Inhibition of protein synthesis, pulmonary localization and pulmonary tumour formation by drug-treated tumour cells as a means of predicting their chemosensitivity

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Mouse mammary carcinoma cells were exposed in vitro to increasing concentrations of doxorubicin hydrochloride (adriamycin (ADR)) or 5-fluorouracil (5-FU). Uptake of [75Se]selenomethionine ([75SeM]) in a methionine-deficient medium measured the resulting inhibition of protein synthesis by the tumour cells. This was compared with the ability of the [75SeM] labelled tumour cells to localize in mouse lungs and to form pulmonary tumours following intravenous (i.v.) injection into isogenic hosts. These parameters were also related to the ability of the drugs to inhibit pulmonary tumour formation in vivo when injected into mice which had received tumour cells i.v. Results from five different tumours were pooled for analysis. At the highest drug concentration (10μg/ml ADR, 100μg/ml 5-FU) inhibition of protein synthesis was significantly related to the in vivo action of the drugs in limiting formation of pulmonary tumours (P<0.02 using the rank difference coefficient). There was also a direct relationship between pulmonary localization of tumour cells following exposure to drugs, their ability to form tumour nodules (P<0.025) and the in vivo action of the drugs in inhibiting tumour formation (P<0.05). Thus inhibition of protein synthesis in vitro and pulmonary localization following i.v. injection may be of value in predicting the in vivo effect of cytotoxic drugs.

Introduction

Tests to predict the sensitivity of tumours to cytotoxic drugs are of several types. Clonogenic assays compare the ability of tumour cells to form colonies in soft agar with or without drug treatment [7]. Non-clonogenic assays measure the incorporation of radioisotopes [19], the exclusion of a vital stain, for example, Fast Green [20], or the ability to form spheroids [10, 15] as an indication of tumour cell viability. Isotope incorporation assays are either short-term, when the inhibition of specific biochemical processes is assessed within a few hours of tumour removal [19], or long-term, when cell proliferation is determined after tissue culture for from 2 to several days [9, 13, 16]. A disadvantage of each technique is the additional uptake of isotope by non-tumour cells present in the cell suspensions made from solid tumours. The tumour cells in the experiments described in this paper were obtained by using a new density gradient centrifugation medium, Nycodenz [17], which allows relatively pure suspensions of tumour cells to be separated from mixed cell populations [5].
Materials and methods

General plan of the experiments

Suspensions of mouse mammary tumour cells separated on a Nycodenz column were exposed in vitro to increasing concentrations of ADR or 5-FU. They were then cultured in methionine-free medium, with $^{75}$Se)selenomethionine ($^{75}$SeM) added, to measure protein synthesis, in terms of isotope uptake. Groups of mice received an intravenous (i.v.) injection of cells from the same tumours. Seven days later these animals received either doxorubicin hydrochloride [adriamycin (ADR)] 5-fluorouracil (5-FU) or no treatment. After a further 7 days the mice were killed and the number of lung tumours was counted. Additional aliquots of the tumour cells were exposed in vitro to drugs and then labelled with $^{75}$SeM. They were injected i.v. into further isogenic mice. Some mice were killed after 1.5 h and the number of tumour cells in the lungs was calculated from the uptake of radioactivity. Other mice from the same group were killed 21 days later and the numbers of experimental pulmonary tumours were counted. A relationship was sought between drug inhibition of protein synthesis, the ability of the drugs to inhibit localization of the tumour cells in the lungs following i.v. injection and the anti-tumour action of drugs given in vivo. Comparison was also made between the inhibition of pulmonary tumour cell localization following exposure to drugs and the ability of the injected tumour cells to form lung tumours.

Tumours and mice

Five mouse mammary carcinomas were studied. One arose spontaneously in an (A x CBA)$F_1$ female mouse ($F_1$) and was in its 45–50th passage in isogenic hosts. The other four tumours ($A_1$, $A_2$, $A_3$ and $A_4$), arose in A-strain female mice and were in their 1–15th passage when used. Mice of the same sex, isogenic with the tumours, were used in a given experiment.

Preparation of tumour cell suspensions

Fragments of macroscopically viable tumour were minced with scissors and enzyme disaggregated in 50 ml of Medium 199 (Gibco) containing 2 mg/ml of collagenase (Type II) and 0.2 mg/ml of DNase (Sigma) at 37°C for 1 h with continuous stirring [8]. The tumour suspension was filtered through 100-mesh stainless-steel gauze and then centrifuged at 200 g for 5 min. The pellet was resuspended in 5–8 ml of Medium 199, depending on the size of tumour used. A four-step column was prepared using 3 ml of undiluted Nycodenz (27.6 per cent, w/v) overlaid by 3 ml aliquots of Nycodenz diluted 2 : 1, 1 : 1 and 1 : 2 with a balanced salt solution [17]. The column was tilted at 45° for 45 min to produce a linear density gradient. A 1 ml volume of the cell suspension was layered on to the top of the column. Following centrifugation at 1500 g for 45 min at room temperature, tumour cells in relatively pure suspension separated as the top cell band on the column. The top cell band was removed, washed twice (170 g for 5 min) and resuspended in Medium 199. The number of viable cells present was counted 5 min after 1 : 20 dilution with 0.17 per cent (w/v) trypan blue, using a haemocytometer. The viable cells are those excluding dye [14]. Cytospin preparations were made from the separated tumour cells and stained with May–Grunwald Giemsa stain.