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Author(s)	浜田, 辰巳; 山崎, 武; 三浦, 貴士
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# Effects of Mitomycin C and X-Irradiation on Colony-Forming Ability of Mammalian Cells Grown in Vitro

By

Tatsumi Hamada, Takeshi Yamazaki and Takashi Miura

Department of Clinical Research, Research Institute for Microbial Diseases, Osaka University, Osaka  
(Director: Assist. Prof. T. Miura)

培養細胞のコロニー形成能におよぼすマイトマイシンCおよびX線の作用

大阪大学微生物病研究所放射線科（医長：三浦貴士助教授）

浜田辰巳 山崎武 三浦貴士

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動物細胞の放射線生存率曲線が sigmoid になることはよく知られており、またアルキル化剤についても同様の作用曲線が示された。

我々は HeLa 細胞のコロニー形成能を指標としてマイトマイシンCの濃度および作用時間に対する生存率曲線を描き、sigmoidal なることを見いだした。同時に X線との併用実験を行なつた

が、マイトマイシンC処置後X線照射した場合の生存率はそれぞれ単独の作用の積となり、X線生存率曲線の外挿値にも勾配にも変化はなかつた。逆にX線前処置はマイトマイシンC生存率曲線の外挿値を僅かに低下せしめたが、勾配には変化なく、いずれにしても明らかな相乗作用は得られなかつた。

## Introduction

It is well known since Puck et al.<sup>7,8)</sup> that sigmoidal survival curve is obtained when mammalian cells are X-irradiated. Levis et al.<sup>9)</sup>, Walker et al.<sup>10)</sup> and Marin et al.<sup>5)</sup> showed that alkylating agents such as nitrogen and sulfur mustards also produced similar survival curves.

In an earlier paper, we reported that dose-response curves for X-rays and mitomycin C measured by mouse thymic and splenic weight resembled each other in shape<sup>6)</sup>. Therefore, we expected that survival of single cells exposed to mitomycin C would also yield sigmoidal curve when plotted against its dose. Using single cell techniques, we studied effects of mitomycin C on the colony-forming ability of mammalian cells. At the same time we carried out combination experiments with X-rays to see whether synergistic effect existed.

## Materials and Methods

### I. Cell line

A clonally isolated subline of HeLa cells was used. The cells were routinely propagated in culture bottles at 37°C. Growth medium consisted of Eagle MEM<sup>11)</sup> containing 10 per cent calf serum.

The cells were always harvested from log-phase cultures, and single cell suspensions were made by the usual trypsinization procedures. Samples from cell suspensions were properly diluted in growth medium containing 20 per cent calf serum and plated in 60 mm glass petri dishes. The dishes were incubated for 12—14 days at 37°C in a humidified atmosphere containing 5 per cent CO<sub>2</sub>, stained with crystal violet

and scored for viable cells. All colonies consisted of more than 50 cells were counted as survivors.

### 2. Mitomycin C treatment

$10^5$  cells were suspended in test tubes containing 5 ml of growth medium added with varying doses of mitomycin C. The cells were kept at 37°C for 30 min, washed, diluted in fresh full growth medium and plated for survival measurement. In an experiment, concentration of the drug was constant and duration of its treatment was varied.

### 3. X-irradiation

The source of X-rays was a Shimadzu therapy unit, operated at 200 kvp and 15 ma. The beam was filtered with 0.3 mm Cu and 0.5 mm Al. The samples were irradiated at a constant dose rate of 120 R/min at room temperature.

### 4. Kinetic analysis of survival curves

Lethal effects of X-rays and mitomycin C were measured by loss of colony-forming ability of HeLa cells. The surviving fraction,  $s$ , was given by the equation within the range of experiments

$$S = 1 - (1 - e^{-D/D_0})^m$$

in which  $D$  was X-ray or drug dose,  $D_0$  was the mean lethal dose or the dose which reduced survival by a factor of  $1/e$  ( $\approx 0.37$ ) in the exponential region of survival, and  $m$  was the extrapolation number.

## Results

### 1. Survival curve for X-rays

Survival curve of this clone for X-rays showed sigmoidal type of response as many other mammalian cells (Fig. 1). The linear portion of the curve had a slope corresponding to a value for  $D_0 = 80$  R, and extrapolation number  $m$  was 4.0

Fig. 1. Survival curve for X-rays.

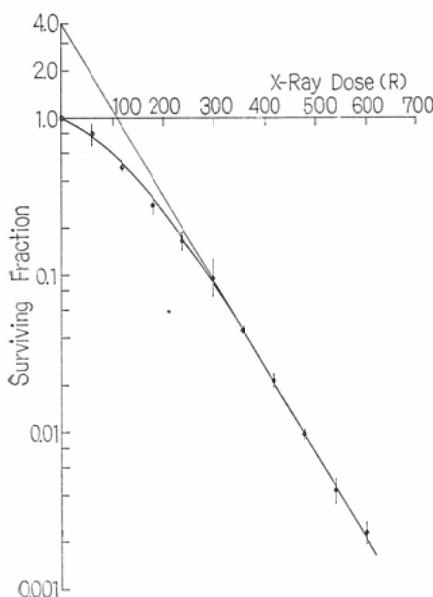


Fig. 2. Survival curve for mitomycin C. Duration of the treatment was constant (30 min).

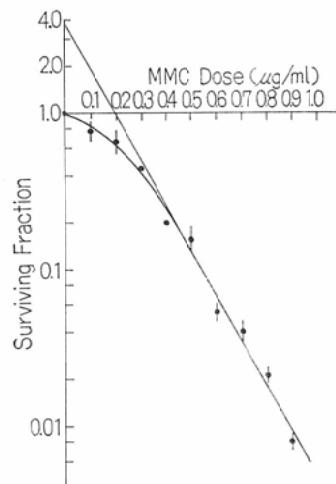


Fig. 3. Survival curve for mitomycin C. Concentration of the drug was constant(0.5  $\mu\text{g}/\text{ml}$ ).

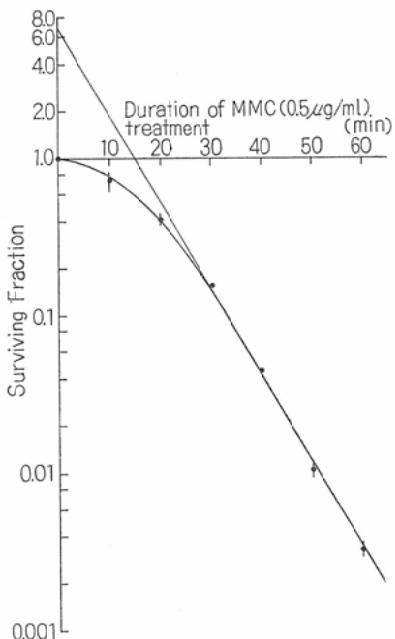
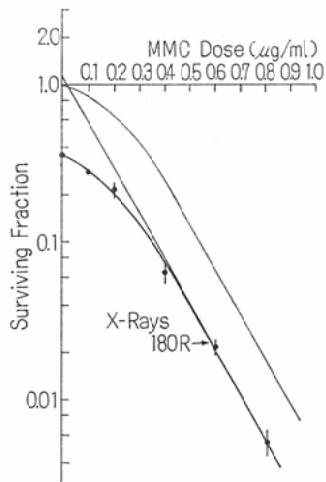


Fig. 5. Survival curve for varying doses of mitomycin C pretreatment. X-ray doses were constantly 180 R.



## 2. Survival curve for mitomycin C

Fig. 2 shows the survival curve obtained by exposing cells to varying concentrations of mitomycin C for 30 min at 37°C. The curve showed an initial shoulder which extended to about 0.4  $\mu\text{g}/\text{ml}$ , followed by a linear portion in which survival was an exponential function of dose.  $D_0$  was about 0.15  $\mu\text{g}/\text{ml}$  and  $m$  was about 3.8

Fig. 4. X-ray survival curves pretreated with mitomycin C.

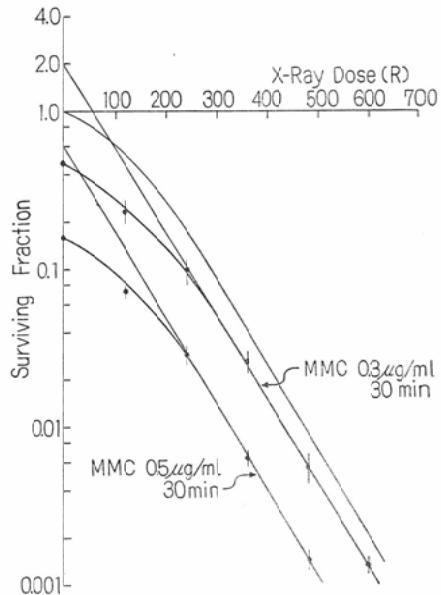
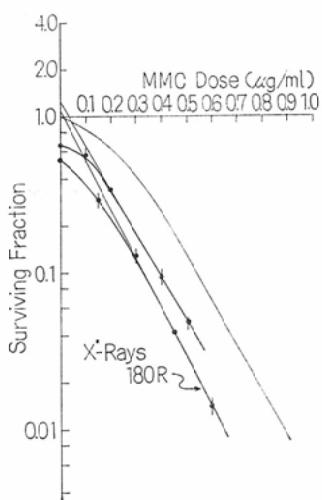


Fig. 6. Mitomycin C survival curves pretreated with X-rays.



When cells were exposed to 0.5  $\mu\text{g}/\text{ml}$  of the drug for varying duration, the survival curve was also of sigmoidal type as shown in Fig. 3.  $D_0$  was about 7.8 min and  $m$  was about 7.0.

Though the extrapolation numbers of both mitomycin C survival curves were different, rough reciprocal relation was found between the concentration of the drug and the duration of its treatment.

### 3. Interaction of mitomycin C and X-rays

Survival curves for X-rays pretreated with 0.3  $\mu\text{g}/\text{ml}$  and 0.5  $\mu\text{g}/\text{ml}$  of mitomycin C for 30 min are shown in Fig. 4. Each curve had the same extrapolation number and  $D_0$  as those of untreated one. The same result is also seen in Fig. 5, in which mitomycin C doses were varied and X-ray doses were constantly 180 R.

Reversely, pretreatment with X-rays slightly lowered the extrapolation number of mitomycin C survival curve, but did not alter its slope as shown in Fig. 6.

### Discussion

Our results demonstrated that, when exponentially growing HeLa cells were exposed to mitomycin C, the survival curve was of the sigmoidal type. This type of response shows that the cells have essential structure sensitive to the agent and damage to it must be accumulated before a lethal response is produced as generally accepted for X-rays.

Iyer, V.N. et al.<sup>2,3)</sup> and Szybalski, W. et al.<sup>9)</sup> reported that exposure of bacterial or mammalian cells to mitomycins or porfiromycins resulted in covalent cross-linking of the complementary DNA strands, a reaction concurrent with cell death. They also reported that the degree of cross-linking by mitomycin C depended on the concentration of the antibiotic, on the duration of its contact with cells and on the temperature. These do not seem to contradict our mitomycin C survival curve.

Combination experiments of the drug and X-rays were carried out to see whether synergistic effect existed between the two agents. Pretreatment with a single dose of mitomycin C did neither abolish the shoulder of the X-ray survival curve nor alter its slope, and the effect of the combination of the two agents was nearly equal to the product of each effect. In the reverse experiments, extrapolation number of the mitomycin C survival curve was somewhat reduced by pretreatment with X-rays, but the slope was not changed. Thus no distinct synergism was found.

### Summary

A subline of HeLa cells growing in vitro was exposed to mitomycin C and the surviving fraction of cells was measured by their ability to form macroscopically visible colonies. Survival thus obtained yielded sigmoidal curves when plotted against the concentration of the drug as well as the duration of its contact with cells. Combination experiments of the drug and X-rays failed to show synergism between the two agents.

### Acknowledgements

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