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EFFECTS OF CALMODULIN ANTAGONISTS ON HUMAN OVARIAN CANCER CELL PROLIFERATION IN VITRO

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Examined were effects of calmodulin antagonists (W-5 and W-7) on proliferation of two kinds of human cell lines, designated HR and KF, derived from serous cystadecarcinoma of the ovary. Although both W-5 and W-7 inhibited their cell proliferation in vitro, the degree of inhibition was more marked with W-5 rather than with W-7. HR cells had higher sensitivity to cisplatinum than KF cells, while KF cells had higher sensitivity to adriamycin. Combinations of calmodulin antagonists and anti-cancer drugs resulted in adjuvant effects with regard to the inhibition of their cell proliferation in vitro.

Calcium ion and calmodulin has been considered to play important roles in regarding cell proliferation (1). It has also been reported that extra-cellular calcium is necessary for DNA synthesis and cell proliferation in normal cells but not in cancer cells (2). Although the reason(s) for the uncontrolled cell proliferation in tumors is not understood, it has been suggested that cancer cells have increased amounts of calcium binding proteins (e.g., calmodulin), through which the initiation of DNA synthesis can be permanently activated without the extra-cellular calcium surge serving as a trigger (3). Recently, it has been reported that a positive correlation exists between the growth rate of 3 Morris hepatomas and their corresponding levels of calmodulin, suggesting the involvement

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Abbreviations used are; W-5, N-(6-aminohexyl)-1-naphthalene-sulfonamide. W-7, N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide.

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of calmodulin in tumor cell growth regulation (4). Subsequently, calmodulin antagonists (W-5 and W-7) have been proved to be inhibitors of cell proliferation (5, 6).

In this study, to further clarify the mode of action of W-5 and W-7 on cancer cell proliferation, we examined inhibitory effects of W-5 and W-7 on cell proliferation and their combined effects with anticancer drugs by using two kinds of cell lines derived from human ovarian cancers.

MATERIALS AND METHODS

Materials

N-(6-aminohexyl)-1-naphthalenesulfonamide (W-5) and N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) were purchased from Rikaken Co., Ltd. Nagoya, Japan.

Cells and cell culture

HR cells were established from ascites of patient with serous cystadenocarcinoma of the ovary on January 1, 1983, and the passage number is about 63. KF cells were established from tissue of patient with serous cystadenocarcinoma of the ovary on November 20, 1982, and the passage number is about 71. Tumorigenicities of both HR and KF cell lines in the nude mouse were 100%. The doubling time and the plating efficiency of the fomer cells were 19 hr and 49%, respectively, while those of the latter cells were 16 hr and 27%. Both cell lines were cultivated as reported previously (7). Briefly, cells were incubated in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin and streptomycin (100 units/ml and 100 µg/ml, respectively: Grand Island Biological Co.) in a 5% CO_2 atmosphere at 37°C. The medium was changed every 3 days, and the cells were passed when confluency was achieved. Morphological features of both cell lines are presented in Fig. 1.

In vitro treatment

For determining the effects of W-5 and W-7 on the proliferation of both cell lines, 10^4 cells were seeded in 24 well Nunc multidishes (Nunc, Roskilde, Denmark), and incubated in a humidified atmosphere of 5% CO_2 at 37°C. After 24 hr of culture, various concentrations of calmodulin antagonists and/or anticancer drugs were added to the medium. Cells in each well were harvested after 3 days of additional culture, and counted using a hematocytometer. All counts were done in triplicate and the viability was assessed by trypan blue dye exclusion. Each drug was dissolved in fresh medium and filtered through a 0.22 µm Millipore filter (Millipore Corporation, Bedford, MA.). Concentrations and contact times with cells of W-5 and W-7 were selected according to the report of Hidaka et al (5). Cisplatinum and adriamycin were selected since each of these drugs has been shown to have some effect in patients with

ovarian cancer (8, 9). Concentrations of these drugs were defined by referring to reports of Morasca et al (10). To examine adjuvant effects by calmodulin antagonists, combinations of calmodulin antagonists and anticancer drugs were also tested. All values are presented as percent of controls.

RESULTS AND DISCUSSION

Morphological features of two kinds of permanent cell lines established from human ovarian cancers are shown in Fig. 1. Characterizationa of these cells were described in "MATERIALS AND METHODS". As shown in Fig. 2, calmodulin

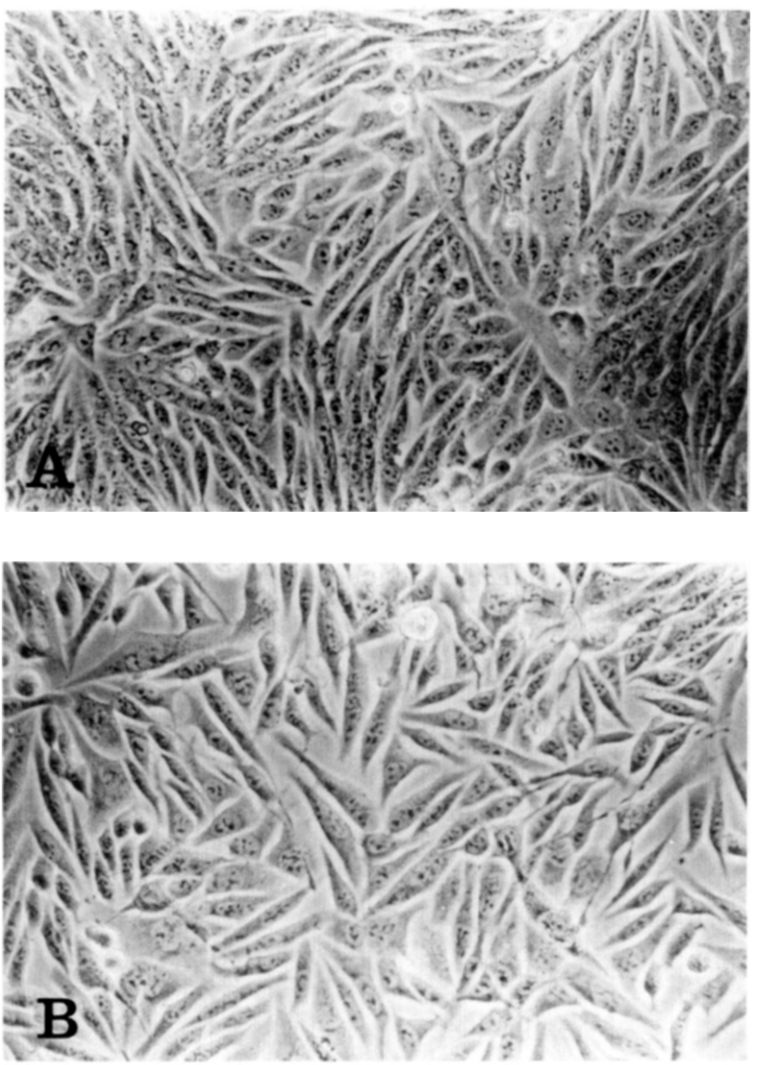

Fig. 1. A; HR cells. Confluent state. Phase contrast, x 200.
B; KF cells. Confluent state. Phase contrast, x 200.

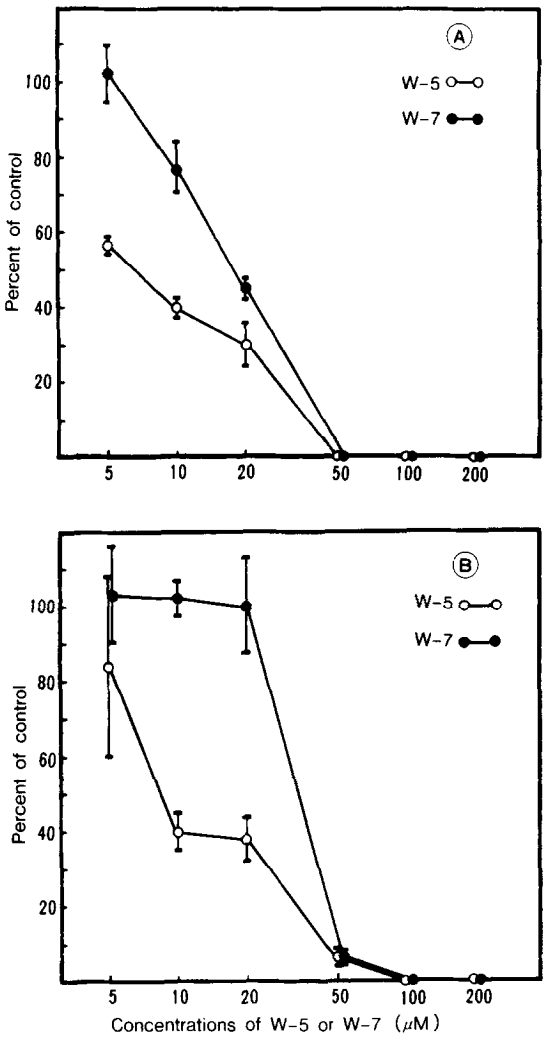

Fig. 2. Inhibitory effect of W-5 and W-7 on HR and KF cell proliferations. A; HR cell line, B; KF cell line. After incubating 10^4 cells in 24 well Nunc multi-dishes for 24 hr, W-5 or W-7 was added to give the final concentrations indicated. After 3 days of additional culture, cells in each well were harvested and counted using a hematocytometer. The number of cells in wells containing fresh medium alone was 15.5×10^4 for HR and 31.3×10^4 for KF and these numbers were used as controls. Each point represents the mean of 3 experimental cultures. Bars show S.D.

antagonists (W-5 and W-7) proved to be inhibitors of the proliferation of these cells in culture. Concentrations of W-7 required for 50% inhibition of the proliferation of HR and KF cells were 18 and 32 μM, respectively. These results are similar to previous reports (5, 11). On the other hand,

those of W-5 were 6.4 μM for HR cells and 8.6 μM for KF cells. W-7 did not inhibit KF cell proliferation below 20 μM (Fig. 2B). These results revealed that W-5 is a more effective inhibitor of both HR and KF cell proliferations than W-7. Previous similar experiment used chinese hamster ovary cells demonstrated that W-5, a chlorine-deficient analogue of W-7 that interacts with calmodulin only weakly, inhibited cell proliferation to lesser extent compared to W-7 (5). Although the mechanism which W-5 inhibits cell proliferation remains unclear, the present results differ from those reported by others (5, 12) possibly because the human cell lines used in the present study differ from chinese hamster ovary K_1 cells in several respects (e.g., biological features and heterogeneity). As shown in Fig. 3, the concentrations of cisplatinum producing 50% inhibition of cell proliferation were 0.17 μg/ml for HR cells and 0.31 μg/ml for KF cells, while those of adriamycin were 0.013 μg/ml for HR cells and 0.004 μg/ml for KF cells. These results demonstrate that HR cells are more sensitive to cisplatinum while KF cells are more sensitive to adriamycin. In addition, we attempted to determine whether both W-5 and W-7 have adjuvant effects to these anticancer drugs. Concentrations of W-5 or W-7 that did not significantly affect cell proliferation were added to the medium with cisplatinum or adriamycin, and after 3 days of additional culture the cell number was counted and compared to cell number in well treated with anticancer drug alone. Adjuvant effects of W-5 and W-7 with adriamycin seemed to be greater than with cisplatinum (Fig. 4). With respect to the inhibition of KF cell proliferation, the adjuvant effect of W-7 with cisplatinum was greater than that of W-5, and as the concentrations of cisplatinum

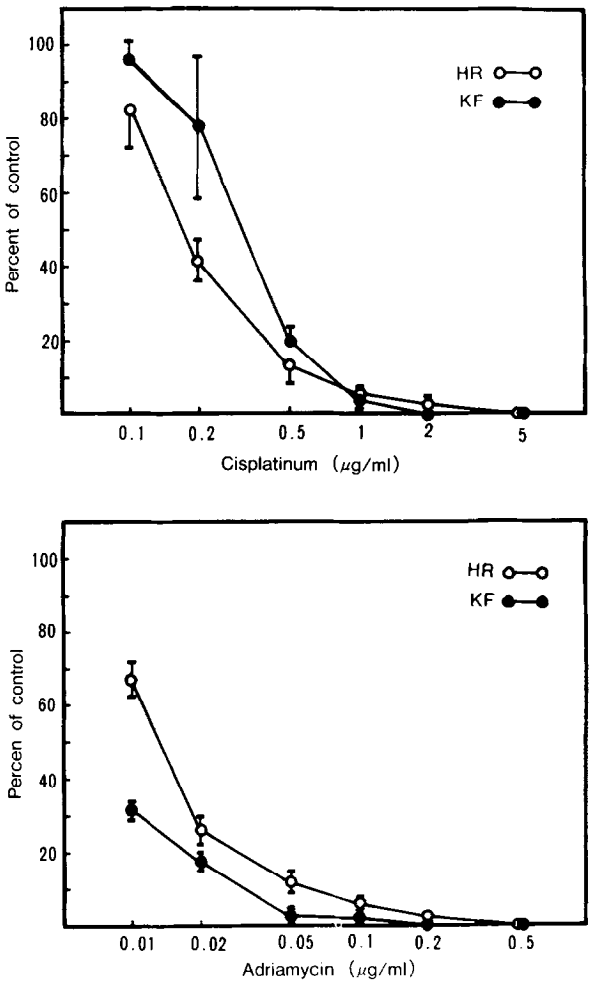

Fig. 3. Effect of cisplatinum and adriamycin on the proliferation of HR and KF cells in vitro. Cells were treated as described in Fig. 2.

increased, the adjuvant effects were greater. Regarding the HR cell proliferation, the adjuvant effect of W-5 to cisplatinum tended to be greater than that of W-7. On the other hand, at each concentration of adriamycin used, about 50% inhibition of proliferation of both HR and KF cells was observed on addition of W-5 or W-7. Calmodulin antagonists such as W-5 and W-7 are considered to penetrate the cell membrane and probably interact selectively with calmodulin. Boynton et al (13) have reported the importance of the

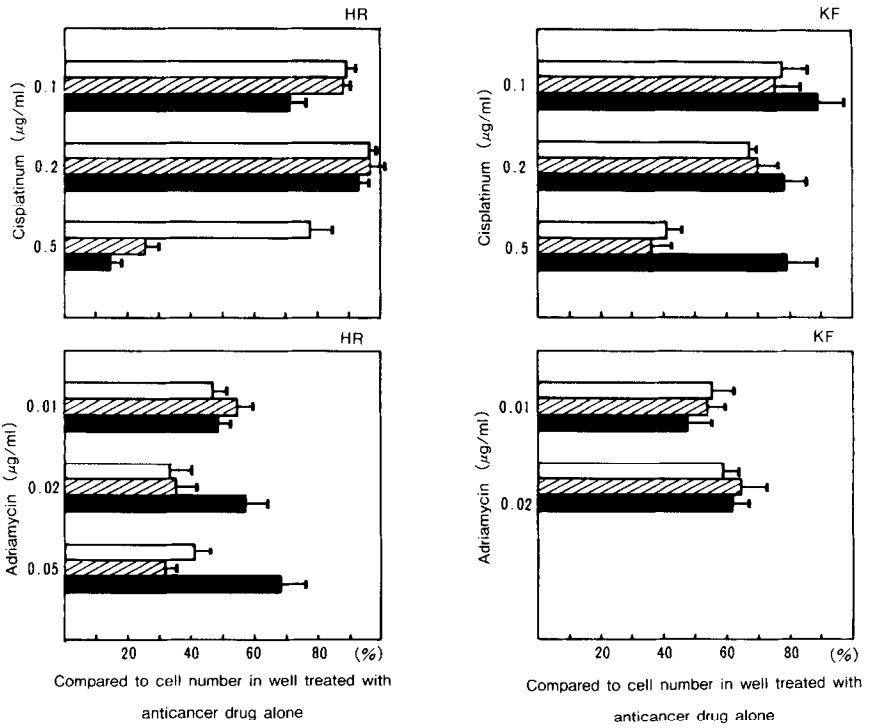

Fig. 4. Adjuvant effects of W-5 and W-7 with cisplatinum and adriamycin in the inhibition of HR and KF cell proliferation in vitro. Each point is presented as the percentage of the cell number in wells treated with anticancer drug alone. To examine adjuvant effects of W-5 and W-7 to cisplatinum and adriamycin, the concentrations of W-5 and W-7 which seemed to have no effect on cell proliferation with alone were used. ☐ ; W-7 (2 μM), ▨ ; W-7 (5 μM), ▩ ; W-5 (2 μM).

Ca^{2+}-calmodulin complex in DNA synthesis of rat liver cells. Thus, W-5 and W-7 may act by inhibiting the formation of the Ca^{2+}-calmodulin complex essential for the initiation of DNA synthesis in cancer cells.

The finding that both W-5 and W-7 not only inhibit cell proliferation but also enhance the inhibitory effects of cisplatinum and adriamycin may prove useful in the treatment of patients with cancer cells resistant to the anticancer drugs.

ACKNOWLEDGEMENT

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