Enhancement of the Cytotoxic Effect of Mitomycin C by β -Adrenergic Stimulants in Rat Ascites Hepatoma AH130 Cells

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Abstract—Among several sympathomimetic drugs, isoproterenol, epinephrine and norepinephrine significantly potentiated the cytotoxic effect and the uptake of MMC into rat ascites hepatoma AH130 cells in *in vitro* experiments, but salbutamol, tulobuterol and phenylephrine did not. The enhancement of MMC uptake into AH130 cells by isoproterenol and norepinephrine was diminished by propranolol but not by phentolamine. These results indicate that the cytotoxic effect and the uptake of MMC is potentiated by the stimulation of β -adrenoceptors, but not by stimulation of β_2 - and α -receptors.

We have recently shown that isoproterenol, a β -adrenergic stimulant, enhanced the cytotoxic effect of mitomycin C (MMC) on HeLa cells and some rat ascites hepatoma (AH) cells (1, 2). Furthermore, it has been suggested that cyclic adenosine 3':5'-monophosphate (cyclic AMP) in AH44 and AH130 cells elevated by this drug increased the uptake of MMC (3). The present paper deals with the effects of several sympathomimetic drugs on the cytotoxicity and the uptake of MMC in AH130 cells.

Agents obtained from commercial sources were mitomycin C (MMC, Sankyo Co.); l-isoproterenol hydrochloride, l-epinephrine bitartrate, l-norepinephrine hydrochloride, and dl-propranolol hydrochloride (Sigma Chemicals Co.); and dl-phentolamine mesylate (Ciba-Geigy Japan Ltd.). dl-Salbutamol hemisulfate and dl-tulobuterol hydrochloride were kindly provided by Hokuriku Seiyaku Co., Katsuyama.

Rat ascites hepatoma AH130 cells were maintained serially by the intraperitoneal passage at weekly intervals in female Donryu rats (weight 100–150 g, Shizuoka Laboratory Animal Center). The cells were withdrawn from the abdominal cavity of a rat at 7 to 9 days after the their implantation, washed with Hanks' solution to remove red blood cells

and suspended in Eagle's minimum essential medium (Eagle's MEM) to a concentration of about 4×10^5 cells/ml. The cells were preincubated with an adrenergic agonist for 15 min and then exposed to MMC for 30 min at 37°C. The treated cells were washed with Hanks' solution, suspended in Eagle's MEM-supplemented 10% fetal calf serum and cultured in 35-mm plastic petri dishes (Falcon 3001) in a 5% CO₂ incubator for 2 days at 37°C. The viable cells were assayed by the staining method with 0.2% Trypan Blue.

Cells were pretreated with an adrenergic agonist in the presence or the absence of an antagonist in phosphate-buffered saline for 15 min. After cells were further incubated with MMC (10 μ g/ml) for 30 min, the contents of MMC in the supernatant of the incubation mixture were measured by analytical high performance liquid chromatography [HPLC, Yanaco L-5000 liquid chromatograph equipped with a Yanaco M-315 spectromonitor; Yanapak ODS-A stainless column, 25 cm $\times$ 4.6 mm i.d.; mobile phase: 0.01 M phosphate buffer (pH 6.0): methanol (70:30, V/V); flow rate: 1.0 ml/min; wave length: 363 nm] according to the method of Hartigh and Oort (4); and the amounts of MMC taken up into the cells

were determined from the decreased amount of MMC in the supernatant as described elsewhere (3).

Figure 1 shows the effects of sympathomimetic drugs on the cytotoxic effect and the uptake of MMC. Isoproterenol, epinephrine and norepinephrine significantly potentiated the cytotoxicity of MMC, and

1×10^{-7} M of each drug was the most effective dose. However, salbutamol and tulobuterol, which were reported to act as β_2 -agonists (5), and phenylephrine, an α -agonist, hardly influenced the cytotoxic effect of MMC (Fig. 1A). The dose-response curve of MMC was shifted to the left by the pretreatment with isoproterenol, epinephrine or norepinephrine (data not shown). The effects of these drugs on the uptake of MMC are shown in Fig. 1B. The cells treated with isoproterenol, epinephrine or norepinephrine took up MMC about twice as much as non-treated cells. However, salbutamol, tulobuterol and phenylephrine did not change the uptake of MMC. Thus, it was confirmed that the enhancement of the cytotoxic effect of MMC was related to the increase of the uptake of MMC by β -adrenergic agonists, except for β_2 -agonists. It is known that rat liver cells contain only β_2 -receptor among the subtypes of β -adrenoceptor (6). On the other hand, we have suggested that rat ascites hepatoma cells may possess some receptors having similar properties to the mammalian β_1 -receptor because their adenylate cyclase hardly responded to β_2 -agonists, and the enzyme activity stimulated by isoproterenol was more selectively inhibited by β_1 -antagonists in hepatoma cells than in normal rat liver cells (7). This study also indicates that β_2 -agonists are not potent for enhancing the cytotoxic effect of MMC and not potent for increasing the uptake of MMC.

Our recent papers (1, 2) have indicated that isoproterenol elevated intracellular cyclic AMP via β -adrenoceptors, and MMC prolonged the term of the high cyclic AMP level, suggesting that the intracellular cyclic AMP elevated by the combined action of isoproterenol and MMC enhanced the uptake and the antitumor effect of MMC. The enhancement of MMC uptake by isoproterenol or norepinephrine was diminished by the β -antagonist propranolol, but not diminished by the α -antagonist phentolamine (Fig. 2). Phenylephrine which acts through α -adrenoceptors was not effective on the cytotoxicity and the uptake of MMC (Fig. 1). Consequently, this study indicates that the enhanced cytotoxicity of MMC is obtained

Fig. 1. Effect of sympathomimetic drugs on the cytotoxicity and the uptake of MMC to AH130 cells. Cells [4×10^5 cells/ml in (A) and 10^7 cells/ml in (B)] were incubated with various concentrations of each sympathomimetic drug for 15 min and then exposed to MMC [$0.03 \mu\text{g/ml}$ in (A) and $10 \mu\text{g/ml}$ in (B)] for 30 min. ●: isoproterenol, ▲: epinephrine, ■: norepinephrine, □: salbutamol, ×: tulobuterol, △: phenylephrine.

Fig. 2. Influence of propranolol and phentolamine on the effect of isoproterenol and norepinephrine on the uptake of MMC in AH130 cells. An adrenergic antagonist (10^{-5} M) was added to the cells (10^7 cells/ml) and then they were incubated with an agonist (10^{-7} M) for 15 min. The pretreated cells were exposed to MMC ($10 \mu\text{g/ml}$) for 30 min. Values are the mean $\pm$ S.E. of 3 experiments. * $P < 0.05$: Significantly different from the control.

through an increase in the uptake of the antitumor agent by the stimulation of β -adrenoceptors, but not by the stimulation of β_2 -receptors in rat ascites hepatoma cells.

References

- 1 Miyamoto, K., Sanae, F., Iwasaki, M. and Koshiura, R.: Effect of β -adrenergic stimulants on cytotoxicity of mitomycin C in HeLa cells. Japan. J. Pharmacol. **32**, 1019–1026 (1982)
- 2 Matsunaga, T., Iwasaki, M., Miyamoto, K. and Koshiura, R.: Relationship between enhancement of cytotoxic effect of mitomycin C and increase of intracellular cyclic adenosine 3':5'-monophosphate by isoproterenol in rat ascites hepatoma cells. J. Pharmacobiodyn. **6**, 25–32 (1983)
- 3 Miyamoto, K., Matsunaga, T., Waki, Y. and Koshiura, R.: Enhancement of mitomycin C uptake by isoproterenol in rat ascites hepatoma. J. Pharmacobiodyn. (in press)
- 4 Hartigh, J.D. and Oort, W.J.: High-performance liquid chromatographic determination of the antitumor agent mitomycin C in human blood plasma. Anal. Chim. Acta **127**, 47–53 (1981)
- 5 Minneman, K.P., Hegstrand, L.R. and Molinoff, P.B.: The pharmacological specificity of beta-1 and beta-2 adrenergic receptors in rat heart and lung in vitro. Mol. Pharmacol. **16**, 21–33 (1979)
- 6 Minneman, K.P., Hedberg, A. and Molinoff, P.B.: Comparison of beta adrenergic receptor subtypes in mammalian tissues. J. Pharmacol. Exp. Ther. **211**, 502–508 (1979)
- 7 Miyamoto, K., Matsunaga, T., Takemoto, N., Sanae, F. and Koshiura, R.: Studies on responsiveness of hepatoma cells to catecholamines: II Comparison of β -adrenergic responsiveness of rat ascites hepatoma cells with cultured normal rat liver cells. Japan. J. Pharmacol. **38**, 101–108 (1985)