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MODIFICATION OF THE SENSITIVITY OF CHO CELLS TO MITOMYCIN C BY DIBUTYRYL CYCLIC AMP

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SUMMARY: The survival of CHO cells exposed to mitomycin C was decreased three times that of the cells treated with 1 mM dibutyryl cyclic AMP before mitomycin C treatment, as compared to the absence of treatment with this cyclic nucleotide. The sensitization effect began at 3 - 4 hours after the start of pre-treatment, reached a maximum at around 10 hours and continued to be effective. Post-treatment with the cyclic nucleotide for more than 12 hours increased the survival of CHO cells exposed to mitomycin C.

Certain hypoxic cell radiosensitizers have an enhanced effect on some chemotherapeutic agents prescribed for patients with cancer (1,2). On the other hand, it has been reported that sensitivity of cells to ionizing radiation is modified by the treatment of the cells with drugs such as methylxanthine derivatives and dibutyryl cyclic AMP(DB-cAMP), which are known to increase the intracellular cyclic AMP levels (3-9). Thus, the sensitivity of cells to the chemotherapeutic agents may be modified also by compounds which alter intracellular cyclic AMP levels. We now report that DB-cAMP sensitized the cell-killing effect of MMC, while post treatment with this nucleotide increased the survival of these cells.

MATERIALS AND METHODS

Cell culture: CHO cells originally derived from a Chinese hamster ovary, were obtained from Dr.N.Inui of the Biological Experimental Center, Japan Salt and Tobacco Monopoly Corporation, and were cultured in MEM supplemented with 10 % calf serum (Flow Laboratories).

Chemicals and treatment of cells: Cell treatment with MMC was performed as follows: 6 x 10^4 cells were seeded in a 35 mm

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in diameter plastic dish (Corning Grass Works), and incubated for 2 days. MMC, kindly provided by Kyowahakko Co.Ltd. was added to the culture medium and the incubation continued for another 1 hour. DB-cAMP, kindly provided by Yamasa Co.Ltd. was added to the culture at a final concentration 1 mM and the incubation continued. The cells were then rinsed twice with MEM plus 10 % calf serum. Effect of MMC and modifying action of DB-cAMP were determined by the colony forming ability of the treated cells cultured in a plastic dish (60 mm in diameter), for 10 days.

RESULTS

The survival of CHO cells treated with various concentrations of MMC, and modification by pre-treatment with DB-cAMP are shown in Fig.1. Survival of the CHO cells was little altered with concentrations of MMC less than 1 x 10^{-6} M, but was exponentially decreased at higher concentrations, that is about 0.5 % at the concentration of 1 x 10^{-5} M. When the cells were

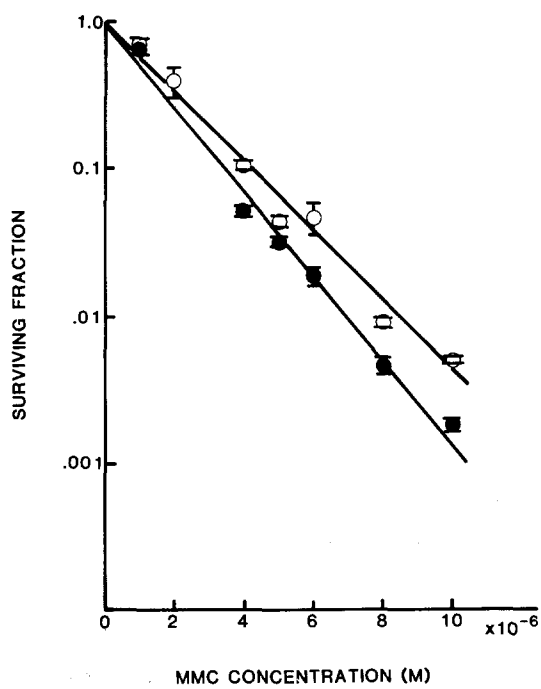

Fig. 1. Effect of pre-treatment with DB-cAMP on survival of CHO cells exposed to MMC in various concentrations. Survival of the cells treated with MMC alone (o) and pre-treated with 1mM DB-cAMP before MMC treatment (●) was determined as described in the text. Pre-treatment with 1mM DB-cAMP was begun 16 hours before MMC treatment. Each point represents the mean ± S.E. for 3 experiments. Values are normalized to the mean of the non-treated controls.

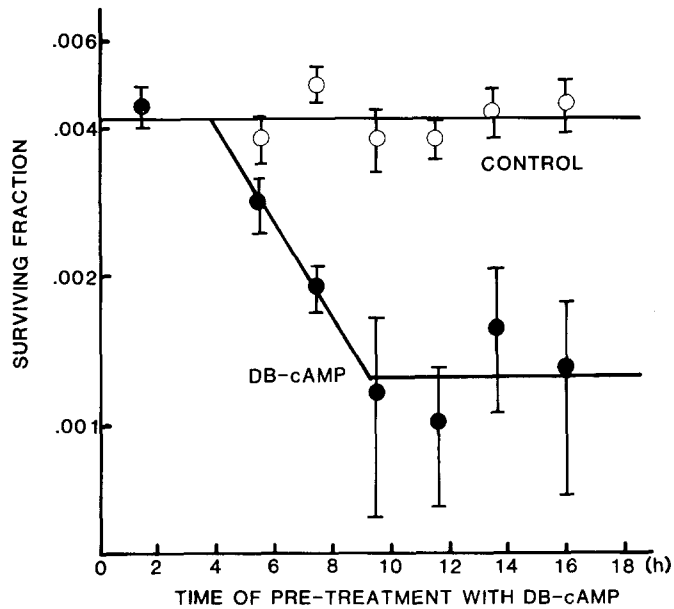

Fig.2. Relationship between the effect of pre-treatment with DB-cAMP on survival of CHO cells exposed to MMC and the time of pre-treatment. Survival of the cells treated with MMC in the concentration of 1 x 10^{-5} M (o) and those treated with MMC at various times after pre-treatment with 1mM DB-cAMP (●) were determined as described in the text. Each point represents the mean ± S.E. for 3 experiments. Values are normalized to the mean of the controls with no treatment.

treated with 1 mM DB-cAMP for 16 hours before the MMC treatment, the survival was remarkably enhanced compared with cells treated with MMC alone. The survival of the cells pre-treated with DB-cAMP was about 1/3 of the non-pre-treated cells at 1 x 10^{-5}M of MMC.

When the intervals between the start of pre-treatment with DB-cAMP and subsequent exposure with 1 x 10^{-5} M MMC were changed, the results were as shown in Fig.2. When the intervals were over 3 to 4 hours, the survival began to decrease, and the effect of the pre-treatment with DB-cAMP was maximal at the interval of about 10 hours then reached a plateau.

The effects of the post-treatment with 1 mM DB-cAMP on the survival of cells treated with MMC are shown in Fig 3. The survival began to increase 6 to 12 hours after the treatment with

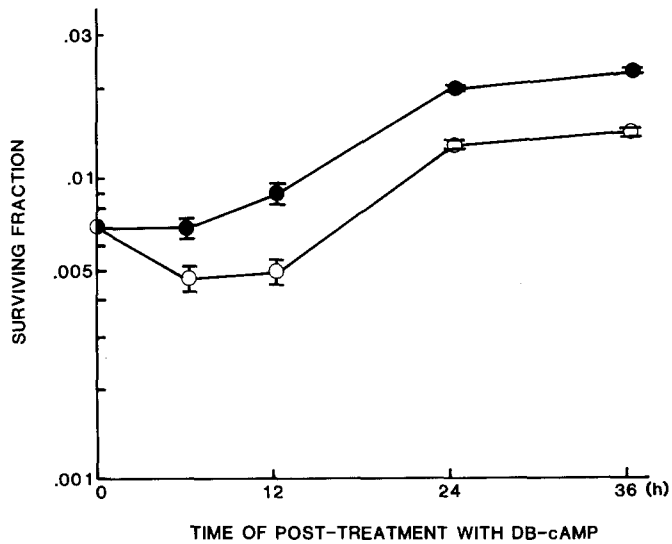

Fig.3. Relationship between the effect of post-treatment with DB-cAMP on survival of CHO cells exposed to MMC and the time for the post-treatment. Survivals of the cells treated with MMC in the concentration of 1 x 10^{-5} M alone (o) and those treated with 1mM DB-cAMP for various times after MMC treatment (●), were determined as described in the text. The cells were cultured for the post-treatment with DB-cAMP before trypsinization to determine the colony forming ability. Each point represents the mean ± S.E. for 3 experiments. Values are normalized to the mean of the controls non-treated with MMC.

MMC and such may reflect the recovery of cells from the MMC induced damage. Increases in the survival in case of post-treatment with DB-cAMP were greater than the case without the treatment. The increase in the survival due with post-treatment with DB-cAMP was statistically significant.

DISCUSSION

Pre-treatment with DB-cAMP decreased the survival of the CHO cells exposed with MMC. It was obvious that 1 mM DB-cAMP itself had no influence on the survival of CHO cells, so it may be said that the pre-treatment with DB-cAMP sensitized the cell killing effect of MMC. On the other hand, post-treatment increased the survival.

Other workers found that the intracellular cyclic AMP levels were sharply increased by adding DB-cAMP to the culture of CHO

cells (10). We also found that the increase of intracellular cyclic AMP level by DB-cAMP treatment began immediately after the treatment, and reached a peak after 2 hr, the increase being 25 times over the controls. The level then rapidly decreased, reached several times the control level 4 hr after the treatment, and thereafter decreased gradually (11). Thus, the maximum sensitization to the effect of MMC by the pre-treatment of CHO cells with DB-cAMP may be attained 8 hours after the intracellular cyclic AMP level reaches a maximum. Therefore, intracellular events, occurring 1 - 6 hours after the maximum cyclic AMP level is attained, may relate to this sensitization. The effect of MMC may be exerted to a greater extent when the chromatin structure of the cell is relaxed as the mechanism of action of MMC apparently involves cross-linking (12). Two mechanisms of this sensitization effect of DB-cAMP are proposed, based on the above considerations. The first is that the increase of intracellular cyclic AMP level may alter the cell cycle, and the cells may be arrested at the S phase where the chromatin structure becomes loose for DNA replication. The second is that the structure of chromatin is relaxed by the intracellular mechanisms followed by the increased cyclic AMP level in the cells. In case of the second assumption, Langan et al. (13) proposed a model for change of the chromatin structure by phosphorylation of histone when the intracellular cyclic AMP level was increased. If such a relaxed configuration of chromatin does occur, the enhanced effect of MMC with DB-cAMP can probably be explained.

Post treatment with DB-cAMP appears to accelerate the recovery of cells from the damage induced by MMC. With regard to X-ray (8) and UV irradiation (14), the recovery of JTC-11 or CHO cells was not induced only by the post-treatmemt with DB-cAMP,

but by both pre- and post-treatment with this nucleotide. Thus, modification of the effects of MMC by DB-cAMP may differ from that of the effects caused by X-ray and UV irradiation.

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