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Detection of Induced Resistance to Cytosine-Arabinoside with a Short-Term Test

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Abstract—*By treatment of the mouse leukemia L* 1210 *with cytosine arabinoside (ara-C;* 120 mg/kg *per* week) *a tumour cell line was developed which was resistant to this cytostatic agent. The sensitive and resistant tumour cell line could be distinguished and the temporal development of resistance followed using an* in vitro *short-term test.*

INTRODUCTION

WHEREAS some tumours show proliferation-dependent resistance to chemotherapy, others develop resistance only after treatment with cytostatics. In order to determine whether acquired resistance is also detectable by means of a short-term test, an ara-C-resistant tumour cell line was developed from mouse leukemia L 1210 cells. Preliminary results with adriamycin have indicated that the development of secondary resistance is detectable with the short-term test [1]. If this finding is confirmed for other substances, it would be possible to carry out resistance tests during the course of treatment with cytostatics (e.g., of leukemias) with a view to identifying and eliminating those drugs which have become inactive.

MATERIALS AND METHODS

Animals

Female NMRI mice (from Ivanovas, Kißlegg, F.R.G.) weighing between 20 and 25 g were maintained under standard conditions: 21°C room temperature, 65% humidity, macrolon cages, water and 'Altromin' standard diet (Altrogge, Lage, F.R.G.) *ad libitum.*

Tumours

The leukemia L 1210 cells were grown in ascites form and transplanted at 7 day intervals (1.5×10^7 cells/mouse).

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Cytostatics

Actinomycin D (Lyovac–Cosmegen, Sharp and Dohme, München, F.R.G.), adriamycin (Adriblastin, Montedison Farmaceutica, Freiburg i.Br., F.R.G.), amethopterin (Methotrexat, Lederle, Müchen, F.R.G.), cytosinarabinoside (Alexan, Mack, Illertissen, F.R.G.), daunomycin (Daunoblastin, Montedison Farmaceutica, Freiburg i.Br., F.R.G.), 5-fluorouracil (5-fluorouracil, Hoffmann-La Roche, Grenzach, F.R.G.).

Radioactive substances

(Methyl-^{3}H)-thymidine (spec. act. 20 Ci/mmole, NEN), (G-^{3}H)-uridine (spec. act. 11 Ci/mmole, Amersham–Buchler), deoxy-(^{3}H)-uridine (spec. act. 18 Ci/mmole, Amersham–Buchler).

Development of a resistant tumour cell line

Ara-C was applied i.p. at a concentration of 120 mg/kg weekly. This pretreatment was carried out for 35 weeks. The animals were always treated on the 4th and 5th days after tumour transplantation.

Determination of tumour cell count

The animals were killed, the ascites were completely removed by puncture and aliquots counted using a Coulter Counter (Coulter Electronics, Harpenden, England).

Short-term test

The short-term test was carried out as previously described [1].

Statistical analysis

For the detection of differences between the drugs, the Kruskal–Wallis test with multiple comparisons according to Dunn was applied to the results of animal experiments and the Friedmann test to the results of short-term tests.

RESULTS

Detection of a cytosine-arabinoside (ara-C)-resistant tumour cell line in vivo

Ara-C shows a clear inhibitory effect on the original tumour (Fig. 1). Similar therapy carried out with the ara-C-pretreated tumour cell line did not lead to any reduction in cell count. This means that the pretreated tumour had developed resistance to ara-C.

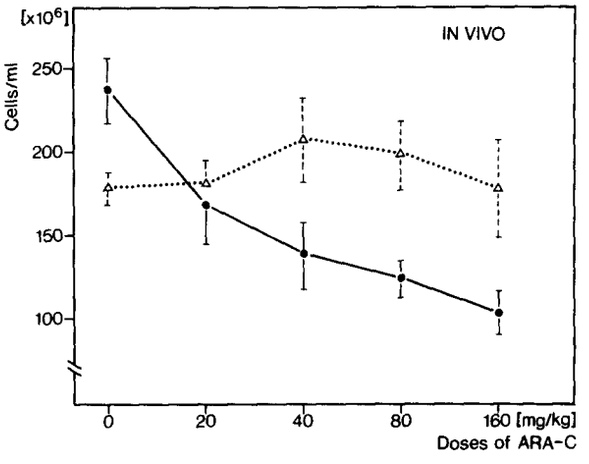

*Fig. 1. Effect of ara-C on untreated (sensitive) and ara-C-pretreated (resistant) cell lines of the leukemia L 1210. Abscissa: different dose level of ara-C (total doses are given). Ordinate: number of cells/*ml *(average values with confidence intervals from 12 animals). Treatment was on the 4th and 5th days, determination of cell counts on the 7th day after tumour transplantation. (●——●) Sensitive original cell line; (△ . . . . △) ara-C-pretreated tumour line (21st passage). n = 120 animals.*

Detection of resistance to ara-C in the short-term test

Short-term *in vitro* tests to detect resistance were carried out in parallel with the *in vivo* experiments. Tumour cells from the sensitive or resistant cell lines were incubated with different concentrations of ara-C ranging from 0.003 to 300 μg/ml. As can be seen from Fig. 2, the ara-C-resistant cells can be distinguished from the sensitive cells. No effect is observed on tumour cells of the resistant line at the three lowest dose levels used (0.003, 0.03, 0.3 μg/ml). At a concentration of 3 μg/ml, however, the incorporation rate of ^{3}H-thymidine falls to 80% of the control

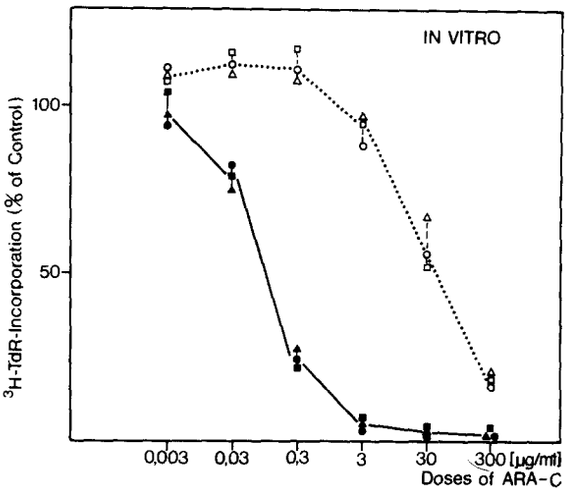

Fig. 2. Dose–response curve for ara-C with untreated (sensitive) and ara-C-pretreated (resistant) cell lines of the leukemia L 1210 in the short-term test (3 experiments). Abscissa: different dose levels of ara-C. Ordinate: incorporation of ^{3}H-*TdR in percentage of control values. Closed symbols: sensitive original tumour cell line. Open symbols: ara-C-resistant tumour cell line. Each point represents the average from 6 values obtained by taking 2 samples from each of 3 incubations.*

value. On the other hand, in the sensitive tumour cell line, ^{3}H-thymidine incorporation is reduced to 75% of the control level by a concentration of only 0.03 μg ara-C. A concentration of 3 μg ara-C/ml led to the complete suppression of ^{3}H-thymidine incorporation.

Development of resistance to ara-C

An attempt was made to follow the development of resistance with time by means of the short-term test. During the experiment to induce ara-C resistance, cells of the original tumour and of the ara-C-pretreated line were incubated *in vitro* in short-term tests as described in Fig. 2. The results of this investigation for *two in vitro* dose levels (0.3 μg and 3 μg ara-C/ml) are shown in Fig. 3. Thymidine incorporation in both untreated and pretreated tumour cells is inhibited by these concentrations of ara-C until the ninth passage. Thymidine incorporation subsequently increases in the pretreated tumour cells until by the 15th passage they show no further reaction to treatment with 0.3 μg and 3 μg/ml ara-C. It is therefore possible, using the short-term test, to follow the development of resistance to cytostatics.

Detection of cross-resistance in the short-term test

An investigation was carried out of the effects of the antibiotics actinomycin D, adriamycin and daunomycin and also of the

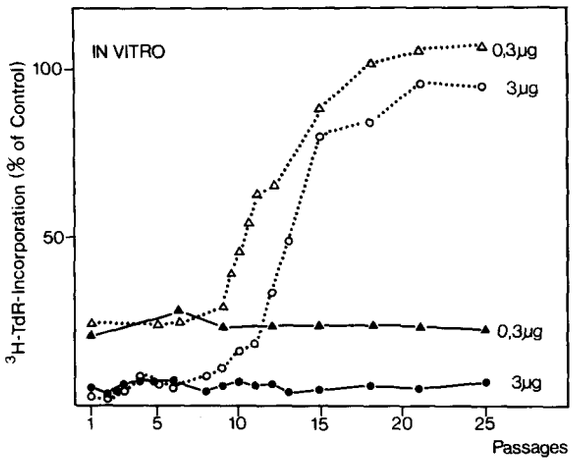

Fig. 3. Effect of ara-C for two dose levels in the short-term test on untreated (sensitive) (●——●, ▲——▲) and ara-C-pretreated (resistant) (○ — — — — ○, △ - - - - - △) cell lines of the leukemia L 1210 at different passages. Concentration of ara-C in vitro: 0.3 µg *and* 3 µg/ml. *Abscissa: number of passages. Ordinate: incorporation of* ^{3}H-*TdR in percentage of control values (control = 100%). Each point represents the average from 6 values obtained by taking 2 samples from each of 3 incubations.*

antimetabolites 5-fluoroucil and methotrexate on ara-C-sensitive and resistant tumour cells between the 26th and 30th passages after starting to develop resistance. The results show that resistance is exclusively against ara-C. For all other drugs investigated, the response in the short-term test was the same with both ara-C-resistant and -sensitive tumour cell lines (unpublished results). In order to check the dependability of resistance detection using the short-term test, a corresponding series of animal experiments was then carried out. The dose-dependent response to the different cytostatics was also the same for both cell lines when tested *in vivo*.

DISCUSSION

The resistance of tumours to cytostatic agents still remains an unsolved problem in the treatment of cancer patients by chemotherapy. It would be extremely useful to know in advance whether a particular tumour is likely to respond to therapy.

For these reasons, various groups have developed test systems designed to measure the sensitivity or the resistance of tumours to cytostatic agents [2–6]. Since such test systems are often too complicated for routine clinical use, a simple, short-term *in vitro* test has been developed in which the effects of the cytostatic on nucleic acid synthesis using radioactive precursors are measured. In earlier studies it was shown that this short-term test can be used to determine the proliferation rate of tumours on which the activity of cytostatic agents is largely dependent. For both animal and human tumours a good correlation has been observed between test results and the success of therapy [1, 7].

In order to determine whether induced resistance was also detectable, an ara-C-resistant tumour cell line was developed. The resistance which was demonstrated *in vivo* could also be detected *in vitro* by the short-term test. The development of resistance in leukemia L 1210 cells with duration of treatment was followed using the short-term test. The ara-C-resistant tumour cell line did not show resistance to any other drugs tested, *in vivo* and *in vitro*, in agreement with results presented by other authors [8, 9].

The essential criterion for a useful pretherapeutic test for tumour resistance, namely a good correlation between the results obtained *in vitro* and *in vivo*, was fulfilled in all experiments.

The development of secondary resistance can be caused by changes in the proliferative state of tumours, i.e., by a decrease in the growth fraction. Some other mechanisms must be responsible for the development of resistance by the L 1210 leukemia cells, however, since the sensitive and resistant tumour cell line grow at the same rate. The possible influence of changes in the host organism can also be excluded, since the tumour cells were transplanted weekly into other animals. The mechanisms by which resistance develops must therefore lie within the tumour cells [9, 10]. It can be concluded that the short-term test is therefore suitable for detecting induced biochemical resistance to ara-C in animal tumours.

Whether this *in vitro* short-term test is also applicable for the detection of induced resistance in human tumours or whether a purification of the cell populations is necessary prior to testing as for example in the clonogenic assays [3], must be cleared up in further studies.

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