LETHAL EFFECT OF MACROMOMYCIN ON HELA CELLS

Sir:

Macromomycin (MCR)[1], an antitumor antibiotic, isolated from the culture filtrate of *Streptomyces macromomyceticus,* is an acidic polypeptide with a molecular weight of 12,500[2]. It exhibited marked antitumor activity against various murine tumor systems[3,4,5]. In connection with its cytotoxicity, KUNIMOTO *et al.*[6] have demonstrated that brief incubation of MCR-treated cells with proteolytic enzymes abolished MCR toxicity suggesting that the antibiotic had bound primarily to the membrane of cultured mammalian cells and interfered with their proliferation. SUZUKI *et al.*[7] later demonstrated that MCR caused strand scission in the DNA of cultured cells, which may result in the inhibition of mitosis and/or DNA synthesis.

The lethal effect of MCR on mammalian cells has not been characterized as yet in terms of the dose-survival and the time-inactivation relationship. This communication describes the pattern of lethal action of MCR on HeLa cells and briefly touches on its clinical implication.

HeLa S_3 cells were grown in F10 medium supplemented with 10% calf serum (both components from Flow Laboratories, USA) and 0.05% heart infusion broth (Difco, USA) in a CO_2 incubator at 37°C. Exponentially growing cells were dispersed with 0.1% trypsin (1: 250, Difco) solution and 10^5 suspended cells were then plated into 60-mm plastic dishes. After incubating for 2 days, the cultures ($2.5 \sim 3.5 \times 10^5$ cells/dish) were treated with various concentrations of MCR for the desired period at 37°C. In two-dose experiments, the cultures were treated with MCR (0.01 μg/ml) for 120 minutes (the 1st treatment), rinsed twice with HANKS' solution to remove the antibiotic and then the 2nd treatment at the same concentration for 60 minutes was given at intervals of 0 to 180 minutes. In this experimental procedure, the second dose is only used as a test dose to detect a possible change in the damage induced by the first (conditioning) dose.

The survival of cells was determined by the following colony formation assay. After drug exposure, the cells were rinsed twice with HANKS' solution, trypsinized and an appropriate number of cells ($10^2 \sim 10^4$ cells/dish) were plated in triplicate 60-mm dishes. The cultures were incubated in a CO_2 incubator at 37°C for 12 to 14 days for colony formation. The colonies which developed were counted after fixing and subsequent staining. Surviving fractions were obtained from the ratio of the tested group to the untreated control group. Under these conditions, the plating efficiency of the trypsin-dispersed control cells was normally 70 to 90%.

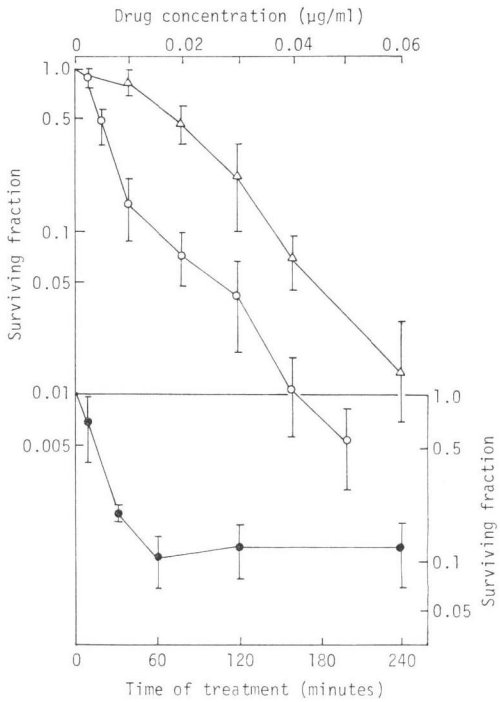


Fig. 1. Lethal effect of MCR and NCS on HeLa cells.

The upper panel shows dose-response curves of HeLa cells treated with MCR (○) and NCS (△) at various concentrations for 60 minutes. The lower panel shows the time-inactivation curve of the cells treated with MCR (●) at a concentration of 0.01 μg/ml for 10 to 240 minutes.

Each of the points and bars represents the mean ±SD of 3 or 4 experiments.

MCR was prepared in our Laboratories and neocarzinostatin (NCS) was purchased from Yamanouchi Pharmaceutical Co., Tokyo. Both antibiotics were dissolved in 0.9% NaCl solution and then diluted in F10 medium supplemented with 28 mM HEPES buffer (pH 7.2) just prior to use.

The dose-survival curve of HeLa cells treated with MCR at various concentrations for 60 minutes at 37°C is shown, in comparison with cytotoxicity of NCS, in the upper panel of Fig.

1. Both antibiotics killed the cells, in roughly exponential fashion, with increasing concentrations of the drug. The 10 percent survival dose was about 0.015 μg/ml for MCR and about 0.035 μg/ml for NCS. Therefore, the cell lethality of MCR is about 2-fold stronger than that of NCS.

Time-inactivation of the cells treated with MCR (0.01 μg/ml) is shown in the lower panel of Fig. 1. The survival decreased exponentially with increasing periods of exposure for 60 minutes, then stayed essentially constant until 240 minutes. The flat region of the time-inactivation curve was not due to thermal denaturation of the drug, because 80% of antimicrobial activity of MCR still remained after 120-minute incubation at 37°C (data not shown). To examine whether or not resistant cells induced by MCR are genetically determined, the cloned cells which were isolated from the survivors after exposure to MCR (0.01 μg/ml, 180 minutes), were tested for their sensitivity to MCR in comparison with that of untreated normal cells. As shown in Fig. 2, the survival-response was essentially the same between normal and coloned cells, demonstrating that the progeny of the resistant cells is not genetically determined. If one assumes a discrete phase of resistance to MCR during the HeLa cell cycle, the insensitivity of the surviving cells after an exposure to the low concentration (0.01 μg/ml) of MCR may be interpreted. To test another possibility, the two-dose fractional treatment was conducted as shown in Fig. 3. The first dose usually reduced survival down to about 0.1 as given by broken line in Fig. 3. The two-dose fractional survival plotted in the inset revealed the immediate 60% reduction of survival followed by nearly constant values over a 3-hour interval. TERASIMA *et al.*[8,9] have reported that bleomycin induces resistance in mammalian cells but the resistance gradually disappears when the antibiotic is removed. However, a similar reversal of resistance was not observed with MCR. Therefore, a possibility of cell cycle dependent sensitivity difference still remains to be determined.

It has been reported that MCR binds to the membrane of mammalian cells resulting in the inhibition of DNA synthesis and the cytotoxicity of MCR can be abolished by a brief treatment of the cells with trypsin[6,10,11], although SUZUKI

Fig. 2. Comparison of survival-response of normal HeLa cells and their resistant clone to MCR.

Normal cells (○) and resistant cells (●) obtained from the cells treated with MCR (0.01 μg/ml) for 180 minutes were exposed to graded concentrations of MCR for 60 minutes.

Each point represents the mean of 2 experiments.

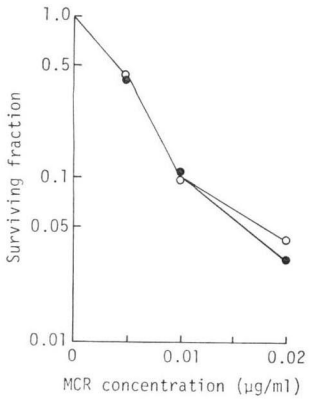
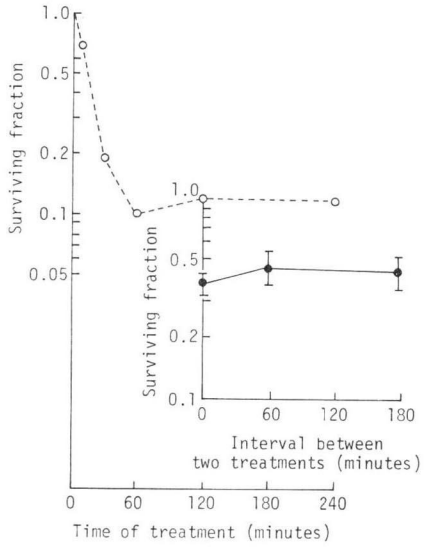


Fig. 3. Survival changes in the two-dose fractionation.

After exposure of cells to the 1st dose (0.01 μg/ml, 120 minutes) and subsequent rinsing, the 2nd dose (0.01 μg/ml, 60 minutes) was given at intervals of 0 to 180 minutes. The two-dose fractional survival is shown in the inset. The broken line indicates the single-dose survival curve obtained from Fig. 1 for reference.

Each of the points and bars represents the mean ±SD of 3 experiments.



et al.[7] did not obtain evidence that cell growth recovered after the treatment of MCR-exposed cells with trypsin. On the other hand, like NCS[12,13] and AUR[14,15], it has been demonstrated recently that MCR contains a non-protein chromophore which can be removed from the protein moiety of MCR and that the biological activity of MCR is due to its chromophore[16,17]. They have suggested that the action of the protein moiety may be related to the permeability of the cells and stabilization of the activity of the chromophore. Based on these findings, the additional decrease in survival by the 2nd treatment (Fig. 3) might be interpreted by assuming that a part of the MCR protein bound to the cell surface was released by washing the cells with buffered saline, resulting in the partial recovery of susceptibility of cells to the antibiotic. In this connection, the binding-saturation model proposed by URANO *et al.*[18] which assumes saturation and dissociation of drug molecules on the critical target of a cell, may be useful. In order to understand the mechanism of the lethal action of MCR, the interaction of the MCR chromophore and its protein with mammalian cell organelles needs further study.

In view of the essentially exponential nature of the dose-survival curve of MCR, the lethal effect on *in vivo* tumor cells should be proportional to the concentration of the drug used. However, present time-inactivation results indicate that continuous infusion of the drug prevents further sterilization of tumor cells. Further investigation of methods to release tumor cells from insusceptibility induced by the antibiotic is needed.

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