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AN ANTAGONISTIC EFFECT BETWEEN AZIRIDINE AND DIAZIRIDINE ON THEIR CYTOTOXIC ACTIVITIES AGAINST L-1210 MOUSE LEUKEMIA CELLS

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The effect of the combination of 1-methyl-2-p-chlorophenylaziridine and 1,2-dimethyl-3-p-chlorophenyldiaziridine on cytotoxic activities against L-1210 mouse leukemia cells was studied. Both compounds clearly showed an antagonistic interaction. The results supported our hypothesis that nitrosomethane formed by the fragmentation of aziridine via the N-oxide in a cellular system causes the cytotoxic behavior of the N-methyl aziridines.

In the preceding paper,[1] we reported that aziridine gave nitroso and olefinic compounds and diaziridine gave amino and carbonyl derivatives in the fragmentation reaction induced by enzymatic action of rat liver microsomes in vitro. In the reaction, the fragmentation of aziridine was oxidative and that of diaziridine was reductive. Both reactions clearly showed an antagonistic interaction with each other when both compounds coexisted in a single enzymatic system, suggesting that a competition occurred for the active center of cytochrome P-450. These results offered evidence confirming our hypothesis that nitrosomethane formed by the fragmentation of aziridine via their N-oxide in the cellular system causes the cytotoxic behavior of the N-methyl aziridine derivatives.[2]

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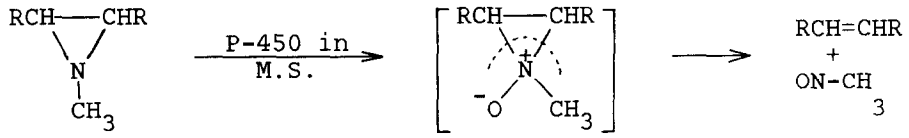
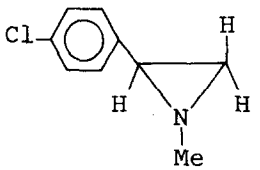


Thus, we supposed the addition of diaziridine to the experimental system for the study of cytotoxic activity of aziridine would depress the formation of nitrosomethane derived from aziridine on the assumption that the antagonistic interaction by both compounds observed in microsomal solution occurs also in a cellular system. Consequently, an antagonistic interaction should also appear in their cytotoxic activities.

To confirm this, we studied the effect of the combination of 1-methyl-2-p-chlorophenylaziridine (1) and 1,2-dimethyl-3-p-chlorophenyldiaziridine (2) on their cytotoxic activities and report our findings here.



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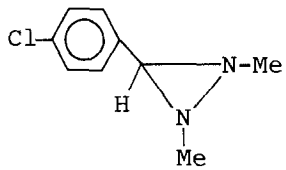

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Experiments and Results

The in vitro cytotoxic effect of aziridine derivatives was tested against L-1210 mouse leukemia cells in free-floating culture. L-1210 cells were maintained in Eagle's minimum essential medium supplemented with 10% fetal calf serum.

The aziridines, 1 and diaziridine 2, were dissolved in absolute ethanol at a concentration of 500 mg/ml and subjected to serial tenfold dilutions with the culture medium.

For determination of the effects on proliferative activity, the cells (6 x 10^4/ml) were exposed to the serial-diluted agents. Three culture tubes thus prepared were used as a set for each dose. After 72 h of incubation, the cell population was measured using a TOA micro-cell counter (TOA Electronics, Kobe, Japan).

The growth inhibition percent (GI%) was calculated from the following formula: GI percent = 100 - $(T-C_0/C-C_0)$ x 100 where C = final cell number in control, T = final cell number in treated tube, and C_0 = cell number in the tube at the time of sample addition.

The standard error of the cytotoxic activity determined in these studies was approximately 3%. Before attempting to

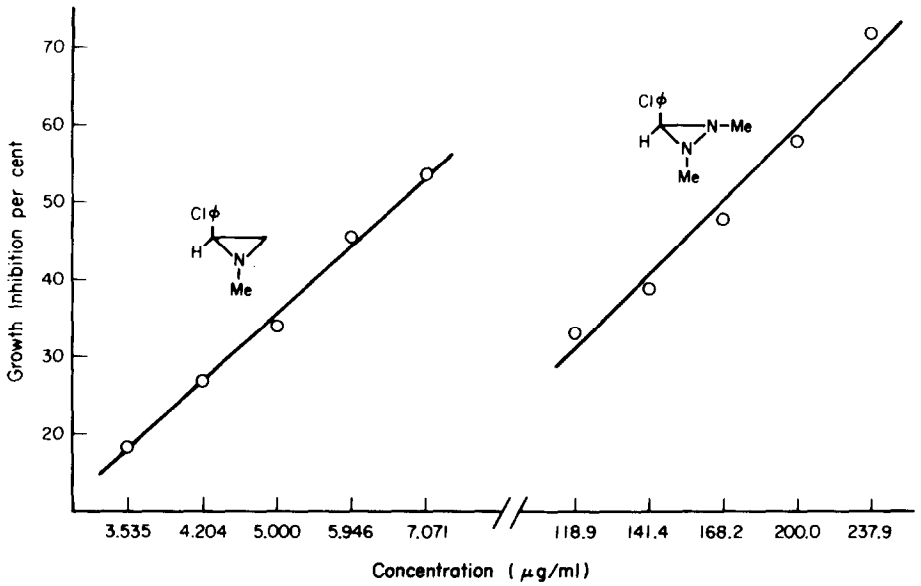

Figure 1. Growth inhibitory effects of 1-methyl-3-p-chlorophenylaziridine (1) and 1,2-dimethyl-3-p-chlorophenyldiaziridine (2) on L-1210 cells.

measure the effects of combination treatment with 1 and 2, the effects of the individual agents had to be measured. Typical dose-response GI% obtained with 1 and 2 are shown in

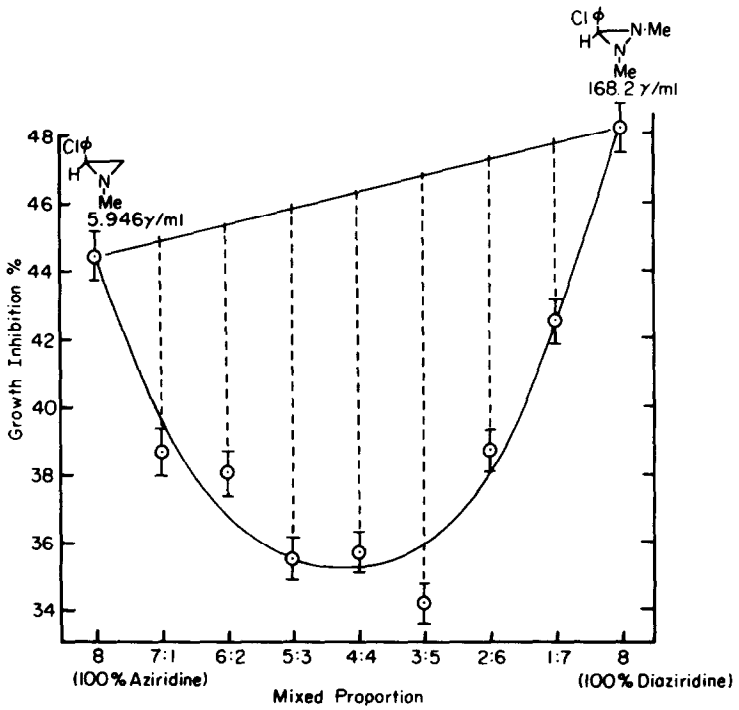

Figure 2. Effect of the combination of 1-methyl-2-p-chlorophenylaziridine and 1,2-dimethyl-3-p-chlorophenyldiaziridine on the cytotoxic activity against L-1210 mouse leukemia cells.

Figure 1. From the nearest ED_{50} (effective dose for 50% growth inhibition) values, the GI% obtained after exposure of cells to the compounds was 45.4% for aziridine 1 (5.946 μg/ml) and 47.8% for diaziridine 2 (168.2 μg/ml). These two doses were used in combination for the treatment of L-1210 cells. The solutions of 1 (5.946 μg/ml) and 2 (168.2 μg/ml) were prepared immediately prior to use and mixed in various ratios. The results of experiments with 1 and 2 given alone and in combination are shown in Figure 2.

Theoretically, GI% of a binary system should appear on the straight line through both points corresponding to pure aziridine and pure diaziridine. Actually however, as shown in Figure 2, our results obtained with mixtures of both compounds indicated a regularly lower GI% value. Especially, a mixture of five-eighths of 1 and three-eighths of 2 was most antagonistic against the cytotoxicity to L-1210 cells.

Discussion

Our results clearly showed that aziridine 1 and diaziridine 2 in combination regularly interact antagonistically in their effect on cultured L-1210 cells. To our knowledge, this is the first example of such an antagonistic effect on cytotoxic activity. In the preceding paper, we reported that aziridine 1 and diaziridine 2 antagonistically affect each other in the fragmentation reaction induced by the enzymatic action of rat liver microsomes.[1] Assuming that the same reaction by both compounds observed in the microsomal solution occurs in the cellular system, the results obtained here strongly support our hypothesis described above. However, the mechanisms of the antagonistic interaction on the enzymatic system or the cytotoxic activity are not clear at present. Further experiments are necessary to elucidate the relationships of these results.

References

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