Difference in Effects of Vinblastine and Vincristine on the Dog Platelet Aggregation

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TAKANO, S. Difference in Effects of Vinblastine and Vincristine on the Dog Platelet Aggregation. Tohoku J. exp. Med., 1981, 135 (1), 79-85 — The present study was attempted to determine difference in effects of vinblastine and vincristine on ADP-induced aggregation of dog platelets. Vinblastine manifested biphasic influence depending upon concentrations, i.e., in low concentrations (0.2-20 \(\mu\)g/ml) it promoted the aggregation, and in a high concentration (100 \(\mu\)g/ml) it inhibited the aggregation with increasing incubation time. Vincristine in any concentrations, however, neither promoted nor inhibited the aggregation. In scanning electron micrographs, platelets were gradually damaged as revealed by their surface appearances with increasing incubation time in the presence of vinblastine (100 \(\mu\)g/ml). There existed a parallel relationship between the inhibition of the aggregation by vinblastine and the increase in number of severely damaged platelets. However, there was no such a parallel relationship between them in the presence of vincristine.

Vinca alkaloids, including vinblastine and vincristine, have been used for clinical therapeutics of neoplastic diseases. The alkaloids are known to affect cell mitosis through an inhibition of the spindle mechanism (Parmer et al. 1960). Overdosage of these drugs damages primarily organs known to be rich in microtubules such as neurons (Vy et al. 1967) and leukocytes (Bensch and Malawista 1969).

In platelets, vinblastine and vincristine are reported to destroy platelet microtubules in moderate concentrations (White 1968). Vincristine obliterated the second wave of ADP- or epinephrine-induced aggregation of human platelets (Hicsonmez 1974; Steinherz et al. 1976; Hicsonmez and Buyukpamukcu 1977). In rat platelets, vincristine caused at least 25% increase in aggregation as compared with the control (Hicsonmez 1974).

The present paper describes difference in effects of vinblastine and vincristine on ADP-induced aggregation of dog platelets in different concentrations and various incubation time as well as platelet surface alteration after exposure to both alkaloids.

MATERIALS AND METHODS

Mongrel dogs of both sexes about 10 kg in weight were anesthetized with pentobarbital sodium (30 mg/kg) intramuscularly. Blood was withdrawn from the carotid...
artery with a syringe containing one tenth volume of 3.8% trisodium citrate. The
platelet-rich plasma (PRP) was prepared by a low speed centrifugation at 700–800 rpm
for 13 min. The platelet count was adjusted to $6 \times 10^5/mm^3$ of plasma. The platelet
aggregation was measured using an Evans aggregometer at 37°C. After 3 min preincuba-
tion at 37°C, the vincala alkaloids (0.1 ml) in different concentrations were added to PRP (0.8
ml) and incubated for different periods. Then, ADP (0.1 ml, 4 x $10^{-4}$M in the final concentra-
tion) was added to this PRP solution.

Specimens of platelets for the scanning electron microscopy were made as follows:
Platelets were fixed at 37°C in 1% glutaraldehyde in 0.1 M phosphate buffer (NaH$_2$PO$_4$-Na$_2$
HPO$_4$ system; pH 7.4) for 1 hr. The platelets, after washed with the buffer, were dehydrated
progressively with graded solutions of acetone from 50 to 100%. After infiltration with
100% isoamylacetate, the platelets were dried using a critical point drying apparatus (Hitachi,
HCT-H). The platelets were placed on a thin glass plate, which was attached to
copper specimen stubs, and rotationally coated in a vacuum evaporator with a thin coat of
gold. These preparations were examined using a JSM-SL type scanning electron micro-
scope (JEOL Co., Ltd.) with accelerating voltage of 10 kV.

**Results**

*Effects of vinblastine and vincristine on the ADP-induced aggregation*

Vinblastine in low concentrations (0.2–20 µg/ml) accelerated ADP-induced platelet aggregation (Fig. 1), but vincristine in comparable concentrations (0.2–20
µg/ml) had no effect. Augmentation of ADP-induced aggregation reached a
maximum with 2 µg/ml vinblastine; 46.3±6.9% ($p<0.05$) as against 26.5±5.4%
in control. Two µg/ml was assumed to be about the same as blood vincala alkaloid
levels in vivo at clinical dose. Incubation of PRP with vinblastine at 100 µg/ml
for 30 min significantly depressed ADP-induced platelet aggregation to 14.7 ±2.4%
as against 31.8±4.8% in control ($p<0.01$) and 29.0±6.7% in vincristine-treatment
($p<0.05$, see Fig. 2).

*Effects of vinblastine and vincristine on the platelet morphology*

Platelets in 80 to 90% of the population were disc and oval with 5 to 30 min
incubation in 0.9% NaCl (Figs. 3, 4, 5 and 6). Some of them had short pseudopods

![Graph](image-url)

**Fig. 1.** Effects of low concentrations of vinblastine (Δ—Δ) and vincristine (□—□) on ADP-
induced aggregation of dog platelets. PRP was incubated with each of the alkaloids or
0.9% NaCl for 5 min. *p<0.05.
Vinblastine and Vincristine on Platelet Aggregation

Fig. 2. Effects of incubation time on ADP-induced aggregation in the presence of vinblastine (100 µg/ml, Δ—Δ), vincristine (100 µg/ml, ○—○) or 0.9% NaCl (□—□).

Fig. 3. Change in distribution of platelet surface appearance observed by scanning electron microscopy after 5 min incubation with vinblastine (100 µg/ml), vincristine (100 µg/ml) or 0.9% NaCl. The data show the mean of 3 experiments.

- smooth edged disc form;
- platelets with a few pseudopods;
- swollen platelets;
- bumpy form or "bubbling and boiling" appearance.

Fig. 4. Change in distribution of platelet surface appearance observed by scanning electron microscopy after 15 min incubation with vinblastine (100 µg/ml), vincristine (100 µg/ml) or 0.9% NaCl. Vertical lines indicate standard errors of the means of 6 experiments. See Fig. 3 for further details.
and swollen form, but there were no platelets with surface bumps or folds such as those frequently seen in vinblastine-treated platelets (Fig. 7). When the platelets were incubated with 100 µg/ml vinblastine, they were deformed into bumpy forms. The longer the incubation time, the more were the platelets deformed (Figs. 3, 4, 5 and 7). After 30 min incubation with 100 µg/ml of vinblastine, the bumpy form platelets were increased to 46.0±3.5 per 100 platelets (p<0.02), which were more than those with vincristine (30.4±3.8/100 platelets, Fig. 5). Furthermore, the platelets with smooth edged disc form were decreased to 5.3±1.6, which were less than those with vincristine (16.7±3.9/100 platelets, Fig. 5). At this time aggregation of the platelets which were treated with vinblastine was significantly inhibited as compared with those treated with 0.9% NaCl or vincristine (Fig. 2). Furthermore, whereas there was a similarity in the distribution pattern of platelet surface alteration between vinblastine-treated platelets for 15 min and vincristine-treated platelets for 30 min, the former did not sufficiently respond to ADP but the latter aggregated in response to ADP (Figs. 4 and 5).

![Fig. 5. Change in distribution of platelet surface appearance observed by scanning electron microscope after 30 min incubation with vinblastine (100 µg/ml), vincristine (100 µg/ml) or 0.9% NaCl. Vertical lines indicate the standard errors of the means of 6 experiments. See Fig. 3 for further details.](image)

![Fig. 6. Scanning electron micrograph of dog platelets incubated in 0.9% NaCl for 30 min at 37°C. × 3,000.](image)
DISCUSSION

It has been shown that of vinca alkaloids, vinblastine exerted biphasic influences on ADP-induced aggregation of dog platelets depending upon concentrations, whereas vincristine showed little effect in any concentration. White (1968) observed that vinblastine and vincristine abolished the second wave of ADP- or epinephrine induced aggregation and that intact microtubules in the platelets disappeared by exposure to the alkaloids. In human leucocytes and L-strain fibroblasts, both alkaloids are reported to combine with microtubular protein (Bensch and Malawista 1969). Hicsonmez (1974) reported that vincristine (3 µg/ml) potentiated aggregation of rat platelet induced by ADP after 30 min of incubation. However, vincristine are reported to inhibit aggregation of human platelets both in vivo and in vitro (Hicsonmez 1974; Steinherz et al. 1976; Hicsonmez and Büyükpamukcu 1977).

In dog platelets, the effect of 2 µg/ml vinblastine in accelerating platelet aggregation seems to be an action on biophysical aspect of the platelet membrane, because short incubation and/or low concentration of the drug exerted no influence on platelet surface appearance.

At 100 µg/ml in concentration and 30 min in incubation time, vinblastine
depressed the aggregation, whereas vincristine did not. Blood platelets contain circumferential bundles of microtubules which support the discoid shape of unaltered cells. Aggregation of dog platelets was depressed by vinblastine with increasing incubation time. As scanning electron micrographs show (Figs. 3, 4 and 5), 5 min incubation with vinblastine already induced platelet surface alteration (Fig. 3) such as swollen forms and "bubbling and boiling" appearances (Fig. 7) without affecting the property of the platelets to aggregate in response to ADP. Vincristine-induced platelet surface alteration was always less than vinblastine-induced one. Thirty min incubation with vinblastine increased the dendritic-form platelets significantly as compared with those with vincristine. The altered platelets having such a "bubbling and boiling" appearance suggest destruction of platelet microtubules by vinca alkaloids and loss of the capacity of platelets to aggregate. Born (1967) reported that in measuring platelet aggregation using the turbidimetric method, the initial increase in transmission is a measure of formation of large aggregates from small aggregates, and of contraction of such large aggregates. The platelets of which microtubules are injured severely with vinblastine do not appear to make such large aggregates, or to contract enough to increase transmission.

The pattern of distribution of platelet surface alteration was similar between platelets incubated with vinblastine for 15 min and those incubated with vincristine for 30 min. The capability of the platelets to aggregate, however, was far different between them, vinblastine-treated platelets failed to aggregate in response to ADP but vincristine-treated platelets did. Accordingly, there appeared to exist parallelism between inhibitory action of vinblastine on aggregation which became manifest with increasing incubation time and the number of severely damaged platelets. But there was no such parallelism between them in the presence of vincristine.

In the above discussion, vincristine seems to produce platelet surface alteration without affecting the capability of platelets to aggregate. The fact suggests that platelet deformation may not be always necessary for inhibition in platelet aggregation.

References
