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Abstract

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KEYWORDS: cytochalasin B, Ehrlich ascites tumor cells, zeiosis, scanning electron microscopy.

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A SCANNING ELECTRON MICROSCOPIC STUDY OF THE TWO-STEP EFFECT OF CYTOCHALASIN B ON EHRLICH ASCITES TUMOR CELLS

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Abstract. The effect of cytochalasin B (CB) on the surface structure of Ehrlich ascites tumor cells was investigated using the scanning electron microscope. The effect occurs in two steps: formation of zeiotic knobs on the cell surface and subsequent grouping of the knobs at one pole of the cell. The early step of zeiotic knob formation occurs at low concentrations of CB (0.5-1 μg/ml) at 37°C and at high concentrations of the drug (5-10 μg/ml) at low temperature but within 1 min at 37°C. This step is only partially inhibited by 5 x 10^{-3} M sodium azide. The subsequent grouping of zeiotic knobs lasts for more than 2 min at 37°C and occurs only in the case of high concentrations of CB. It is inhibited by sodium azide and is often associated with grouping of the microvilli, which are then lost from all of the cell surface except the area of knob-grouping.

Key words : cytochalasin B, Ehrlich ascites tumor cells, zeiosis, scanning electron microscopy.

We previously reported that Con A-induced cap formation in Ehrlich ascites tumor cells was accompanied by grouping of microvilli and that cytochalasin B (CB) had no marked effect on this cap formation though it caused morphological changes in the cell (1). The principal change conbisted of zeiosis (2,3), which was identified by transmission electron microscopy.

Cytochalasins induce many morphological changes including zeiosis or blebbing (4). Clustering of zeiotic knobs (3,5-8) and the withdrawal of microvilli (6,9) were reported by several workers. With a few exceptions (3,7,8,10), these reports concerned cells attached to a substratum at 37°C.

In the present study, the effect of CB on Ehrlich ascites tumor cells was studied using scanning electron microscopy. The effect consisted of two steps related to the dose response, temperature dependency, and the time course. The result obtained from the experiment using sodium azide also coincided with the two-step effect of the drug.

MATERIALS AND METHODS

Cells. Ehrlich ascites tumor cells were harvested from the abdomen of ddY mice, washed three times with Dulbecco's phosphate buffer saline (PBS) (11), and suspended in the same solution (1).

Drugs. Cytochalasin B was purchased from Sigma Co. and dissolved in dimethyl sulfoxide (DMSO) at 1 mg/ml. Poly L-lysine was from the Nakarai Co. (Kyoto) and a 0.1 % solution was used for coating the glass surface on which fixed cells were attached.

Experimental procedure. Cells (2 x 10^6/ml) were preincubated at 37°C for 10 min and incubated with 10 μg/ml CB at 37°C. After 10-15 min, an equal volume of 5 % glutaraldehyde solution in PBS was added. The incubation time, the incubation temperature and the concentration of CB were varied in each experiment. The details are described in Results and the figure legends. In the control, 10 μl/ml DMSO was added instead of CB.

Fixed cells were washed with PBS and placed on poly L-lysine coated glass (5 x 5mm^2) and left for 30 min at room temperature in a moist chamber. The piece of glass with attached cells was gently shaken in PBS to remove excess cells, dehydrated in a graded alcohol series, immersed in isoamyl acetate and dried by the critical point method with carbon dioxide as the transition fluid using a Hitachi HCP-1 critical point dryer. The cells were then coated with Pt-Pd using an Eiko Ion Coater (Model LB-3) and viewed in a Hitachi HHS-2R or a JEOL LSM-U_3 scanning electron microscope. Photos were taken with Fuji Neopan SS-12.

RESULTS

Cells (2 x 10^6/ml) were preincubated at 37°C for 10 min and further incubated for 10 min with each concentration of CB (0.5, 1, 5, 10 μg/ml). Control cells treated only with DMSO had long thin microvilli (Fig. 1). These microvilli were distributed evenly in most cells, but some grouping of microvilli was occasionally observed in a small proportion of the cells (up to 5 % of cells). When the cells were incubated with a low concentration of CB (0.5-1 μg/ml) at 37°C for 15 min, zeiotic knobs occurred on the whole cell surface and these knobs were small and of different sizes (Fig. 2). At 5-10 μg/ml CB, these knobs enlarged and decreased in number, and gathered at one pole of the cell. At the same time, a smooth surface area appeared (Figs. 3,4). Microvilli shortened and gathered in small patches (Fig. 3), or a protrusion, which consisted of knobs and microvilli, was observed, the remaining cell surface being smooth (Fig. 4). The grouping of knobs was preceded by the formation of knobs all over the cell surface, microvilli being distributed among the knobs.

Cells were preincubated at 37°C for 10 min and further incubated with 10 μg/ml CB at 37°C. After 1, 2, 5, 10 and 15 min the reaction was stopped with an equal volume of 5 % glutaraldehyde solution. The rate of zeiotic knob formation was very rapid and was observed after 1 min (Fig. 5). Microvilli were seen among diffusely distributed zeiotic knobs at this stage. After 2 min, the grouping of these knobs was already evident in some cells and was accompanied by the loss of microvilli from the cell surface (Fig. 6). The difference in surface structure be-

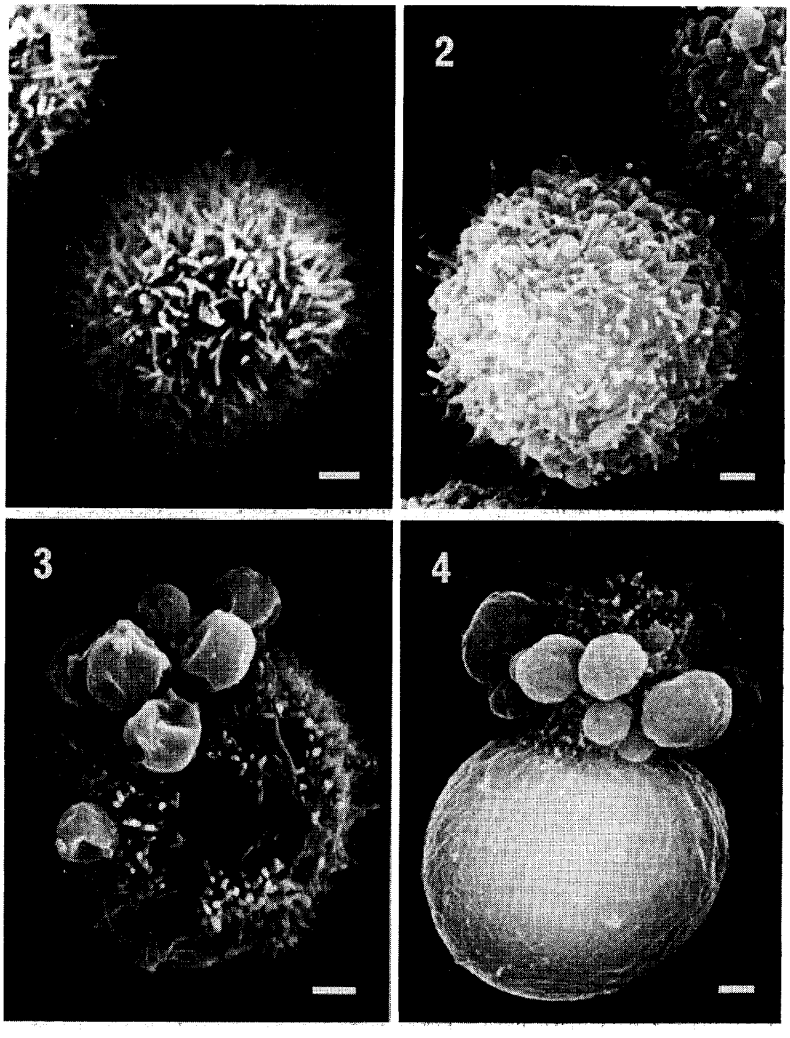

Fig. 1. Ehrlich ascites tumor cells preincubated at 37°C for 10 min and subsequently incubated with 1 % DMSO at 37°C for 15 min. The cells have long thin microvilli. Bar 1 μm.

Fig. 2. Ehrlich ascites tumor cells treated with 0.5 μg/ml CB at 37°C for 15 min. This results in the formation of small zeiotic knobs which are distributed diffusely around cell surface. The diameter of these knobs is less than 1 μm. Bar 1 μm.

Figs. 3-4. Incubation of cells with 10 μg/ml CB at 37°C for 10 min results in the formation of zeiotic knobs which cluster at one pole of the cell. Knobs increase in diameter up to 1.5 μm or more concomitantly with a decrease in number. The knobs are round or oval in thin sections. Microvilli are found in small patches, between which the cell surface appears smooth (Fig. 3) or a protrusion consisting of knobs and microvilli is observed, the other part of the cell being smooth. Bar 1 μm.

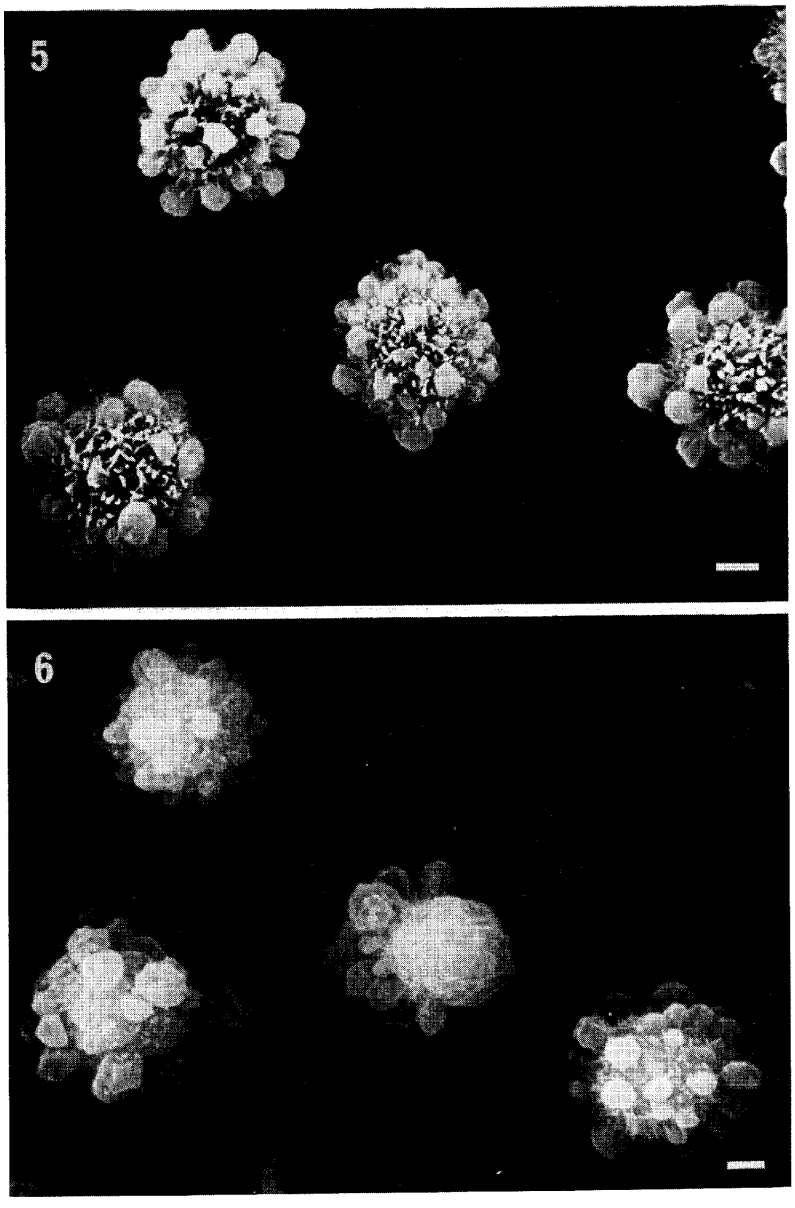

Fig. 5. Cells incubated with 10 μg/ml CB at 37°C for 1 min. Zeiotic knobs appear around the cell surface. Microvilli remain among knobs. Bar 2 μm.

Fig. 6. Cells incubated with 10 μg/ml CB at 37°C for 2 min. The grouping of knobs is already observable and is accompanied by the loss of microvilli from the cell surface. Bar 2 μm.

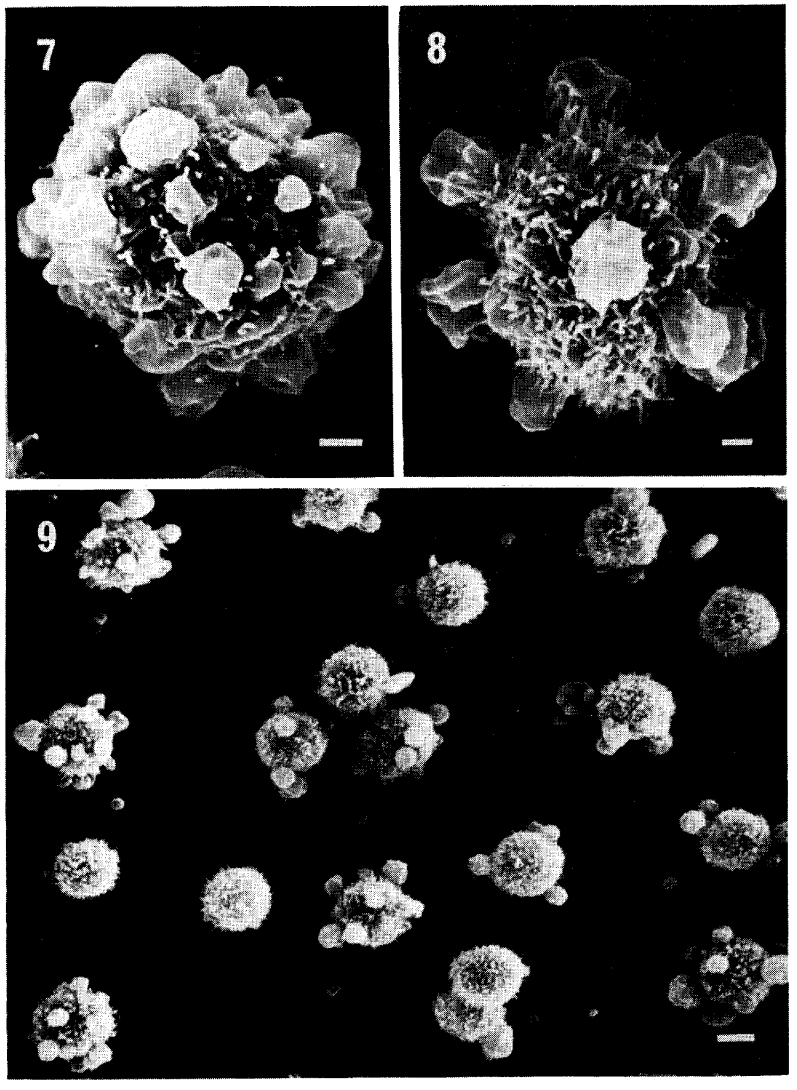

Fig. 7. Cells preincubated at 15°C for 10 min and subsequently incubated with 10 μg/ml CB at 15°C for 10 min. Knobs and short microvilli are observed around the cell surface. Bar. 1 μm.

Figs. 8-9. These cells are treated with 5 x 10^{-3}M sodium azide at 37°C for 10 min. CB is then added to the cell suspension (10 μg/ml final) and incubation continued at 37°C for 10 min. Knob formation itself is partly, and knob grouping is completely, inhibited. Microvilli are observed among the knobs. Bar 1 μm (Fig. 8), 5 μm (Fig. 9).

tween the cells which initiated the grouping of zeiotic knobs and the cells which maintained these knobs around the cell surface was clearly demonstrated (Fig. 6). The grouping of knobs was completed within 5 min.

At low temperatures, knob formation was not as extensive as at 37°C, but enlarged knobs were sometimes observed at 10°C and the grouping of knobs was totally inhibited under 20°C (Fig. 7).

Cells were preincubated in the presence of 5×10^{-3} M sodium azide at 37°C for 10 min and further incubated with 10 μg/ml CB for 10 min. Sodium azide at a concentration which inhibits Con A-induced cap formation in these cells (1), only partially inhibited knob formation, but completely prevented the clustering of knobs (Figs. 8,9). Short microvilli were observed among the knobs.

DISCUSSION

That cytochalasins disrupt intracellular contractile systems was first proposed because of a correlation between their inhibitory effects on cell motility and structural changes of microfilaments (12). Observation of the inhibitory effect of CB on sugar transport led to the suggestion that the cell membrane was the site of action of the drug (13), and binding assays demonstrated that at least two binding sites (high and low affinity binding sites) were present in human red blood cells (14) and in other cell types (15). Most high affinity binding sites were thought to be involved in sugar transport and low affinity binding sites in cell motility and morphology (15).

Zeiosis, one of the most striking effects of CB on cell morphology, was observed in Ehrlich ascites tumor cells. In the early phase of CB action, zeiotic knobs occurred at the cell surface and microvilli were distributed diffusely among the knobs (Figs. 2, 5, 7-9). Godman *et al.* thought that the knob formation occurred because of disruption of a microfilament net in the cell cortex and herniation of the cytoplasm (3).

In a later phase, migration of knobs occurred, a process identical to the formation of the bouquet or crown previously described in cultured cells (3,4), and, microvilli had disappeared from the cell surface except at the knob-grouping area or scattered as small patches on the cell surface (Figs. 3, 4, 6). The disappearance of microvilli had been reported previously (4, 6, 9), but in our experiments on Ehrlich ascites tumor cells, it was a process occurring concomitantly with the grouping of the knobs. The presence of a protrusion consisting of grouped knobs and microvilli (Fig. 4) and the presence of a protrusion consisting of grouped microvilli after withdrawal of CB (not shown in Figures.) suggest that the microvilli plasma membrane was used for the additional surface area of the knobs. Also in these cells, a large amorphous mass which lacked organelles was found by transmission electron microscopy at the base of grouped knobs as reported in other cells (3, 4, 6), thus the grouping of the knobs and the presence

of this mass seem to be closely associated. In Ehrlich ascites tumor cells, the grouping of knobs and of microvilli may be a similar event although the effect of CB on microvilli varies with the cell type (4). As previously described (1), Con A-induced cap formation in Ehrlich ascites tumor cells is accompanied by grouping of microvilli, and Con A binding sites have been reported to gather into blebs or ruffling in CB-treated cells (7, 8). In Ehrlich ascites tumor cells, CB in itself does not alter the distribution of Con A receptors as reported by Glenney *et al.* (16).

The present study shows that CB alters the shape of microvilli and causes clustering of microvilli as in ConA-induced cap formation. The rapid response to the drug in our experiments compared with that reported for cultured cells may be due to the fact that the latter were associated with a substratum (17).

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