Cytotoxicity of Anti-Cancer Drugs to Vascular Endothelial Cells and Inhibitory Effect of PGE₁

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Summary In this study, the degree of in vitro cytotoxicity of anti-cancer drugs, including 5-fluorouracil, doxorubicin, cis-diamminedichloroplatinum, and mitomycin C, to vascular endothelial cells collected from the carotid artery of cows was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide) assay. The results indicated that the viability of vascular endothelial cells decreased with the addition of all of the above anti-cancer drugs, and a positive correlation was found between cytotoxicity and the drug concentration, as well as between cytotoxicity and the length of incubation with the anti-cancer drugs. The cytotoxicity of doxorubicin, cis-diamminedichloroplatinum, and mitomycin C became evident very rapidly and was stronger than that of 5-fluorouracil. The protective effect of prostaglandin E₁ on the cytotoxicity caused by anti-cancer drugs was examined. The results also indicated that the addition of prostaglandin E₁ inhibited the decrease in the viability of vascular endothelial cells which had been induced by 5-fluorouracil. These findings suggested that anti-cancer drugs cause endothelial cell injury and that prostaglandin E₁ can protect this type of injury at least when induced by 5-fluorouracil.

Key Words: cytotoxicity of anti-cancer drugs, vascular endothelial cells, prostaglandin E₁, MTT assay

Recent advances in catheterization technique enabled to inject anti-cancer drugs continuously to the specific target organ. Although the use of intrahepatic arterial injection chemotherapy allows advanced liver cancer to be effectively treated, this therapy must often be discontinued due to the high incidence of thrombotic obstruction of the hepatic artery. Direct injury to vascular endothelial...
cells (ECs) by anti-cancer drugs as well as catheter-induced mechanical damage is thought to play a part in this thrombogenesis [1]. Accordingly, protection of ECs from cytotoxic action by anti-cancer drugs leads to a prolongation of arterial patency and an enhancement of transarterial infusion chemotherapy.

Prostaglandin E₁ (PGE₁) has recently been attracting attention from the standpoint of its cytoprotective effect [2, 3]. However, there has been no report which proved the protective effect of PGE₁ from cytotoxicity induced by anti-cancer drugs.

In this in vitro study, we attempted to evaluate the characteristics of the cytotoxicity of anti-cancer drugs on ECs. The inhibitory effect of PGE₁ was also examined.

MATERIALS AND METHODS

Cells. ECs from the carotid artery of cows (HH, a gift from the Japanese Cancer Research Resources Bank Cell, Tokyo) were used. They had been cultured for 8 to 12 generations in minimum essential medium (MEM) (Sigma Chemical Co., St. Louis, MO) to which 10% fetal bovine serum (FBS) (GIBCO BRL, Gaithersburg, Maryland, MD) had been added. The cells were seeded in 96-well microplates at a rate of 5 × 10⁴ cells/100 µl/well and were cultured under conditions of 37°C, and 5% CO₂ and 95% air.

MTT assay. The MTT assay [4] was employed to measure the viability of cultured ECs by using 3-(4,5-dimethylthiazol-2-yl)-3,5-Biphenyl tetrazolium bromide (MTT). After being cultured for 24 h, the cells were placed in MEM containing 400 µg/ml of MTT (Sigma Chemical Co.), and further cultured for 4 h. After the medium was removed by suction, a 100 µl/well of dimethyl sulfoxide (Wako Pure Chemical Industries Ltd., Osaka) was added to dissolve the resulting formazan, and the absorbance was measured at a wavelength of 545 nm with a control wavelength of 630 nm using an ELISA reader (Wellreader SK601, Seikagaku Corporation, Tokyo).

Effect of concentration and incubation time of various anti-cancer drugs on EC viability. The ECs were seeded into 96-well microplates at a rate of 5 × 10⁴ cells/100 µl/well, and were cultured for 24 h. Then the medium was replaced with a medium to which 5-fluorouracil (5Fu), doxorubicin (ADM), cis-diamminedi-chloroplatinum (CDDP), or mitomycin C (MMC) had been added. The concentrations of each of these anti-cancer drugs were 5, 25, or 200 µg/ml, respectively. ECs were further cultured for 1, 3, 6, 12, 24, and 48 h, and then an MTT assay was conducted according to the procedure described above.

Effect of PGE₁ on ECs. ECs were seeded into 96-well microplates at 5 × 10⁴ cells/100 µl/well, and then cultured for 24 h. The medium was then changed to a medium to which PGE₁ (Prostandin, Ono Yakuhin Kogyo Co., Ltd., Osaka) had been added to be 10⁻¹, 1, 10, 10², 10³, or 10⁴ ng/ml. The ECs were further cultured for 24 h, and an MTT assay was conducted.
Effects of PGE_1 on cytotoxicity of anti-cancer drugs to ECs. The ECs were seeded in 96-well microplates at $5 \times 10^4$ cells/100 µl/well, and then cultured for 24 h. The medium was changed to a medium to which the various anti-cancer drugs (5, 25, or 200 µg/ml) and PGE_1 (1 or 100 ng/ml) had been added. The ECs were further cultured for 12, 24, and 48 h, and an MTT assay was conducted. Controls were prepared by culturing ECs for 0 h in an MEM/10% FBS medium after the medium exchange.

Influence of 5Fu on capability of ECs to produce prostaglandin I_2 (PGI_2) and effect of PGE_1. ECs were seeded in 96-well microplates at $5 \times 10^4$ cells/100 µl/well, and cultured for 24 h. Then the medium was changed to a medium to which 5Fu and PGE_1 had been added to be 5 µg/ml and 1 ng/ml, respectively. ECs were further cultured for 12, 24, and 48 h, and then washed 3 times with phosphate-buffered saline (PBS). After the ECs were allowed to stand for 15 min in the medium, calcium ionophore A23187 (Sigma Chemical Co.) was added to the medium to be 5 µg/ml. After standing for 15 min, the concentration in the medium of 6-keto PGF_1α, a stable metabolite of PGI_2, was measured by RIA. Controls were prepared by culturing ECs for the same lengths of time (12, 24, and 48 h) in an MEM/10% FBS medium after the medium exchange. The results were expressed as a percentage of viability level of the control. Analysis of variance was used to test for significant differences.

RESULTS

Influence of anti-cancer drugs on ECs

We first examined a correlation between the number of ECs and absorbancy of MTT assay which indicates cell viability, and confirmed a significantly positive correlation between them under our experimental conditions.

Viability of the control cells gradually increased up to 48 h, reflecting an increase in the number of cells during the incubation period.

When ECs were incubated in the presence of 5Fu for 6 h, cell viability did not decrease significantly. However, when they were further incubated for 12, 24, and 48 h, a decrease in the viability was evident as compared with that of the control at all concentrations examined. The cell viability decreased in a dose-dependent manner (Fig. 1).

Figure 2 shows the effect of ADM on the viability of ECs. As can be seen in this figure, there was significant decrease in the viability when ECs were incubated with the anti-cancer drug for 3 h at the concentration of 200 µg/ml. At 6, 12, 24, and 48 h after the incubation, a significant decrease was found in the viability at all concentrations tested. The decrease in the cell viability occurred in a dose-dependent manner (Fig. 2).

The result with CDDP is shown in Fig. 3. After incubating ECs with CDDP for 3 and 6 h, a significant decrease in the viability was observed at 25 and 200 µg/ml. At 12, 24, and 48 h after the incubation, the cell viability decreased at every
concentration of the drug and in a dose-dependent manner (Fig. 3).

Figure 4 shows the effect of MMC on the viability of ECs. After 3 h, there was a significant decrease in the cell viability at the concentrations of 25 and 200 µg/ml of MMC. At 6, 12, 24, and 48 h, the cell viability was decreased significantly at

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Fig. 3. Effect of CDDP on the viability of ECs. Conditions and symbols are the same as described in the legend for Fig. 1 except that CDDP was used as a drug in place of 5Fu. Mean±SD (n=8). **p<0.01 (vs. controls).

Fig. 4. Effect of MMC on the viability of the endothelial cells. Conditions and symbols are the same as described in the legend for Fig. 1 except that MMC was used as a drug in place of 5Fu. Mean±SD (n=8). *p<0.05, **p<0.01 (vs. controls).

all the concentrations examined in a dose-dependent manner (Fig. 4). The decrease was especially marked at 200 μg/ml MMC.

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All concentrations ranging from 10\textsuperscript{-1} to 10\textsuperscript{-4} ng/ml, PGE\textsubscript{1} itself had no significant effects on the viability of ECs (data not shown).

After incubating ECs for 12 h with 5Fu at the concentration of 5, 25, or 200 µg/ml, change in cell viability was not found in the presence or absence of PGE\textsubscript{1}, indicating that PGE\textsubscript{1} has no effect on cell viability at this incubation period. During further incubation with 5Fu for 24 and 48 h in the presence of PGE\textsubscript{1}, a significant improvement against the decrease in cell viability due to 5Fu was found (Fig. 5). Such a protection by PGE\textsubscript{1} tended to be greater at high 5Fu concentration, whereas there was no significant difference in the effects of PGE\textsubscript{1} between low

![Graph showing the influence of PGE\textsubscript{1} on cytotoxicity of 5Fu to ECs.](image)

Fig. 5. Influence of PGE\textsubscript{1} on cytotoxicity of 5Fu to ECs. ECs were incubated with 5Fu in the presence or absence of PGE\textsubscript{1} and cell viability was expressed as a percentage of viability level before anticancer drug was added. Mean ± SD (n = 8). ■, Without PGE\textsubscript{1}; □, 1 ng/ml PGE\textsubscript{1}; ■, 100 ng/ml PGE\textsubscript{1}. *p < 0.05, **p < 0.01.

and high concentrations (Fig. 5).

On the contrary, no clear protective effect of PGE₁ on ECs was observed when ECs were incubated in the presence of either ADM, CDDP, or MMC (data not shown).

**Capability of PGI₂ production**

As a control test, ECs were incubated with or without the addition of PGE₁ in the absence of 5Fu, and then the release of 6-keto PGF₁α into the medium was examined after the addition of the ionophore A23187. It was found that PGE₁ itself did not affect on the release of 6-keto PGF₁α under the conditions employed.

When ECs were incubated with 5Fu, the concentration of 6-keto PGF₁α was decreased as a function of time. However, this decrease was significantly prevented by the addition of PGE₁ at 24 and 48 h after incubation (Fig. 6).

**DISCUSSION**

In a series of our study [5] on intra-hepatic arterial injection chemotherapy, we observed that the patency rates of blood vessels with self-retaining catheters employing the implanted ports were 64.1% after 1 year and 57.6% after 2 years, with an average patency period of 311.9 days; these results are far from satisfactory. There have been reports that the survival time of patients receiving intra-hepatic arterial injection chemotherapy increases with the number of repeated treatments [6]. To improve the effectiveness of this therapeutic modality, it is crucial to clarify the mechanism of endothelial cell injury induced by anti-cancer drugs.

There are hardly any reports regarding the cytotoxicity of anti-cancer drugs to vascular endothelial cells.
ECs; only a few scattered reports deal with the influence of anti-cancer drugs on cell regeneration following damage to the endothelium [7]. Among the 4 drugs examined in this experiment, the effects of the cytotoxicity of ADR, CDDP, and MMC became evident very rapidly, and cytotoxicity was stronger with higher concentrations of the anti-cancer drugs, as well as with longer periods of incubation. The anti-cancer drugs used in this study primarily suppress cell division and growth by inhibiting DNA and RNA synthesis of the tumor cells and normal cells [8]. However, since the normal in vitro cell cycle of cultured cells is 12 to 24 h, it seems unlikely that the inhibition of DNA and RNA synthesis is due to the cytotoxicity observed at only 3 h. It may be considered that such a mechanism involves the direct effects of anti-cancer drugs on the cell membranes [9, 10]. Since we used these anti-cancer drugs at a clinically feasible concentration, 5 μg/ml, it seems likely that cytotoxicity due to this kind of mechanism occurs in vivo. In the case of 5Fu, the inhibition of DNA and RNA synthesis could be one of the factors contributing to the cytotoxicity, since the effect of cytotoxicity becomes evident after 12 h of incubation with 5Fu. The results indicated that the protective effect of PGE\textsubscript{1} toward ECs was evident only with respect to 5Fu. This was presumably due to the fact that the cytotoxicity induced by ADR, CDDP, and MMC appeared quickly and strongly, whereas the cytotoxicity induced by 5Fu was comparatively mild. Thus, the PGE\textsubscript{1} could exert its cytoprotective effect, even though ECs were incubated with 5Fu for 24 and 48 h. In order to demonstrate the functional aspect of this protective effect, calcium ionophore was used to measure the capability of ECs to produce PGI\textsubscript{2}. PGI\textsubscript{2}, which is produced in vivo in the vascular ECs, has physiological properties such as vasodilation and the inhibition of platelet aggregation and is a substance essential to the maintenance of microcirculation [11]. In this study, we observed that the capacity for PGI\textsubscript{2} production was significantly increased in ECs upon treatment with PGE\textsubscript{1} as compared with cells without PGE\textsubscript{1} after 24 and 48 h incubation with 5Fu, while there was no difference between the values of 24 and 48 h incubation in either in the presence or absence of PGE\textsubscript{1}. When compared the results shown in Figs. 5 and 6, it is conceivable that PGI\textsubscript{2} production certainly related with cell measured by the MTT assay. These results suggest that the protective effect of PGE\textsubscript{1} toward the cells in vitro is a direct effect. As the mechanism underlying the effect, the increased production of cyclic AMP [12] and the inhibition of defluxion of lysosomal enzyme [13] are thought. However, further studies are required to clarify the mechanism.

In conclusion, we believe that PGE\textsubscript{1} could be clinically beneficial to protect vascular ECs from the injury caused by 5Fu.

REFERENCES

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