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CROSS RESISTANCE BETWEEN ACTINOMYCIN-D, ADRIAMYCIN AND VINCRISTINE IN A MURINE SOLID TUMOUR IN VIVO

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Abstract

In experimental tumour systems, patterns of cross-resistance to the cytotoxic drugs of the anthracycline group, the vinca alkaloids and actinomycin-D have been well documented, (1 - 3). Early work suggested that resistance to these agents resulted from failure of drug uptake (4 - 6), and in addition changes in membrane glycoprotein content have been identified as important factors underlying this decrease in membrane permeability in resistant tumour cells (7). However, recent evidence from in vitro experiments suggests that a common mechanism of enhanced active efflux of these drugs, rather than of impaired influx, may underly the development of cellular drug resistance (8, 9).

Previous in vivo studies of cross-resistance have been performed using murine tumours in the ascites form, either sublines of P 388 leukaemia (3, 10) or of the Ehrlich ascites tumour (2). In this study, resistance to actinomycin-D was developed in vivo in a solid tumour, the Ridgway osteogenic sarcoma (ROS) in AKR mice, and patterns of cross resistance to adriamycin, vincristine and cyclophosphamide were then examined. In addition a preliminary investigation of in vivo drug uptake and retention in sensitive and resistant ROS tumour sublines has been performed, by estimating concentrations of radiolabelled actinomycin-D and vincristine in tumour digests at intervals following injection.

Materials and Methods

Actinomycin-D, adriamycin, vincristine and cyclophosphamide were purchased respectively from Merck, Sharp and Dohme Ltd., Pharmitalia Ltd., Eli Lilly Ltd. and W.B. Pharmaceuticals Ltd. Tritiated actinomycin-D (specific activity 11 - 16 μCi/μg) and tritiated vincristine (specific activity 3.5 μCi/μg) were obtained from Radiochemicals, Amersham. The ROS tumour, originally supplied by Dr. F.M. Schabel, Jr. of the Southern Research Institute, Birmingham, Alabama, U.S.A. was propagated by serial subcutaneous passage in inbred AKR mice. Untreated 1 gm tumours grew with an overall doubling time of approximately 3 days to 10-15 gm in weight in 10-14 days. Tumour response to chemotherapy was assessed at the point of maximal tumour regression (10-14 days after treatment) by caliper measurements, from which an estimate of tumour weight was derived. The lower limit of palpation was 60 mg, although reproducible measurements were not possible on tumours with an estimated weight of less than 100 mg. The parent ROS tumour is exquisitely sensitive to actinomycin-D (11), a dose of 0.5 μg/gm consistently causing complete regression of tumours up to 2 gm in weight. Resistance to actinomycin-D was derived by suboptimal treatment (0.25 μg/gm q 4 W x 4) followed by repeated passage of tumours, treated at gradually increasing doses. After 7 transplant generations tumours continued to grow despite treatment with 0.8 μg/gm of actinomycin-D, which is the LD_{50} of the drug, established in separate studies. This resistant tumour subline was designated ROS/ADX and has been maintained using tumours treated with 0.5 μg/gm of actinomycin-D prior to passage. No differences in histological appearance, mass doubling time or tumourogenicity (take-rate) were apparent between the ROS/ADX and the parent ROS tumours.

Logarithmically-spaced doses of actinomycin-D were injected intra-venously (via the tail vein) into groups of 6 mice bearing ROS and ROS/ADX tumours, 1 to 1.5 gm in weight, and response was assessed 10 days after treatment. In the same way response to adriamycin and cyclophosphamide was measured, 10 days and 14 days respectively after single dose intravenous treatment of ROS and ROS/ADX tumours of equivalent size (1 to 1.5 gm). For vincristine a q 3 d x 3 schedule was given intravenously, and response measured 14 days after the last injection.

For the drug disposition studies, a dose of 0.5 μg/gm of actinomycin-D (containing 0.1 μCi per μg of ^{3}H-actinomycin-D) or 1 μg/gm of vincristine (containing 0.025 μCi per μg of ^{3}H-vincristine) was injected intravenously into groups of 5-6 mice bearing ROS or ROS/ADX tumours 1 - 2 gm in weight. Plasma samples were obtained 3 to 72 hours later by retro-orbital puncture, animals were then killed and tumours were removed, cleared of all necrotic and haemorrhagic debris, weighed and digested overnight at room temperature in 4 volumes of 33% KOH. In each case, 0.1 ml plasma samples were also digested with equal volumes of 33% KOH. Aliquots (0.2 ml) were then neutralized with 1.0 ml of 1.5 M HCl, 10 mls of scintillation fluid was added and radioactivity measured on a Packard tricarb liquid scintillation counter (Model 3390) with external standardization, after allowing 48 hours for decay of chemiluminescence. An estimate of tumour blood content (1.2 $\pm$ 0.2% per gm (S.E.M. of 5 mice)) was made separately using ^{51}Cr-labelled red cells, and a correction was thus possible of each estimate of tumour drug concentration for drug present in blood in the tumour, using the measurement of radioactivity in plasma samples taken at the time of tumour removal.

Results and Discussion

The response of ROS and ROS/ADX tumours to each of the 4 drugs is illustrated in Figure 1.

Doses (not shown) of 1 μg/gm of actinomycin-D, 40 μg/gm of adriamycin and 2 μg/gm of vincristine resulted in 83-100% animal mortality. For all these 3 drugs it is evident that while the maximal tolerated doses produce virtually complete regression of ROS tumours, there is a clear lack of response of the ROS/ADX tumours. Treatment with 250 μg/gm of cyclophosphamide also results in complete regression of ROS tumours, but with this dose, treatment of ROS/ADX tumours results in inhibition of tumour growth to 5% of untreated control weight, indicating that sensitivity to cyclophosphamide has to a considerable extent been retained. A similar pattern of cross-resistance to actinomycin-D, adriamycin and vincristine, but not cyclophosphamide, was also noted in previous studies using resistant sublines of P 388 leukaemia (3).

Tumour drug concentrations following injection of ^{3}H-actinomycin-D and ^{3}H-vincristine are shown in Figure 2.

In both cases it is apparent that the initial (3 hour) drug levels are similar in ROS and ROS/ADX tumours, the major point of difference, particularly with respect to actinomycin-D, being the more rapid fall of drug concentrations in ROS/ADX tumours than in ROS tumours from 3 to 72 hours after treatment. These data support the suggestion of Johnson et al (3), who proposed that the uptake of those drugs expressing a common pattern of cross-resistance may not be markedly impaired in resistant tumours, at least when drug resistance was derived *in vivo* (12). This group have emphasized the importance of active drug efflux from resistant cells, and have noted that previous studies which did report reduced drug uptake (of actinomycin-D and the anthracyclines), were mainly performed on resistant cells in which drug resistance was derived *in vitro* by continuous drug exposure (4, 5). Since resistance acquired *in vivo*, as in the present study, may relate more closely

to the circumstances seen clinically, it is conceivable that failure of drug retention may be the major problem to which new therapeutic strategies should be addressed.

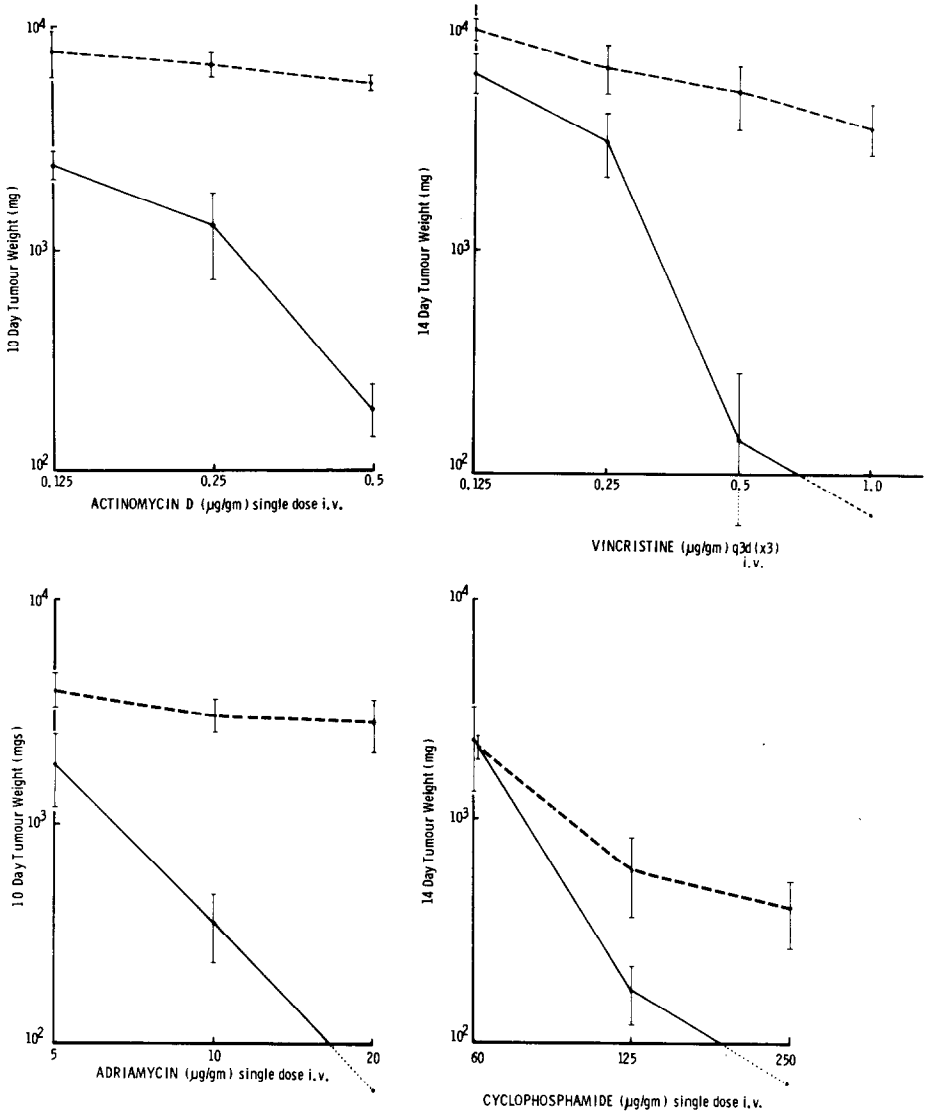

Fig. 1 Dose response curves for 4 cytotoxic drugs, against ROS (_____) and ROS/ADX (-----) tumours. Mean tumour weights with standard error (6 per group) are shown. Untreated mean tumour weights of 6 mice, at 10 days were 1.2 (± 0.1) x 10^4 mg for both ROS and ROS/ADX sublines.

A previous study in vivo of drug disposition in solid murine tumours has indicated that both uptake and retention (of actinomycin-D) may be decreased in resistant tumours (13). In common with the present study, however, these data were obtained from whole tumour samples which were likely to be heterogenous in nature and which were subject to the variations in tumour cell drug accessibility which are associated with the chemotherapy of solid tumours. With respect to the ROS and ROS/ADX tumours, further studies are therefore planned using isolated tumour cells in an attempt to define precisely the biochemical nature of the events which lead to the development of cross-resistance.

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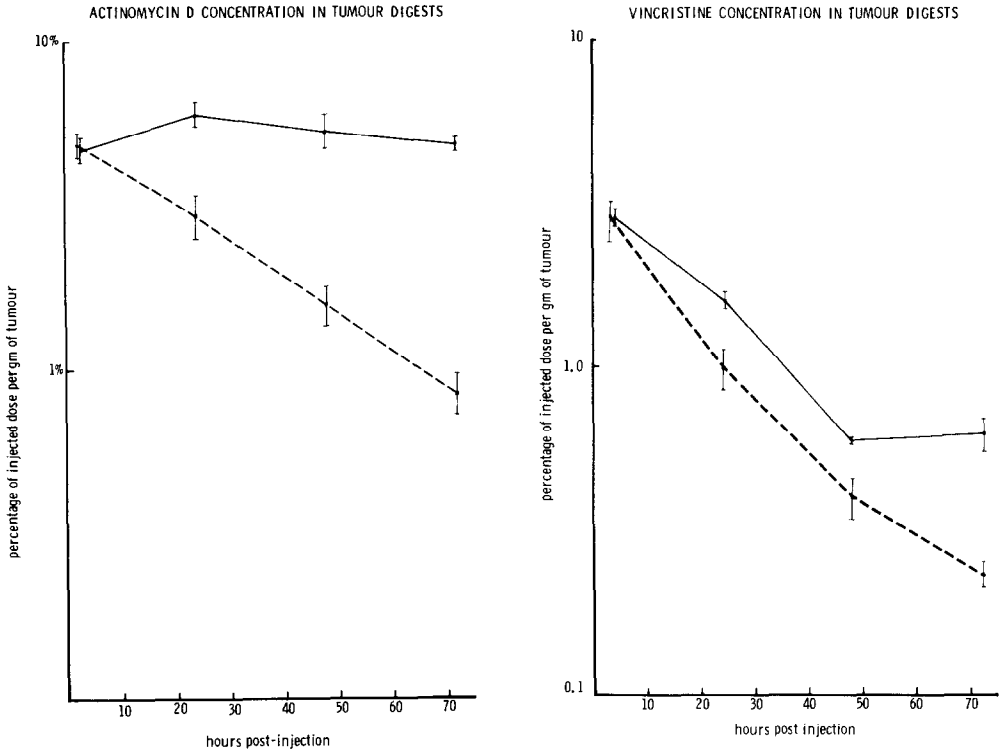

Fig. 2 Concentrations of ^{3}H-actinomycin-D and ^{3}H-vincristine in ROS (_____) and ROS/ADX (-----) tumours from 3 to 72 hours after i.v. injection. All results corrected for tumour blood content, standard error shown.

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