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Methylglyoxal bis(butylamidinohydrazone) exhibits antitumor effect on human malignant melanoma cells but reduces the antitumor action of cisplatin

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The antitumor effect of a polyamine biosynthetic pathway inhibitor methylglyloxal bis(butylamidinohydrazone) (MGBB) on human malignant melanoma (HMG) cells and its combination effect with cisplatin were investigated. The growth of cultured HMG cells was inhibited in a dose dependent manner by either MGBB or cisplatin; complete inhibition of cell proliferation was attained with 5 μg/ml of MGBB or 50 μg/ml of cisplatin. Pretreatment of HMG cells with MGBB diminished the antitumor action of cisplatin. The cultured HMG cells were inoculated in nude mice and aliquots of the resulting solid tumors (HMG tumor) were transplanted. The growth of transplanted HMG tumors in mice was inhibited markedly by cisplatin (3.8 mg/kg) and moderately by MGBB (10 or 20 mg/kg). The *in vivo* antitumor effect of cisplatin was also reduced by combined treatment with MGBB.

Key words: Cisplatin, human malignant melanoma cells, methylglyoxal bis(butylamidinohydrazone).

Introduction

Inhibitors of the polyamine biosynthetic pathway have received considerable attention for possible use in the treatment of cancer.[1–3] Methylglyoxal bis(butylamidinohydrazone) (MGBB) is a potent inhibitor of three polyamine-synthesizing enzymes, i.e. ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase and spermidine synthase,[4] and also inhibits the growth of human erythroid leukemia K 562 cells.[5] In order to achieve more effective chemotherapeutic results, however, the combinations of polyamine biosynthetic inhibitors and other antitumor agents have been investigated.[6]

In the present study, we investigated the antitumor effect of MGBB in combination with cisplatin, which shows effects on a number of solid tumors[7] and malignant melanoma,[8] on human malignant melanoma (HMG) cells.

Materials and methods

Chemicals

MGBB was synthesized as described previously.[9] Purity was checked by nuclear magnetic resonance and infrared spectrophotometric analysis. Cisplatin was obtained from Nippon Kayaku. (Tokyo, Japan) All other chemicals were products of Nacalai Tesque (Kyoto, Japan).

Animals

Female nude mice (BALB/C *nu/nu*) were purchased from Clea Japan (Tokyo, Japan). All animals were maintained in a air-conditioned facility and given food and water *ad libitum*

Cell culture

HMG cells were established in our laboratory.[10] They were grown in RPMI 1640 medium (Gibco, Grand Island, NY) with 20% fetal calf serum at 37°C under a closed system. HMG cells in log-phase growth were harvested after a short exposure to 0.25% trypsin. The cells were diluted to an initial density of 5×10^4 cells/ml for the culture. MGBB dissolved in phosphate buffered saline (PBS) was added to the culture medium. HMG cells were exposed to MGBB for 48 h to attain the polyamine depletion in the tumor cells

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before cisplatin treatment *in vitro*. For the cisplatin treatment, the cells were exposed to cisplatin for 1 h, washed with fresh medium and recultured. Cell number was determined by a hemocytometer.

Evaluation of tumor growth

HMG cells were inoculated on the right shoulder of nude mice. The resulting solid tumors (HMG tumors) were chopped into 3 mm cubes and transplanted s.c. to the same region of nude mice. Eight days later MGBB and cisplatin dissolved in physiological saline were administered i.p. into the HMG tumor bearing mice, at doses of 10 or 20 mg/kg of body weight every day and at a dose of 3.8 mg/kg of body weight every fifth day, respectively. Control mice were given the same volume of the saline by i.p. injection. The size of the tumor was measured with calipers using the formula; length × width^2 × 0.5.

Results

Antitumor effects of MGBB and cisplatin on the HMG cells in culture

Decreasing rates of proliferation of HMG cells were observed with increasing concentrations of MGBB (Figure 1) and cisplatin (Figure 2). The growth of the cancer cells was completely suppressed by the presence of 5 μg/ml MGBB or 50 μg/ml cisplatin. However, the antitumor effect of cisplatin was evidently diminished by pretreatment of tumor cells with MGBB (Figure 3).

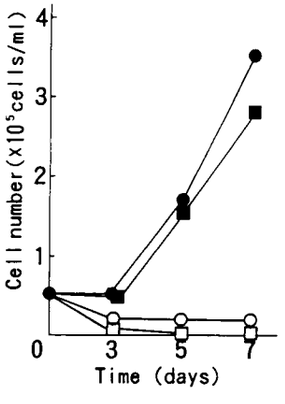

Figure 1. Effect of MGBB on the growth of HMG cells. These cells were diluted to an initial density of 5 × 10^4 cells/ml and grown in the absence (●) or presence of 2.5 (■), 5 (○) and 10 (□) μg/ml of MGBB for the times indicated.

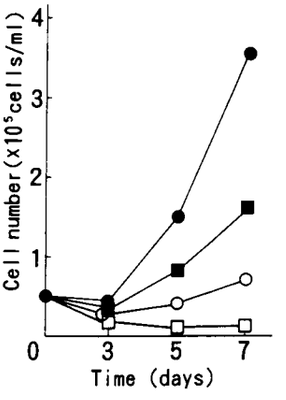

Figure 2. Effect of cisplatin on the growth of HMG cells. These cells were diluted to an initial density of 5 × 10^4 cells/ml and grown in the absence (●) or presence of 5 (■), 10 (○) and 50 (□) μg/ml of cisplatin for the times indicated.

In vivo antitumor effects of MGBB, cisplatin and their combination on transplanted HMG tumors

HMG tumors transplanted in the nude mice was grown for 8 days, and MGBB or/and ciplatin were administered i.p. As shown in Figure 4, the growth

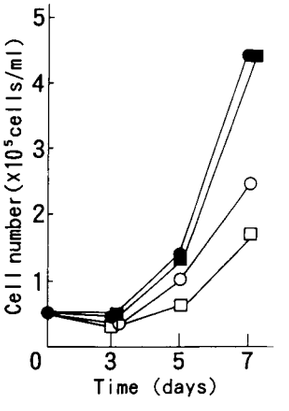

Figure 3. Effect of MGBB on the antitumor effect of cisplatin on cultured HMG cells. These cells were diluted to an initial density of 5 × 10^4 cells/ml and grown in the absence (●, □) or presence of 5 μg/ml (■, ○) of MGBB for 48 h. Then these cells were treated with 5 μg/ml of cisplatin (○, □) for 1 h, washed with fresh medium and then cultivated again in the absence of MGBB and cisplatin. Cell numbers were determined at the times indicated.

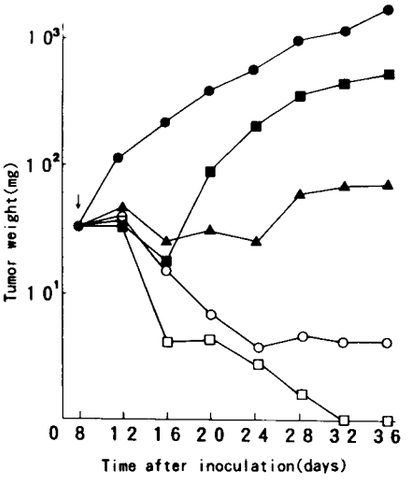

Figure 4. Weights of transplanted HMG tumors in mice treated with cisplatin, MGBB and a combination. Eight days after the transplantation of HMG tumors to nude mice, MGBB and cisplatin were injected i.p. to the mice at a dose of 10 or 20 mg/kg every day and 3.8 mg/kg every fifth day, respectively. Control mice were given the same volume of the saline. Control, ●; MGBB (20 mg/kg), ■; cisplatin (3.8 mg/kg), □; MGBB (10 mg/kg) + cisplatin (3.8 mg/kg), ▲; MGBB (20 mg/kg) + cisplatin (3.8 mg/kg), ○.

of HMG tumors was suppressed by these treatments. However, the antitumor activity of cisplatin was again reduced by combination with MGBB.

Discussion

Inhibitors of polyamine biosynthetic enzymes are known to exhibit antitumor activity on various cancer cells.[1,3] In the present study, MGBB was shown to strongly inhibit the growth of HMG cells *in vitro*, but only moderately inhibit the growth of HMG tumors *in vivo*. This discrepancy of efficacy may be due to the fact that polyamines were supplied from the diet and other tissues to HMG tumors in mice, but not in HMG cells in culture. Therefore, combined treatment with other drugs not related to polyamine metabolism have been employed for the *in vivo* experiment.[6]

In this study cisplatin was investigated in combination with MGBB for human malignant melanoma cell treatment. However, contrary to our expectation, combination with MGBB drastically reduced the antitumor effect of cisplatin.

Oredsson *et al.*[11] have reported that the cytotoxicity of cisplatin was decreased when given together with difluoromethylornithine (DFMO), an inhibitor of ODC, to 9L rat brain cells *in vitro*. Shrestha *et al.*[12] also reported that the cytotoxicity of cisplatin was suppressed when given together with DFMO to ST-2 human gastric cancer *in vivo*. However, other workers[13] have reported enhanced cytotoxicity toward tumor cells when the cells were treated with DFMO after cisplatin treatment. These results suggest that the schedule of administration of these drugs might be important.

Zwelling *et al.*[14] have reported that interstrand cross-linkings are highly toxic to tumor cells. The cisplatin molecule has two replaceable chlorides, which are about 3.3 Å apart. The binding sites of cisplatin on DNA should therfore be around 3.3 Å apart, facilitating cross-linking between the cisplatin and DNA. In a polyamine deficient state caused by treatment with inhibitors of polyamine synthetic enzymes, the structure of DNA is unstable and the DNA strands become movable. Thus, cisplatin can hardly cross-link these movable DNA strands and this results in poor production of antitumor efficacy.[15] Tofilon *et al.* have reported that DFMO reduced DNA interstrand cross-linking with cisplatin. Our results in the present study also suggest that the antitumor activity of cisplatin is reduced by combination with MGBB, diminishing cross-linking formation between cisplatin and DNA.

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