake observed in vivo (Chart 2) may, in part, reflect minor differences in volume of ascitic fluid peculiar to each cell line.

The rate of loss of Daunomycin from previously loaded cells in vivo varied considerably and appeared related to drug response. The striking capacity of P388 cells to retain Daunomycin may explain the observation that survival of animals bearing this tumor was related to the total cumulative administered drug dose; this was also true for animals bearing the L1210 tumor (I. Wodinsky, unpublished data).

The data of Chart 2 suggest that more frequent administration of Daunomycin to animals bearing the L1210 leukemia might lead to an improved therapeutic response. Preliminary studies have not supported this hypothesis (I. Wodinsky, unpublished data).

It is noteworthy that cross-resistance between Daunomycin, a terephthalanilide (NSC 38280) and Vinblastine was found in the present study. Cross-resistance between terephthalanilides, Vinca Alkaloids (2), and actinomycin D (15) had previously been shown suggesting common modes of resistance. In the case of actinomycin D, drug bound to DNA of Ehrlich ascites tumor cells was detached during subsequent DNA synthesis in vivo (11). A similar phenomenon might account for the gradual loss of Daunomycin from tumor cells in vivo. DNA-dependent RNA synthesis in inhibited by Daunomycin (9, 12, 22), but DNA synthesis is also inhibited at high drug levels (3, 6, 12). The rapid loss of Daunomycin from drug-resistant tumor lines might indicate that DNA synthesis was not inhibited by the drug. Alternatively, Daunomycin-resistant cells may have the selective ability to eliminate the drug; such a phenomenon has been implicated in resistance to terephthalanilides in animal tumor cells (23).

Very recent investigations (D. Kessel, unpublished data) have indicated that barriers to drug-DNA interactions may be responsible for resistance to Daunomycin. Daunomycinone, the aglycone of Daunomycin, binds only weakly to DNA (3). Like Daunomycinone, the aglycone was rapidly taken up by tumor cells in vitro and in vivo. But subsequent loss of Daunomycinone in vivo was rapid; all of a 1-mg/kg dose was lost within 4 hours after injection of Daunomycinone into animals bearing the P388, P388/57155, or P388/38280 tumors. The essential differences between Daunomycinone and Daunomycin is presumably the ability of the latter compound to bind to DNA. Rapid loss of Daunomycin from drug-resistant lines might, therefore, be based on barriers to drug-DNA interaction.
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