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Species-specific differences in the toxicity of puromycin towards cultured human and Chinese hamster cells

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The toxicity of the protein synthesis inhibitor puromycin towards a number of human and Chinese hamster cell lines has been examined. In comparison to cells of human origin, Chinese hamster cells exhibited about 25-fold higher resistance towards puromycin. These differences appeared to be species related as all the cell lines from any one species showed similar sensitivity towards puromycin. The incorporation of [^{3}H]leucine in the hamster cell lines was accordingly found to be more resistant to the inhibitory effects of puromycin as compared to human cells. Studies on the cellular uptake of [^{3}H]puromycin showed that in comparison to human cells, the drug uptake/binding in the hamster cell lines was greatly reduced. However, protein synthesis in the extracts of hamster and human cells showed no significant differences in sensitivity towards puromycin. These results show that the observed species related differences in cellular toxicity to puromycin are due to differences in the cellular uptake/binding of the drug.

Cellular toxicity; Puromycin; Species difference; (Mammalian cell)

1. INTRODUCTION

Over the last few years, we have been investigating the mechanism of action of a variety of anticancer drugs in cultured mammalian cells [1–5]. During the course of these studies, it was observed that a number of anticarcinostatic drugs like mithramycin, chromomycin A3, colchicine, vinblastine, maytansine and rhodamine 123 exhibited large differences in their toxicity towards cultured cells from various species [3,5,6]. In general, cells of human origin were much more sensitive to these drugs in comparison to those of hamster/mouse origin [3,5,6]. Here, we report that such differences also exist for the protein synthesis inhibitor puromycin which causes premature chain termination by competing with aminoacyl-tRNA for binding to the nascent peptide chain [7,8]. Our studies show that the observed differences in toxicity of puromycin are caused by differences in the cellular uptake/binding of the drug between cells of the sensitive and resistant species.

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2. MATERIALS AND METHODS

2.1. *Cell lines and culture conditions*

The cell lines used in this study and their species of origin are as follows: CHO WT (wild-type) V79 and M3-1 are Chinese hamster cell lines established from ovary, lung and bone marrow tissues, respectively [5,6]. HeLa (clone S_3) is a human epithelial cell line established from cervical carcinoma [5,6]. HT-1080 is a pseudodiploid human cell line obtained from the American Type Culture Collection (ATCC CCL 121), Rockville, MD [5,6]. M-6 is a human cell line established from malignant melanoma [5,6]. All of these cell lines were routinely grown as monolayer cultures in alpha minimum essential medium supplemented with 5% fetal bovine serum at 37°C in a 95% air-5% CO_2 atmosphere by procedures described earlier [5,6].

2.2. *Determination of cellular toxicity towards puromycin*

Toxicity of different cell lines towards puromycin was deter-

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mined by seeding CHO and HeLa cells into wells of 24-well tissue culture dishes containing different dilutions of the drug as described [5,6].

2.3. *Effect of puromycin on [^{3}H]leucine incorporation*

About 1×10^6 cells from each cell type suspended in 1 ml medium were incubated with different concentrations of puromycin for 15 min at 37°C. 5 μCi [^{3}H]leucine (spec. act. 50 Ci/mmol) was then added and the tubes were further incubated at 37°C for 30 min. At the end of the incubation period, 1 ml of 10% trichloroacetic acid was added to each tube, and the tubes were chilled in ice for 30 min. The precipitate so formed was filtered on GF/C filter papers (Whatman) and washed 3 times with 5% ice-cold trichloroacetic acid. The filters were dried and counted after adding 3–4 ml of aqueous counting scintillation fluid (ACS) (Amersham).

2.4. *Cellular uptake of [^{3}H]puromycin*

For these studies, 1 day prior to the experiments, 5×10^5 cells from each cell line were seeded (in duplicate for each time period and drug concentration) into wells of 24-well tissue culture dishes and incubated overnight at 37°C. Next day, the growth medium was carefully aspirated and 0.25 ml of a solution of [^{3}H]puromycin (spec. act. 11 Ci/mmol; final concentration 3 or 7.5 μCi/ml) in growth medium was added to each well. After the indicated period of incubation at 37°C, the radioactive medium was aspirated and the cells were washed three times with cold phosphate-buffered saline (containing per l: 8 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4 and 0.085 g KH_2PO_4). The cells from each well were dissolved in 0.25 ml of a lysis mixture consisting of 0.5% deoxycholic acid in 0.1 N NaOH and the amount of radioactivity was measured after the addition of 3–4 ml aqueous counting scintillant (Amersham Searle). Nonspecific binding was measured by incubating [^{3}H]puromycin in 24-well tissue culture dishes having no cells. This value was subtracted from the total binding to yield the actual binding/uptake of labelled drug. At the same time, the total number of cells in two wells of each cell line was determined by trypsinization and counting of aliquots in a Coulter counter. The uptake of [^{3}H]puromycin in different cell lines was normalized for a constant cell number.

2.5. *Cell-free protein synthesis*

Protein synthesis in cell-free extracts was carried out as in [10,11] in a final volume of 0.05 ml which contained per ml: 0.6 ml cell extracts, 0.9 μmol ATP, 0.18 μmol GTP, 0.19 μmol creatine phosphate, 1.2 mg creatine phosphokinase, 30 μmol Hepes, pH 7.5, 10 μM dithiothreitol, 89 μmol KCl, 19 unlabelled amino acids 0.12 μmol each, 50 μCi of [^{3}H]leucine (spec. act. 50 Ci/mmol) and the drug at the indicated concentrations. The reaction was started by addition of cell extracts. After 40 min incubation at 34°C, the reactions were terminated by the addition of 0.5 ml of 10% trichloroacetic acid containing 3% casamino acids. The mixtures were heated in a water bath at 95°C for 10 min and then chilled in ice. The precipitates were collected on glass fibre filters, washed with 5% trichloroacetic acid, dried and counted.

2.6. *Drugs and chemicals*

Puromycin hydrochloride was purchased from Sigma (St. Louis, MO). [^{3}H]Puromycin and [^{3}H]leucine were purchased from Amersham (IL). All other chemicals used were of the highest purity available.

3. RESULTS AND DISCUSSION

Fig.1a shows the dose-response curves of a number of different cell lines, viz. HeLa, HT-1080, M-6 (human), CHO WT, V79 and M3-1 (Chinese hamster) towards puromycin. In these experiments, relative plating efficiencies of the above cell lines were determined in growth medium containing different concentrations of the drug. As can be seen, the relative plating efficiencies of all three human cell lines decreased sharply between 0.05 and 0.10 μg/ml of puromycin and at 0.15 μg/ml, the survival was reduced to about 10% of the control (D_{10}) value. In contrast, the cell lines of Chinese hamster origin, viz. WT CHO, V79 and M3-1, were highly resistant (approx. 25-fold) to puromycin (D_{10} approx. 4 μg/ml). Since all the cell lines from any one species showed similar sensitivity to puromycin, it strongly indicated that the observed differences are characteristics of the species from which these cells are derived. The observed species-related differences in cellular toxicity are specific for puromycin as similar differences were not seen for other protein synthesis inhibitors such as emetine (Gupta, R.S., unpublished). To investigate the basis of such species-related differences in cellular toxicity, a number of studies were carried out. Since all the cell lines from any one species behaved similarly, further studies were carried out using only one representative human (HeLa) and Chinese hamster (WT CHO) cell line.

Since puromycin is a specific inhibitor of eukaryotic protein synthesis, the effect of different concentrations of puromycin on the incorporation of [^{3}H]leucine in HeLa and WT CHO cells was determined. Results of these studies depicted in fig.1b show that in HeLa cells, puromycin inhibits the incorporation of [^{3}H]leucine in a dose-dependent manner. Around 5 μg/ml puromycin, [^{3}H]leucine incorporation is reduced to about 60% of control and at 25 μg/ml, to about 10% of control. However, WT CHO cells were highly resistant to puromycin as no inhibition of [^{3}H]leucine incorporation was observed at 10 μg/ml drug, and even at 25 μg/ml, only slight (< 20%) inhibition of incorporation was seen. Thus, the effect of

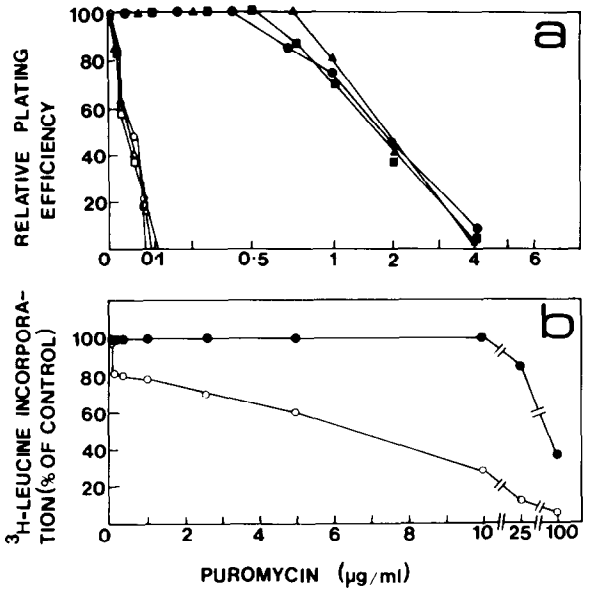

Fig.1. (a) Dose-response curves showing the effect of increasing concentrations of puromycin on the relative plating efficiencies of HeLa (○—○), HT 1080 (△—△), M6 (□—□), WT CHO (●—●), V79 (▲—▲) and M3-1 (■—■). (b) Effect of different concentrations of puromycin on [^{3}H]leucine incorporation in HeLa (○—○) and WT CHO (●—●) cells.

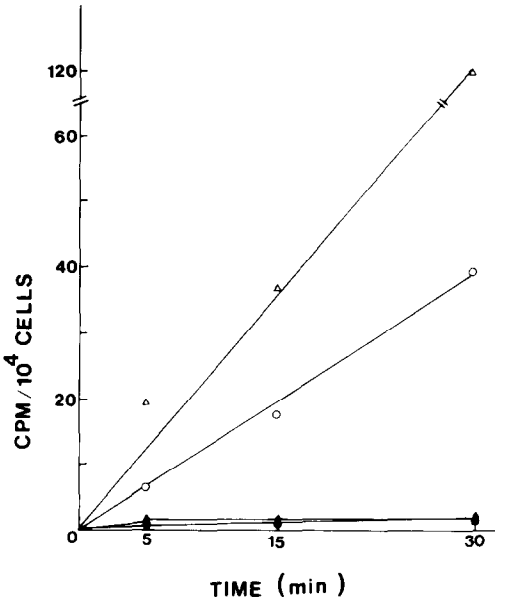

Fig.2. Binding of [^{3}H]puromycin to HeLa (○—○, △—△) and WT CHO (●—●, ▲—▲) cells. [^{3}H]Puromycin diluted in growth medium (3 μCi/ml, circles; and 7.5 μCi/ml, triangles) was added to cells and the uptake was measured as described [9]. HeLa (open symbols), WT CHO (closed symbols).

puromycin on [^{3}H]leucine incorporation paralleled its toxic effect on human and Chinese hamster cell lines.

To understand the biochemical basis of the observed differences in sensitivity towards puromycin, we investigated the cellular uptake and binding of [^{3}H]puromycin to HeLa and WT CHO cells. The results of these experiments are shown in fig.2. As can be seen, there was considerable binding of [^{3}H]puromycin to HeLa cells which occurred in both a dose- and time-dependent manner. The binding at both puromycin concentrations [3 μCi/ml (0.27 μM) and 7.5 μCi/ml (0.68 μM)] increased linearly with time. However, in WT CHO cells, no significant binding of [^{3}H]puromycin at these concentrations of the drug was detected. The results of these studies therefore indicate that the species-related differences in the cellular toxicity of puromycin could be due to differences in the drug uptake/binding between the two cell types.

To determine whether any of the observed differences in cellular toxicity were caused by differences in the sensitivity of the protein synthesis machinery to this drug, the effect of different concentrations of puromycin on in vitro protein synthesis in extracts of human and hamster cells was examined. Results of these studies presented in table 1 indicate that puromycin inhibited incorporation of [^{3}H]leucine into proteins in vitro in both WT CHO and HeLa cell extracts to the same extent. These results provide strong evidence that the observed differences between human and Chinese hamster cells in cellular toxicity of puromycin are not caused by differences in the protein synthetic machinery of the two cell types. In view of these results, the species differences in cellular toxicity to puromycin are most likely caused by differences in the drug's cellular uptake/binding.

In addition to puromycin, cultured cells of Chinese hamster and human origin show large differences in cellular toxicity for a number of other drugs, including colchicine, chromomycin A3, maytansine, mithramycin, vinblastine, taxol and rhodamine 123 [5,6,9]. For most of the drugs for

Table 1

Effects of puromycin on in vitro protein synthesis

[Puromycin] (μg/ml)	Human (HeLa) cells		Chinese hamster cells (CHO)	
	[^{3}H]Leucine incorporated (cpm)	% inhibition	[^{3}H]Leucine incorporated (cpm)	% inhibition
0 (control)	8300	0	14300	0
50	5500	33.8	8300	42
100	3320	60.0	5800	59.5
200	2500	69.9	4400	69.3
500	1700	79.6	3500	75.6

Protein synthesis in cell-free extracts was carried out as described in section 2. The result shown above is an average of three replicates which differed from each other by less than 10%

which species-related differences in cellular toxicity are observed, the mutants exhibiting a multidrug-resistant phenotype have also been reported to show increased resistance [4,12–15]. Recent studies from our laboratory indicate that the species-related differences in toxicity for the above drugs are apparently caused by a similar mechanism to that responsible for the MDR phenotype [16]. This view is supported by the observation that the resistance of hamster cells to the above drugs as well as the reduced drug uptake/binding could be restored to the sensitive human cell's level in the presence of non-toxic doses of verapamil [16], a calcium channel blocking agent which is known to cause reversal of the multidrug-resistance phenotype [17,18].

For many of the drugs for which differences in cellular toxicity are observed between human and Chinese hamster cells viz. mithramycin, chromomycin A_3, olivomycin [6] and cardiac glycosides [9], corresponding differences at the species level have also been observed [9,19,20]. Therefore, it is likely that the sensitivity/toxicity of humans to puromycin may be much greater in comparison to rodent species which are commonly employed to evaluate drug toxicity.

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