Biochemical Pharmacology, Vol. 26, pp. 1094–1096. Pergamon Press, 1977. Printed in Great Britain.

Collateral sensitivity: problems of demonstration in murine leukemia cells

(*Received* 6 *August* 1976; *accepted* 26 *October* 1976)

Development of cellular drug resistance is a common occurrence in chemotherapy of cancer, and is often a major obstacle to the successful drug therapy of neoplasia. Neoplastic cells resistant to a specific drug can also be cross resistant or collaterally sensitive to other efficacious drugs. Collateral sensitivity is the term used to describe a cell population that is resistant to one or more drugs but that is also more sensitive to the inhibitory or lethal effects of one or more other drugs than the parent wild type cell population [1]. Many examples of collateral sensitivity have been demonstrated by different investigators and this literature has been reviewed recently [2]. Since cellular drug resistance commonly occurs in cancer chemotherapy, it would be therapeutically advantageous to exploit the mechanisms of collateral sensitivity in neoplasias that commonly acquire cellular resistance to specific antineoplastic agents. That is, if one could exploit the phenomenon of cellular resistance such that collateral sensitivity occurred, a major obstacle to chemotherapy could be diminished. Using the same drug doses on collaterally sensitive cells with the proper drugs should theoretically increase cellular toxicity to resistant neoplasias without an increased host toxicity. Even though, at the present time, the concept of collateral sensitivity is extremely appealing and does indeed occur, the purpose of this communication is to emphasize that this phenomenon may be a chance occurrence or a laboratory phenomenon. It should not, however, be considered as having clinical relevance nor having general applicability until more extensive experiments substantiate the phenomenon.

Our laboratory has investigated the phenomenon of collateral sensitivity extensively for the past two years. We report here on the collateral sensitivity of two L-asparaginase (EC 3.5.1.1.) resistant murine lymphoblast leukemia cells (L5178Y) to methotrexate (MTX) which has been previously reported [3]. These two cell lines are representative of 8 similar cell lines studied in this laboratory during the past two years. We chose these specific examples because acquired resistance to L-asparaginase develops regularly, thereby limiting its use clinically and because MTX is presently used in the treatment of some leukemias.

In vitro. L5178Y cells were grown in Fischer's medium supplemented with 10% horse serum (GIBCO) [4]. L-Asparaginase-resistant cells were selected in asparagine-free Fischer's medium with 20% horse serum. These cells are designated L5178Y/A. They were not selected by the agar cloning method used by others [5], but selected in suspension culture. L-Asparaginase was not used in the selection process. The cells were maintained in asparagine-free Fischer's medium supplemented with 10% horse serum. These cells were periodically tested for their resistance to L-asparaginase. After three months of continuous culturing of L5178Y/A cells in asparagine-free medium, these cells did not show any dependence on exogenous asparagine. To determine growth doubling times, cells in late exponential growth were diluted in fresh medium to 1×10^5 cells/ml. The cell suspension was then poured into 16 × 125 mm sterile screwcapped polystyrene test tubes (Falcon) and incubated at 37°. At 3, 10, 20, 30, 45, 55, and 70 hr after inoculation the contents of three tubes were pooled and duplicate samples were removed, diluted with "Isoton" and counted with the Coulter Counter Model ZB. To determine resistance or collateral sensitivity either MTX, asparaginase, or saline was added to a freshly diluted cell suspension of 5×10^4 cells/ml and treated as described above. Cell numbers were determined 24 and 48 hr after inoculation. MTX was a gift from Lederle Laboratories, Pearl River, New York. *Escherichia coli* L-asparaginase was obtained from Merck, Sharp and Dohme Research Laboratories.

In vivo. L5178Y cells were carried in 19–23 g BDF_1 mice (Jackson Laboratories). Resistant cells (L5178Y/ASP) were selected *in vivo* with 100 i.u./kg asparaginase as described previously [6]. These cells have been established in this laboratory for seven years. Assays for resistance and collateral sensitivity were performed as reported previously [3]. Twenty-four hours after inoculation of 10^6 cells into the peritoneal cavity, either 100 i.u./kg L-asparaginase or 6 mg/kg MTX or saline was injected i.p. every other day for a total of three injections.

The mechanisms of collateral sensitivity are essentially unknown. It has been postulated that biochemical alter-

Table 1. Effects of L-Asparaginase on the growth of L5178Y and L5178Y/A cells *in vitro* in 24 hours

L-Asparaginase concentration in medium (i.u./ml)	Percent of control growth* L5178Y	L5178Y/A
0.01	34.2 ± 1.06	87.3 ± 4.93
0.10	32.1 ± 1.37	80.5 ± 0.70
1.00	30.4 ± 0.71	50.7 ± 5.81

Cells in late exponential growth were diluted in fresh Fischer's medium to approximately 5×10^4 cells/ml. L-Asparaginase or saline was added to the diluted cell suspension, which was then poured into sterile polystyrene test tubes and incubated at 37°. Twenty-four hr after inoculation, the contents of three tubes were pooled and duplicate cell counts were determined with a Coulter Counter.

* Mean ± S.D. of three independent experiments.

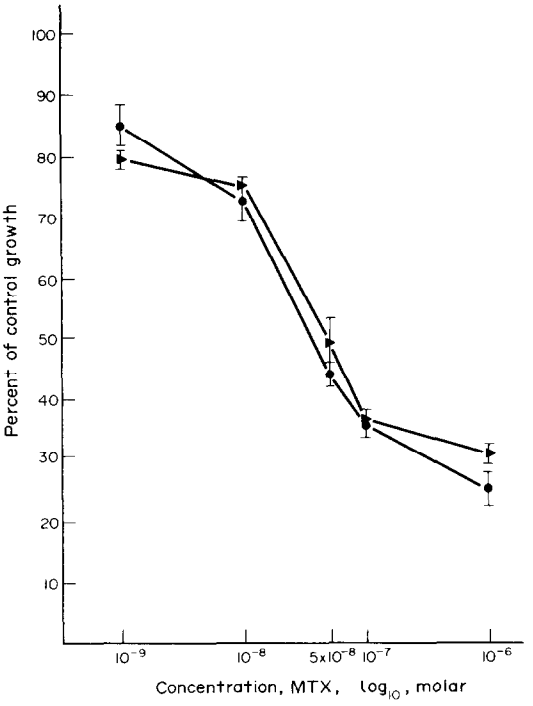

Fig. 1. Effects of methotrexate on the growth of L5178Y and L5178Y/A cells *in vitro*. ●——●, L5178Y cells; ▲——▲, L5178Y/A cells. Cells in late exponential growth were diluted to approximately 5×10^4 cells/ml. MTX or saline was added to the freshly diluted cell suspension. Cells were poured into sterile polystyrene test tubes and incubated at 37°. At 24 hr the contents of 3 tubes were pooled and duplicate samples were counted with a Coulter Counter. Each point is the mean value of three independent experiments with standard deviation.

ations may be responsible for the altered sensitivity of the resistant cells; i.e., altered enzymes in specific drug-sensitive biochemical pathways, altered membrane permeability or transport mechanisms, etc. [2]. In addition, antigenic alteration and/or loss of oncogenic potential of the resistant cells may also account for collateral sensitivity. In order to exclude the possibility of antigenic alteration and loss of oncogenic potential, we attempted to select for drug-resistant cells and study collateral sensitivity *in vitro*. This approach excluded any host influence in the experiments as well as any immunoreactive influence of MTX and L-asparaginase [7, 8].

The observed doubling time for L5178Y and L5178Y/A cell growth was 12 ± 1 hr. The effects of L-asparaginase on the growth of L5178Y and L5178Y/A cells is reported in Table 1. At 24 hr, L-asparaginase, 0.01, 0.10 and 1.0 i.u./ml of medium inhibited the growth of the sensitive cells to 30–34 per cent of control growth. There was a graded reduction of growth of L5178Y/A cells with increasing L-asparaginase concentration. L5178Y/A cell growth was reduced to 87 per cent by 0.01 i.u. asparaginase per ml of medium and to 51 per cent of control growth by 1.0 i.u. per ml of medium. This graded decrease in L5178Y/A growth is probably due to the intrinsic glutaminase activity of *E. coli* L-asparaginase [9]. It has been demonstrated that supplementing the medium with excess L-glutamine reduces the L-asparaginase-induced inhibition of cells grown in asparagine-free medium [5]. Table 1 demonstrates that L5178Y/A cells are resistant to the growth inhibitory effects of L-asparaginase. Figure 1 is the growth survival curve for L5178Y and L5178Y/A cells when exposed to various MTX concentrations at 24 hr. Fifty percent inhibition of cell growth was essentially the same for both cell lines, 4.0×10^{-8} M MTX at 24 hr. At 48 hr inhibition of L5178Y and L5178Y/A cells by MTX was also essentially the same. Fifty percent inhibition of growth by MTX was 1.25×10^{-8} M. L5178Y/A cells are, therefore, not collaterally sensitive or cross-resistant to MTX. These experiments were repeated 10 times.

Failure to demonstrate collateral sensitivity *in vitro* led us to investigate this phenomenon in an *in vivo* system. Table 2 shows that mice bearing L5178Y or L5178Y/ASP cells have mean survival times (MST) of 9 and 10 days, respectively. The MSTs are not significantly different. L-Asparaginase caused an increase in MST of 9 to 20 days in mice bearing L5178Y cells but no increase in mice bearing L5178Y/ASP cells (Table 2). The increase in MST caused by MTX was not significantly different in mice bearing either cell line. The data are from three independent experiments. As in the *in vitro* studies we failed to demonstrate collateral sensitivity *in vivo*.

It is possible that drug-resistant cells exhibiting collateral sensitivity have partially lost their oncogenic potential. Mouse leukemias have been shown to have increased MST in the resistant variants [10]. Earlier studies [3] with L-asparaginase-resistant cells *in vivo* show an increase in MST as compared to sensitive cells. No increase in MST was observed with our resistant cells (Table 2). It should be noted that resistant cells that have increased MST and exhibit collateral sensitivity can also be cross-resistant to other, unrelated drugs to which they were sensitive or they can also be as sensitive to other agents as their parent cell population.

Another possibility is that in determining mechanisms of cellular drug resistance, when one cell population selected for resistance to a specific agent may differ genetically in its mechanism of resistance to that drug, collateral sensitivity may also be randomly expressed in drug-resistant cell mutants. This would explain why, after repeated attempts, we were unable to demonstrate this phenomenon in our laboratory under the conditions expressed above.

Table 2. Effects of L-asparaginase and methotrexate (MTX) on mean survival times of mice bearing L5178Y or L5178Y/ASP cells

Cell line	Treatment	Mean survival time (days)
L5178Y	Saline	9
	MTX (6 mg/kg)	15
	L-Asparaginase (100 i.u./kg)	20
L5178Y/ASP	Saline	10
	MTX (6 mg/kg)	13
	L-Asparaginase (100 i.u./kg)	10

Each animal was inoculated with 1×10^6 cells i.p. Twenty-four hours later treatment was begun. A total of three injections were given i.p. to each group every other day. Each value is the mean from three independent experiments with 8 mice in each group.

It should be emphasized that these experiments were extended over a period of two years and at no time could collateral sensitivity be demonstrated.

A recent study demonstrates collateral sensitivity *in vitro* between MTX-resistant L5178Y cells and adriamycin [11]. Adriamycin reduces the viability of MTX-resistant cells more effectively than in MTX-sensitive cells. The MTX-resistant cells take up more [^{3}H]adriamycin than do the sensitive cells, resulting in an increased cytotoxicity in the resistant cells. These results suggest that collateral sensitivity is not just an *in vivo* phenomenon.

Collateral sensitivity is still essentially an unexploited phenomenon. Determining its mechanism of action may give further insight into the problem of drug resistance observed so often in neoplasia. However, until its universality of occurrence and the definite conditions for its development are established, collateral sensitivity should be treated solely as a subset of cellular drug resistance, i.e., cellular changes that sometimes accompany the development of certain modes of resistance by certain agents in certain cells under certain defined conditions.

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