

A Study of Cytocidal Effects According to the Type of Anticancer Agent Combined with OK-432

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Abstract: This study was performed for the evaluation of combined effects of OK-432 and anticancer agents on K562 cells in vitro.

On the growth curve, an increase of cytotoxic effects was not observed by co-operative action of OK-432 and Cisplatin, but such increase was found with OK-432 and VP-16. It was evident that there are the difference depending on the type of anticancer agent combined with OK-432.

When this difference was investigated on the cell cycle, G₂M phase accumulation was delayed with OK-432 and Cisplatin. On the other hand, this delay was not observed with OK-432 and VP-16. It was suggested that the degree of this G₂M phase accumulation might affect the cytotoxic effects on cancer cells.

Key Words: OK-432, Cisplatin, VP-16, Cell cycle

Introduction

OK-432 is a bacterial preparation of the Su strain of *Streptococcus pyogenes* which was developed based on the antitumor effects of hemolytic streptococci.¹⁾ Subsequent research has shown that the antitumor activity of OK-432 is manifested by activation of the immune system in the cancer bearing host, and it is clear that this drug has both direct effects and host-mediated effects.

Previous papers have reported the direct effects of OK-432, i. e., the clinical effects of intratumor injections of OK-432 alone in stomach cancer,²⁾ liver cancer³⁾ and cancer of the head and neck.⁴⁾ There have also been papers showing marked antitumor effects when OK-432 was used simultaneously with anticancer agents and indicating that these combined effects with anticancer agents are more effective than the administration of OK-432 alone.^{5,6,7)} However, it is still not clear which anticancer agents show the best antitumor effects when used concurrently with OK-432. Therefore, we studied how the combined effects with OK-432 are influenced by the type of anticancer agent using cultured cancer cells.

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Materials and Methods

The K562 cells, a human leukemia cell line, were used in this study. K562 cells were prepared for culture of 1.0×10^6 cells. Then, 10 ml of 0.5 KE/ml solution of OK-432 (supplied by Chugai Seiyaku) dissolved in RPMI1640 medium was placed in 100 mm Petri dishes which were incubated for 24, 48 or 72 hours in a CO₂ incubator (37 °C). The anticancer agents used were Cisplatin (supplied by Nippon Kayaku) and VP-16 (also supplied by Nippon Kayaku). They were added to the medium at same time as OK-432 at a concentration of 1.0 µg/ml of Cisplatin or 0.1 µg/ml of VP-16, and K562 cells treated with continuous exposure in the same way as OK-432. To determine the concentration of the anticancer drugs used, growth curves were made beforehand and the concentrations showing basically the same growth conditions as 0.5 KE/ml of OK-432 were selected. Samples were collected after 24, 48 and 72 hours, and the growth curves were prepared by counting the viable cells using Trypan blue stain.

Next, the samples were subjected to nuclear extraction using 0.1% Triton X-100 by means of the method of Sasaki.⁸⁾ Then, DNA staining was performed for 30 minutes using 50 µg/ml of Propidium iodide containing RNase, about 10,000 cells were measured using a FACScan (Becton Dickinson) and DNA histograms were prepared.

Results

1. Cell growth kinetics

OK-432 at 0.5 KE/ml had cytostatic effects on the growth curve of K562 cells, i. e., the cell proliferation was not completely stopped and the number of cells increased slightly even through there were the inhibition of cell proliferation. When 1.0 µg/ml of Cisplatin was administered concomitantly with this concentration of OK-432, the cell proliferation showed the same growth curve as when Cisplatin was administered alone, and the co-operative effects of OK-432 and Cisplatin were not ob-

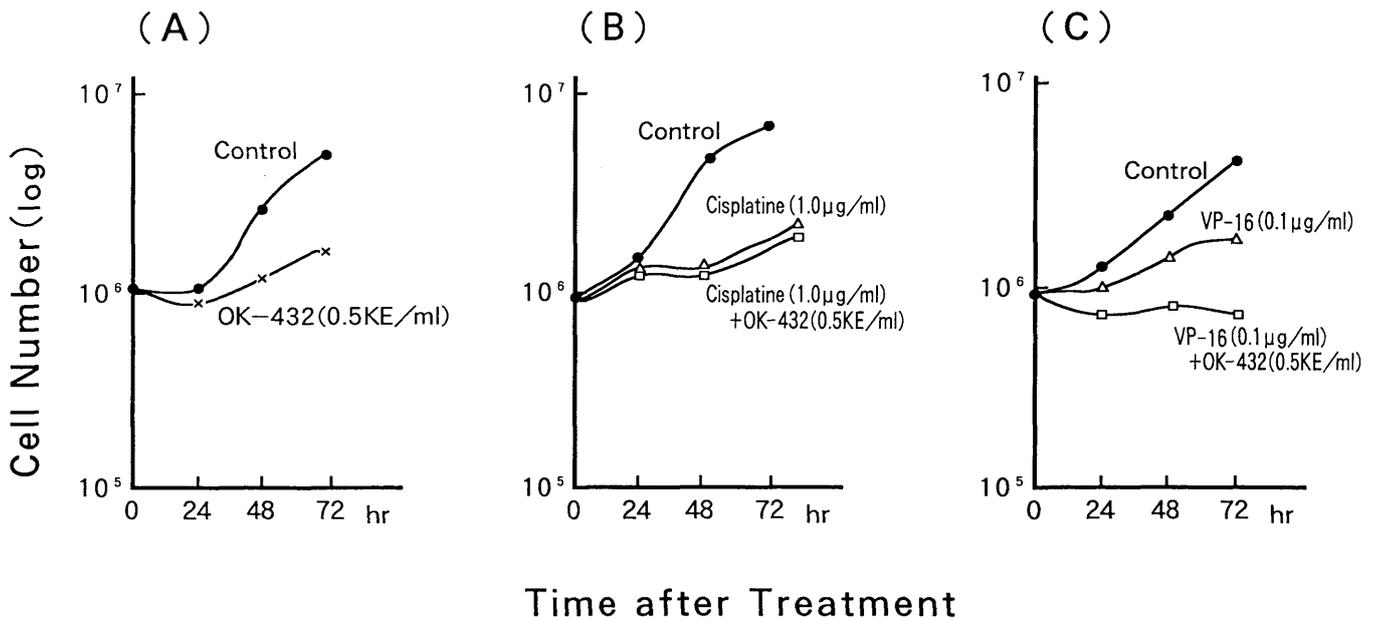


Fig. 1. Growth curve on K562 cells treated with anticancer drugs (A: Control. B: Cisplatin. C: VP-16.)

served. However, when 0.1 µg/ml of VP-16 was administered simultaneously, the inhibitory effects of cell proliferation were greater than those when VP-16 was administered alone (Fig. 1)

2. Cell cycle analysis

The changes, in the cell cycle of K562 cells treated with 0.5 KE/ml of OK-432, were G₁ phase accumulation at 48 hours and accompanied with the decrease of the S and G₂M phases. However, the increase of the S and G₂M phases were again seen at 72 hours when the cell proliferation appeared. After all, the accumulation of the G₁ phase was transient (Fig. 2).

The changes, in the cell cycle caused by Cisplatin alone, were the accumulation of the late S phase and G₂M phase at 24 hours, and more marked the accumulation in the G₂M phase at 48 and 72 hours. When OK-432 and Cisplatin were administered simultaneously, slight accumulation of the G₂M phase was observed at 24 hours. But the same each phase accumulation as in the control were seen at 48 hours. At 72 hours, the middle S phase appeared, but G₂M phase accumulation was not observed (Fig. 3).

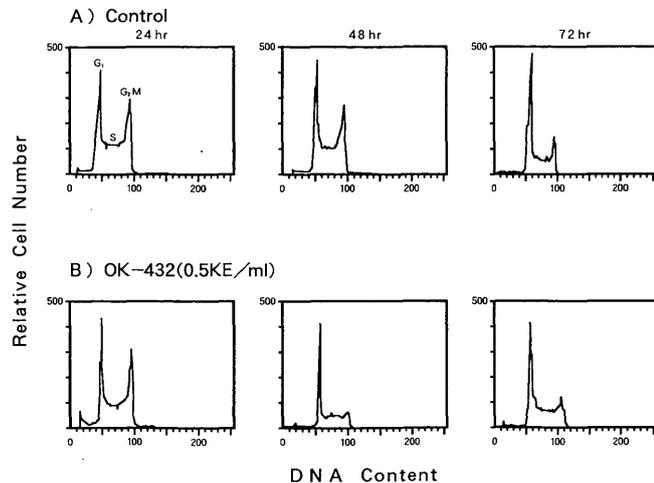


Fig. 2. DNA histograms on K562 cells treated with OK-432 alone (A: Control. B: OK-432.)

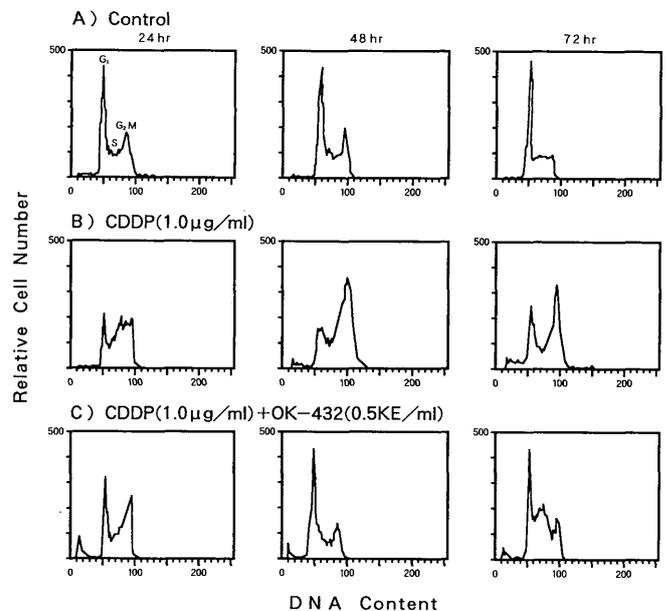


Fig. 3. DNA histograms on K562 cells treated with Cisplatin or Cisplatin combined with OK-432 (A: Control. B: Cisplatin alone. C: Cisplatin + OK-432.)

The changes in the cell cycle caused by VP-16 alone consisted of marked accumulation in the G₂M phase from 24 to 72 hours, with the most marked accumulation seen at 24 hours. When VP-16 and OK-432 were administered simultaneously, marked accumulation in the G₂M phase at 24 hours, about the same as that with VP-16 alone, was seen, and G₂M phase accumulation continued after 48 hours. However, viable cells at 72 hours, when the presence of dead cells was confirmed, showed the same DNA histogram as the controls (Fig. 4)

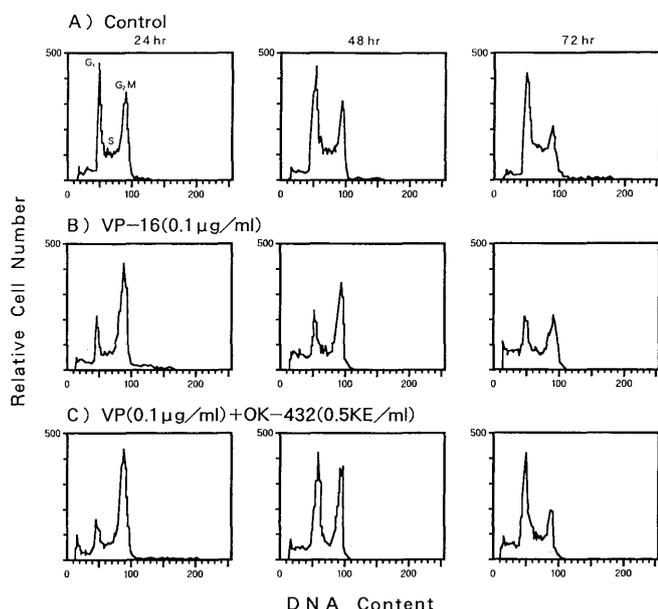


Fig. 4. DNA histograms on K562 cells treated with VP-16 or VP-16 combined with OK-432 (A: Control. B: VP-16 alone. C: VP-16 + OK-432.)

In this study of changes in the cell cycle (G₁ phase accumulation) by OK-432 alone at 48 hours, G₁ phase accumulation with the combined treatment of Cisplatin and OK-432 was the same as that in the DNA histogram for OK-432 alone, but the combination of VP-16 and OK-432 resulted in the same G₂M phase accumulation as with VP-16 alone.

Discussion

Clinical papers on the efficacy of intratumor injections of OK-432 combined with anticancer agents have been reported. The anticancer agents usually used concurrently include Adriamycin, Mitomycin C and Cisplatin,^{5,6,7,9)} and it has been reported that the combination of Adriamycin and OK-432 are highly effective from the standpoint of immunotherapy.⁷⁾ However, there have been no basic studies on which type of anticancer agent used together with OK-432, which shows direct effects, provides the best

co-operative effects. Therefore, we studied the effects on growth curves in the type of anticancer agent combined with OK-432. The reason for such differences by type of anticancer agents, if they exist, was also investigated with respect to influence on the cell cycle. The anticancer agents used in this study were Cisplatin and VP-16, which are both in wide clinical use at present. Since both of these agents are known to cause accumulation of cells in the G₂M phase,^{10,11)} the comparison of changes in the cell cycle was facilitated.

The results indicated that the combination of OK-432 and Cisplatin on the growth curve had about the same cytotoxic effects as each of the two agents alone. However, the combination of OK-432 and VP-16 showed clearly stronger cytotoxic effects than those seen when these agents were administered separately. These findings confirmed that there is a difference in cytotoxic effects in accordance with the type of anticancer agent used simultaneously with OK-432.

Since OK-432 causes accumulation of cells in the G₁ phase, the combined use of anticancer agents with a high sensitivity in the cell of G₁ phase is logical from the standpoint of the cell cycle. Cisplatin shows sensitivity in all phases of the cell cycle, especially in the G₁ phase at high concentrations.¹²⁾ VP-16 appears to show high sensitivity in the late S and G₂ phases.¹³⁾ Therefore, the combination of OK-432 and Cisplatin should have better cytotoxic effects than those of OK-432 and VP-16, but we had obtained the opposite results. In an investigation of changes in the cell cycle by combined use, OK-432 and VP-16 did not show a delay of accumulation to G₂M phase when compared with VP-16 alone. However, combined use of OK-432 and Cisplatin caused a delay of accumulation to G₂M phase when compared with Cisplatin alone. Many points are unclear concerning the significance of accumulation to the G₂M phase, but G₂M phase accumulation is considered to be a useful index in susceptibility tests of anticancer agents.^{14,15)} Since arrest in the G₂M phase lead to cell death, these findings agree with those obtained on growth curves in this study.

In cases where the combination of OK-432 and an anticancer agent can be expected to have direct antitumor effects, the type of anticancer agent use will probably differ depending on the type of cancer. However, it must also be understood that the effect of OK-432 differ depending on the type of anticancer agent.

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