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Evaluation of Drug Sensitivity of Leukemic Cells by O_2 Consumption

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SUMMARY

O_2 consumption method assaying cytotoxic activity of 6MP was compared with other 4 methods; dye exclusion method, colony-formation inhibition method, ^{3}H-thymidine uptake inhibition method and growth inhibition method using L1210 mouse leukemic cells and 6MP. The most sensitive method for cytotoxicity assay was colony formation inhibition method on an agar plate but at least 10 days are necessary for determination. Dye exclusion method was the simplest but sensitivity was the lowest among 5 methods and 16 hours are required for the evaluation. O_2 consumption method was more sensitive and reliable than dye exclusion method. Moreover, O_2 consumption method was the simplest among 4 methods except dye exclusion method and the results could be obtained within 8 hour-treatment with 6MP. These results suggest that O_2 consumption method is an useful and convenient for evaluating drug sensitivity of leukemic cells.

INTRODUCTION

The sensitivity to anti-cancer agents of leukemic cells ob-

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tained from different patients is different from each other as same as of bacterial cells. It should be necessary to estimate the drug sensitivity of leukemic cells for selecting clinically effective anti-cancer agents. Many methods[1-5] have been proposed and tried to applicate to the clinical use. There are two types of methods; (1) assaying the inhibition of tumor cell growth and (2) assaying the inhibition of tumor cell metabolism. The former is more sensitive and reliable than the latter but the culture of tumor cells from a patient is difficult and a long time is necessary to determine drug sensitivity. The latter is simpler and easier than the former but is less sensitive and unreliable. In this paper, we deal with the estimation of drug sensitivity by O_2 consumption inhibition of leukemic cells.

MATERIALS AND METHODS

Mouse leukemic cells. L-1210 lymphoid leukemic cells of DBA/2 mice were kindly supplied by Dr. Masanori Shimoyama, National Cancer Center Hospital. The leukemic cells were maintained *in vitro* in RPMI 1640 medium (Nissui Kagaku Co., Tokyo) supplemented with 20% calf serum (Flow Laboratories, USA), 50 units/ml of penicillin G and 50 μg/ml of streptomycin at 37° C in 5% CO_2 and 95% air. From original cell line, 6-mercaptopurine (6MP)-resistant cell line was obtained by *in vitro* screening. Minimal inhibitory concentrations of 6MP (MIC) of the original and resistant cell lines determined by colony formation inhibition method were 0.3 μg/ml and more than 30 μg/ml, respectively. Doubling time of both cell lines was the same and was about 13.7 hours.

Determination of O_2 consumption. Leukemic cells were suspended in RPMI 1640 medium at an appropriate concentration. A half milliliter of the cell suspension was mixed with 2.7 ml of the reaction mixture and the O_2 consumption was monitored at 37° C with Yanaco oxygenometer Model PO-100A. The reaction mixture

contained RPMI medium, 6MP and 217 umoles of O_2.

Growth inhibition test. L-1210 leukemic cells were cultured in 0.1 ml of 20% calf serum-RPMI medium contained various concentrations of 6MP. After incubation for 48 hours at 37° C, 1/4 volume of 0.4% trypan blue solution was added to the cultured cells and the number of viable cells was counted with a hemocytometer. Minimal concentration of 6MP which inhibited more than 90% of cell growth was determined as MIC.

Colony formation inhibition. One tenth milliliter of L-1210 cell suspension (1×10^7 cells/ml) was spread on an agar plate containing the culture medium and 6MP and the number of colony was counted 2 weeks after the incubation at 37° C.

RESULTS

O_2 consumption by L-1210 cells

Original 6MP-sensitive L-1210 cells cultured in Petri dishes were collected, washed twice with RPMI 1640 medium and suspended in the same medium at 1×10^6, 1×10^7, and 1×10^8 cells/ml. A half milliliter of each cell suspension was loaded in a oxygenometer and O_2 consumption was monitored from 2.5 to 30 min after the incubation. The amount of oxygen consumed with each aliquot linearly increase within 15 min. Fig. 1 shows the mean O_2 consumption at one min during 2.5 to 15 min. O_2 consumption was corresponding to the viable cell number, suggesting that cytotoxic effects of anti-cancer agents on leukemic cells can be determined by O_2 consumption.

Effect of 6MP on growth of L-1210 and O_2 consumption

Original L-1210 cells (1×10^6 cells/ml) were cultured in the culture medium containing 30 μg/ml of 6MP. Cells were removed from the mixture and washed with RPMI at 5, 8, 16 and 24 hours after the 6MP-treatment. The washed cells were divided into 5 groups and assayed (1) viability by trypan blue dye exclusion, (2) oxygen consumption, (3) ^{3}H-thynidine uptake, (4) growth

inhibition after culturing for another 48 hours and (5) colony-forming activity on an agar plate. As shown in Fig. 2, more than 80% of L-1210 cells were viable by dye exclusion within 8 hour-treatment with 6MP. In contrast, cytotoxic effect was observed at 4 or 8 hours after the 6MP treatment with other four assays.

Both original and 6MP-resistant L-1210 cells were cultured in the culture medium containing various concentrations of 6MP and the effects of 6MP on O_2 consumption and growth inhibition were determined. Colony formation inhibition test was also carried out with the sensitive and resistant cell lines on an agar plate containing an appropriate concentration of 6MP. The results are shown in Table 1. These results suggest that O_2 consumption method is able to use practically as a convenient and useful method for assaying drug sensitivity of leukemic cells.

DISCUSSION

Before clinical use, it should be necessary to evaluate the effects of drugs on a patient's tumor cells, because the drug sensitivity of tumor cells in different patients may be different from each other. Colony formation inhibition test is the most reliable and mostly correalated to the clinical efficacy of a drug among the methods proposed.[5] However, primary culture of tumor cells is very difficult and so tumor cells are sometimes proliferated through a xenograft in a nude mouse.[1,4] Neverthless, colony formation inhibition assay requires a long time for the evaluation of a drug. With O_2 consumption method described in this paper, drug sensitivity can be easily assayed in a short time without cell proliferation. But it is a problem of this method that a lot of tumor cells is required for the assay and a micro-O_2 consumption method should be further developed.

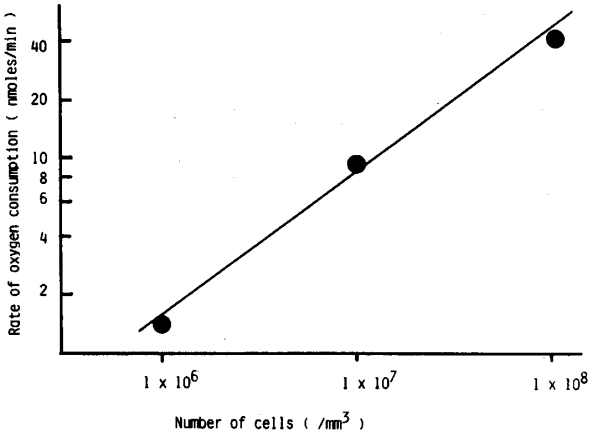

Fig. 1. Oxygen consumption of original L-1210 cells.

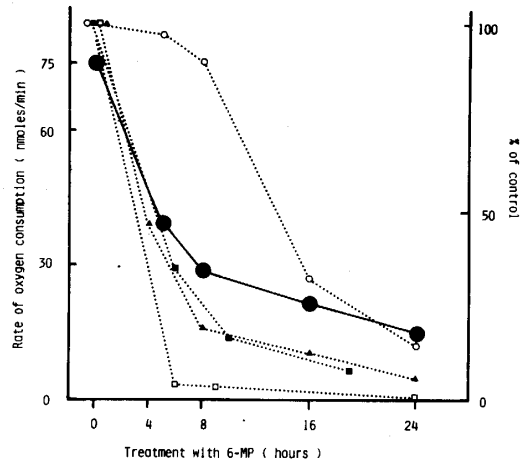

Fig. 2. The effects of 6MP on original 6MP-sensitive L-1210 cells. (●) oxygen consumption, (○) trypan blue dye exclusion, (□) colony formation, (■) ^{3}H-thymidine uptake, (▲) growth inhibition.

Table 1. Comparison of MIC values of 6MP determined by 3 different methods.

Method	MIC (μg/ml)	
	sensitive L1210	resistant L1210
O_2 consumption inhibition	1.0	> 30
Growth inhibition	1.0	> 30
Colony formation inhibition	0.3	>30

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