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Short Communication

Comparison of Injuring Effects of Vesicant, Irritant, and Nonvesicant Anticancer Drugs on Endothelial Cells

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Abstract. Anticancer drugs are classified as vesicant, irritant, and nonvesicant drugs on the basis of frequency of their vascular disorder. In this study, we compared the injuring effects of three typical anticancer drugs of each class on porcine aorta endothelial cells (PAECs). The concentration inducing 50% cell viability inhibition was lower in the order of vesicant, irritant, and nonvesicant drugs. These results suggest that injuring effects of anticancer drugs on PAECs may be relevant as an indicator of frequency of their vascular disorder, and that this experimental model may be useful for the study of vascular disorder.

Keywords: vascular disorder, anticancer drug, endothelial cell

Intravenous injection of anticancer drugs often causes vascular-related adverse reactions such as venous irritation, vascular pain, phlebitis, and necrotizing vasculitis. These vascular disorders limit the continuation of chemotherapy and deteriorate patient's quality of life. Anticancer drugs are classified as vesicant, irritant, and nonvesicant drugs based on the frequency of vascular disorder (1, 2). Vesicant drugs (anthracyclines, taxanes, and vinca alkaloids) are defined as agents that cause local tissue necrosis when they are extravasated. Irritant drugs (platinum agents, topoisomerase inhibitors, alkylating agents, and some antimetabolites) are defined as agents that cause inflammatory reactions without persistent tissue damage at the time. On the other hand, nonvesicant drugs (a part of antimetabolites and monoclonal antibodies) are agents that produce little local tissue necrosis or inflammation (3). The procedure of treatment for extravasation is indicated for each group.

Since vascular endothelial cells are first exposed to drugs administered intravascularly, the endothelial cell dysfunction may contribute to vascular disorders. In fact, the endothelial cell injury is implicated in the pathophysiology of several diseases (4). We have reported that the protease inhibitor gabexate mesilate and the vesicant drug vinorelbine, which often cause severe vascular injury, induce cell injury in porcine aorta endothelial cells (PAECs) (5 – 7). However, comparison of injuring effects of vesicant, irritant, and nonvesicant drugs on PAECs has not been studied. In this study, we selected three typical drugs from each class of anticancer drugs (vesicant: vinorelbine, epirubicin, and actinomycin D; irritant: 5-fluorouracil, cisplatin, and irinotecan; nonvesicant: cytarabine, methotrexate, and nimustine) and compared their injuring effects on PAECs.

Vinorelbine, epirubicin, actinomycin D, 5-fluorouracil, cytarabine, methotrexate, and nimustine were obtained from Wako Pure Chemicals (Osaka). Cisplatin and irinotecan were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

PAECs were obtained from Dainippon Sumitomo Pharmaceutical Co., Ltd. (Osaka). Cells were maintained in Dulbecco's modified Eagle's medium (MP Biomedicals Inc., Irvine, CA, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B (GIBCO BRL, Grand Island, NY, USA) at 37°C in 5% CO_2 – 95% air.

PAECs were seeded at a density of 1.0×10^4 cells/well in 24-well plates (Nalge Nunc International, Rochester, NY, USA) and grown to subconfluence. Cell viability was assessed by measuring the mitochondrial activity that reduces 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (WST-8) to formazan, as described previously (8). Briefly, after treatment with anticancer drugs for 24

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h, the cells were then washed with PBS and incubated with WST-8 assay solution (Cell Counting Kit-8; Dojindo Lab., Kumamoto) for 1 h at 37°C in humidified air supplemented with 5% CO_2. The incubation medium was carefully withdrawn and transferred to 96-well flat-bottom plastic plates (Corning Inc., Corning, NY, USA). The amount of formed formazan was measured from the absorbance at 450 nm with a reference wavelength of 620 nm using a microplate reader (Immuno-Mini NJ-2300; Inter Medical, Tokyo).

Data were expressed as the mean ± S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) followed by the Dunnett test. Statistical significance was defined as $P < 0.05$. The concentration inducing 50% cell viability inhibition (IC_{50} values) was estimated by probit analysis.

As shown in Fig. 1, all anticancer drugs examined except for cytarabine decreased the cell viability dose-dependency. Vesicant drugs vinorelbine, actinomycin D, and epirubicin showed significant decreases at concentrations of ≥ 10 nM, ≥ 100 nM, and ≥ 1 μM, respectively. Irritant drugs cisplatin, irinotecan, and 5-fluorouracil showed significant decreases at ≥ 3 μM, ≥ 3 μM, and ≥ 30 μM, respectively. Nonvesicant drugs methotrexate and nimustine showed significant decreases at ≥ 1 mM and ≥ 3 mM, respectively.

As shown in Table 1, IC_{50} values of vesicant drugs vinorelbine, actinomycin D, and epirubicin were 0.30 μM, 3.04 μM, and 6.11 μM, respectively. Those of irritant drugs cisplatin, irinotecan, and 5-fluorouracil were 28.96 μM, 70.53 μM, and 3.49 mM, respectively. On the other hand, that of the nonvesicant drug nimustine was 3.07 mM, and those of methotrexate and cytarabine were more than 10 mM.

In this study, vesicant drugs vinorelbine, actinomycin D, and epirubicin markedly induced the cell injury, whereas nonvesicant drugs methotrexate and nimustine induced the cell injury at high concentrations and the other nonvesicant drug cytarabine did not induce cell injury. Many clinical reports indicate that vinorelbine and epirubicin induce phlebitis with a high frequency of over 30% (9 – 13). On the other hand, there is no report about phlebitis induced by methotrexate, nimustine, or cytarabine. In addition, there is also no report about actinomycin D–induced phlebitis. Perhaps the reason for this may be because actinomycin D is usually used for the treatment of childhood cancer at a low dose of 0.015 mg/kg (14). The dosage and administration procedure of anticancer drugs vary widely with the therapeutic regimens. Therefore, this makes it difficult to compare the

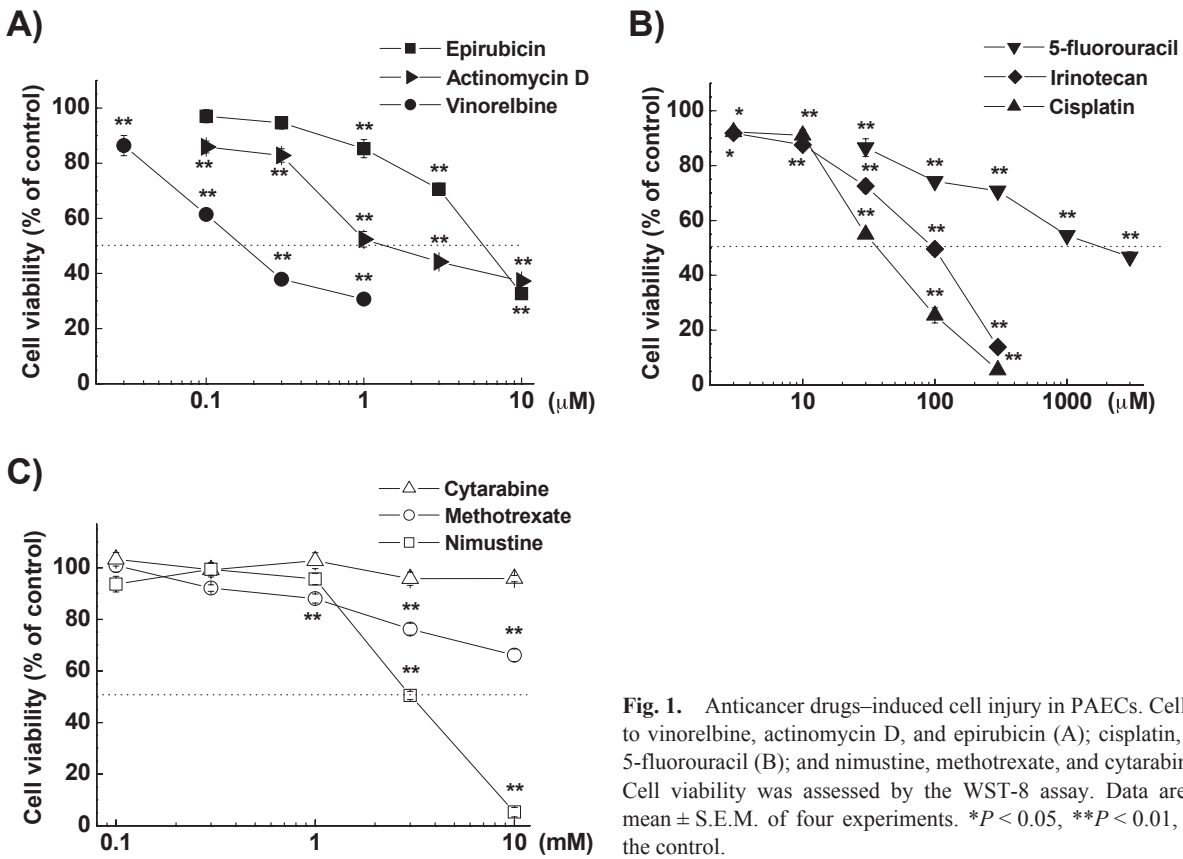

Fig. 1. Anticancer drugs–induced cell injury in PAECs. Cells were exposed to vinorelbine, actinomycin D, and epirubicin (A); cisplatin, irinotecan, and 5-fluorouracil (B); and nimustine, methotrexate, and cytarabine (C); for 24 h. Cell viability was assessed by the WST-8 assay. Data are expressed the mean ± S.E.M. of four experiments. *$P < 0.05$, **$P < 0.01$, compared with the control.

Table 1. IC_{50} values of anticancer drugs in PAECs

Classification	Drug	IC_{50} [M] (IC_{50} [w/v])	Typical dosage and administration procedure
Vesicant	Vinorelbine	0.30 ± 0.90 μM (0.33 ± 0.10 μg/mL)	25 mg/m^2 / 50 – 100 mL/5 – 10 min
	Actinomycin D	3.04 ± 0.27 μM (3.81 ± 0.34 μg/mL)	0.015 mg/kg / 5 mL/5 min
	Epirubicin	6.11 ± 0.34 μM (3.55 ± 0.20 μg/mL)	60 – 100 mg/m^2 / 50 – 100 mL/5 – 10 min
Irritant	Cisplatin	28.96 ± 4.17 μM (8.69 ± 1.25 μg/mL)	60 – 80 mg/m^2 / 500 mL/120 min
	Irinotecan	70.53 ± 4.22 μM (43.95 ± 2.63 μg/mL)	180 mg/m^2 / 250 mL/120 min
	5-Fluorouracil	3.49 ± 0.83 mM (0.45 ± 0.11 mg/mL)	400 mg/m^2 / 50 mL/5 min
Nonvesicant	Nimustine	3.07 ± 0.23 mM (0.95 ± 0.07 mg/mL)	70 mg/m^2 / 100 mL/30 min
	Methotrexate	≥ 10 mM (≥ 4.54 mg/mL)	3 g/m^2 / 500 mL/180 min
	Cytarabine	≥ 10 mM (≥ 2.43 mg/mL)	2 g/m^2 / 500 mL/180 min

injuring effects of anticancer drugs. In the right column of Table 1, the typical dosage and administration procedure of each anticancer drug are shown for the comparison of the injuring effects. It is unlikely that the vesicant drugs induce vascular disorders more easily than irritant and nonvesicant drugs because of higher concentration of injection per min. Although the concentrations of methotrexate and cytarabine per mL are relatively high, their IC_{50} values are more than 10 mM. In addition, IC_{50} values of nimustine were equivalent to those of 5-fluorouracil. However, nimustine showed a significant decrease in cell viability at more than the concentrations of 3 mM, whereas 5-fluorouracil showed that at more than the concentrations of 30 μM. Therefore, the frequency of vascular disorder induced by the drugs may be lower than that induced by other drugs. Taken together, these results indicate that the injuring effects of anticancer drugs on PAECs may be relevant as an indicator of frequency of their vascular disorder in the clinical setting. Since vascular endothelial cells are first exposed to drugs administered intravascularly, the endothelial cell dysfunction may contribute to vascular disorders.

In general, the vascular injury induced by intravenous drugs is related with high or low pH of infused fluid, osmotic pressure, and direct injuring effect. However, an infusion solution of anticancer drugs is usually at adjusted osmotic pressure. Additionally, epirubicin-induced phlebitis is not necessarily related with low pH of the infused fluid (9). Therefore, it is likely that anticancer drugs directly injure vascular vessels.

In our previous report (7), we indicated that vinorelbine increases intracellular reactive oxygen species production in PAECs and antioxidant agents such as glutathione and *N*-acetylcysteine reverse the vinorelbine-induced decrease in cell viability, suggesting that oxidative stress plays an important role in the vinorelbine-induced endothelial cell injury. On the other hand, we found that glutathione and *N*-acetylcysteine had no effect on the vinorelbine-induced cell injury in HepG2 cells, human hepatoma cell lines (T. Yamada et al., unpublished data). Therefore, the injuring effects of anticancer drugs on PAECs may be related more closely to oxidative stress compared with their injuring effects on other cell types.

In conclusion, the study presented here demonstrates, for the first time, that the IC_{50} values of anticancer drugs on PAECs were lower in the order of vinorelbine, actinomycin D, epirubicin, cisplatin, irinotecan, nimustine, 5-fluorouracil, methotrexate, and cytarabine. Moreover, the other nonvesicant drug cytarabine did not induce the cell injury. These results suggest that the injuring effects of anticancer drugs on PAECs may become helpful as an indicator of frequency of their vascular disorder in the clinical setting. Therefore, the present experimental model using PAECs may be useful for the study of vascular disorders. In addition, it is important to consider clinical dosage and administration procedure of anticancer drugs.

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