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SOME DETERMINANTS OF DRUG RESPONSIVENESS IN BOVINE LEUKEMIA CELLS*

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Abstract—Buffy coat cells derived from a cow with lymphosarcoma and grown in culture were studied and levels of enzymes involved in metabolism of selected anti-tumor agents measured. The data are in general agreement with other results obtained using freshly isolated bovine leukemic cells of diverse origin. These data suggest that the bovine leukemias, unlike leukemia in man, have a marked biochemical uniformity. Our results suggest that bovine leukemic cells are considerably more sensitive to fluorouracil, and considerably less to cytosine arabinoside than the 35 similarly derived human leukemic cell types. The bovine leukemic cells contain high levels of a pyrimidine-5′-phosphoribosyltransferase involved in fluorouracil anabolism, and low levels of deoxycytidine kinase, the first enzyme on the pathway of conversion of cytosine arabinoside to the pharmacologically active form.

WE HAVE examined determinants of responsiveness of several antineoplastic agents in leukemic cells of murine and human origin. The data have provided means for assessing probable responsiveness of these cells to methotrexate,[1] cytosine arabinoside,[2] 6-mercaptopurine,[3] fluorouridine,[4] and fluorodeoxyuridine.[5] Both murine and human leukemias varied widely in drug responsiveness, and in levels of enzymatic determinants of such responsiveness. In contrast, a study of several bovine leukemias derived from lymphosarcomas has revealed striking similarities in levels of these enzymes. These similarities were evident also when the bovine data were compared with results obtained using cells from patients with acute lymphocytic leukemia secondary to primary lymphosarcoma.

MATERIALS AND METHODS

Circulating bovine leukemia cells, separated from freshly drawn blood and a cell line, NBC-10, established in culture[6] were used. This cell line had been grown until February 1971 as a suspension culture in McCoy's 5A (modified) medium plus 20 per cent heat-inactivated horse serum which then was replaced by 20 per cent heat-inactivated fetal calf serum. Before being used for these studies, some of the cells in culture were transferred to RMPI medium (Grand Island Biological Company, Grand Island, N.Y.) plus 20 per cent heat-inactivated fetal calf serum and were grown in stationary suspension culture at 37° in half-filled flasks. For comparison, some studies were

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carried out using L1210 cells from CDF_1 mice or from culture (MEM-Eagle's medium plus 10 per cent fetal calf serum). Circulating malignant cells from patients with lymphosarcoma were isolated as described.[1]

Sources and methods for preparation of radioactive drugs have been described.[1-5] Stock concentrations were 10 mM 6-mercaptopurine, 5-fluorouracil, 5-fluorouridine, cytosine arabinoside, adenine, guanine, uridine, thymidine, deoxycytidine, cytidine and adenosine. All were adjusted to yield final specific activities of 10^4 counts per min per microliter when diluted into 10 ml of a liquid phosphor for liquid scintillation counting.

Suspensions of cells (10 mg/ml, wet wt) in a buffered salt medium were incubated for 10 min at 37° with labeled drugs (final level = 0·1 mM). The cells were then collected by centrifugation and suspended in fresh medium at 37° for 5 min to remove diffusible pools of nucleosides (but not nucleotides formed). Chromatographic examination of cell extracts was used to confirm this assumption. The cells were then collected, suspended in 0·5 ml of 0·9 per cent NaCl and diluted with an appropriate phosphor for measurement of retained radioactivity. Conversion of substrates to non-diffusible products was linear with time over the 10-min interval.

In other experiments, cell-free extracts were prepared with a Teflon glass homogenizer. After removal of particulate material by centrifugation at 100,000 *g* for 60 min, the supernatant fluid was used to assay various kinases and the pyrimidine-5′-phosphoribosyl-transferase. Specific procedures have been described.[4,5,7] Protein was measured by the method of Lowry *et al.*[8]

RESULTS

The data summarized in Table 1 provide a comparison between freshly isolated bovine leukemic cells, a line of bovine leukemic cells carried in culture, cells derived from patients with acute leukemia secondary to lymphosarcoma and the L1210 murine leukemic cells. It is noteworthy that the bovine leukemias have a uniform capacity for phosphorylation of the various nucleosides and pyrimidine and purine bases tested, as compared with the widely scattered values obtained with human leukemias. Bovine leukemic cells have a markedly lower capacity for conversion of

TABLE 1. RATES OF PHOSPHORYLATION OF DRUGS BY LEUKEMIA CELLS OF HUMAN, BOVINE AND MURINE ORIGIN*

Compound	All†	Bovine‡	Bovine‡	Bovine‡	Bovine§	NBC-10‖	L1210¶
Cytosine arabinoside	5–100	9	10	4	8	10	190
5-Fluorouracil	2–8	100	150	130	85	125	50
6-Mercaptopurine	12–85	100	70	90	80	65	290

* Experiments were carried out with intact cells as described in Materials and Methods. Values represent the means of three determinations and are expressed as micromoles of drug phosphorylated per kilogram of cells per hr.

† Acute lymphocytic leukemia in patients with lymphosarcoma; range of 30 values collected between 1964 and 1968.

‡ Bovine leukemic cells freshly isolated at Davis, Calif.

§ Bovine leukemic cells freshly isolated at New Bolton Center.

‖ Bovine leukemic cells in culture.

¶ L1210 in culture.

cytosine arabinoside to nucleotides, and a higher capacity for phosphorylation of fluorouracil than do the human leukemic cells.

A more detailed comparison between the bovine cells carried in culture and a similarly grown L1210 murine cell line is shown in Table 2. Conversion of fluorouracil to nucleotides was similar in rate in these two cell types, but the rate of phosphorylation of 6-mercaptopurine and cytosine arabinoside was markedly lower in the bovine cell. The latter also had a lower capacity for phosphorylation of cytosine arabinoside, adenine and guanine, and a much higher capacity for phosphorylation of uridine than did L1210.

TABLE 2. RATES OF PHOSPHORYLATION OF NUCLEIC ACID PRECURSORS AND DRUGS BY BOVINE AND MURINE LEUKEMIC CELLS IN CULTURE*

Compound	Bovine cells NBC-10	L1210 cells
Cytosine arabinoside	10	190
Cytidine	250	230
Deoxycytidine	20	410
Uridine	950	400
Fluorouracil	125	50
Adenine	500	1800
6-Mercaptopurine	65	290
Guanine	350	135
Thioguanine	300	120

* Intact cells were used as described in Materials and Methods. Units are micromoles of compound phosphorylated per kilogram of cells per hr.

In another series of experiments, we measured levels of enzymes involved in phosphorylation of the compounds described above. The bovine leukemic cells lack uridine phosphorylase (Table 3), and therefore depend on high levels of phosphoribosyltransferase for conversion of fluorouracil to nucleotides. Deoxycytidine kinase

TABLE 3. ENZYME LEVELS IN BOVINE LEUKEMIC CELLS, NBC-10 AND L1210 CELLS IN CULTURE*

Enzyme	L1210	Bovine
Uridine kinase	150	300
Uridine phosphorylase	10	0
Fluorouracil-phosphoribosyl-transferase	5	7·5
Deoxycytidine kinase	5	0·1
Thymidine kinase	10	20
Cytidine kinase	35	36
Adenosine kinase	8	11
Deoxyadenosine kinase	4	6

* Experiments were carried out as described in Materials and Methods. Data are means of three determinations, expressed as micromoles of substrate converted to the nucleotide per g of protein per hr.

was essentially undetectable in the bovine leukemic cells; levels of other kinases were clearly demonstrable.

DISCUSSION

The most remarkable observation is the striking similarity of biochemical behavior of the bovine leukemic cells from different sources: cells were obtained from three cows at Davis, Calif., in 1968, one cow at New Bolton Center, Penn., in 1969, and a long-term cell line (NBC-10) carried in culture at the latter location. In contrast, a wide variation was observed when biochemical properties of various lymphosarcoma-derived acute lymphocytic leukemias of human origin were tested. Whether these observations will be confirmed or not by later experiments is unknown, but the data suggest that bovine lymphosarcoma-derived leukemias may represent a single disease of common origin. A viral factor in this disease has been implicated.[9–11]

The examination of specific drugs indicates that bovine leukemia, like LPC-1 plasma cell murine leukemia, may be highly responsive to 5-fluorouracil because of high levels of a pyrimidine-5′-phosphoribosyltransferase although uridine phosphorylase is lacking.[7] The former enzyme appears to be an important determinant of fluorouracil responsiveness. The bovine cell lines examined all showed impaired capacity to convert cytosine arabinoside to nucleotides; in the cultured bovine cell line, this was traced to a lack of deoxycytidine kinase, the pertinent kinase involved in the first step of such phosphorylation.[12]

The therapeutic implications of this study may be of some practical interest; treatment of lymphosarcomatous cattle with fluorouracil is suggested, even though up to now veterinarians have rarely attempted to treat such animals. Treatment, however, could be well worthwhile and economical if the disease were detected in the early stages, when the productive life of a valuable animal might be significantly prolonged. The data shown here, involving a limited number of samples, are compatible with the suggestion that bovine leukemia may be a single disease, or may have a common viral origin.[13]

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