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Goniodomin A, an Antifungal Polyether Macrolide, Increases the Filamentous Actin Content of 1321N1 Human Astrocytoma Cells

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Abstract

Goniodomin A, a polyether macrolide isolated from the dinoflagellate *Goniodoma pseudogoniaulax*, caused morphological change in 1321N1 human astrocytoma cells.

Observation by scanning electron microscopy revealed that the shape of the cell became refractile from flat in response to goniodomin A. This morphological change was accompanied by the outgrowth of a needle-like structure from the cell surface. Goniodomin A increased the intracellular content of filamentous actin in a concentration- and time-dependent manner. It also caused the change in filamentous actin distribution in the cells. The elongation of filamentous actin was observed in goniodomin A-treated cells. These results suggest that goniodomin A induces morphological change by increasing the content of filamentous actin in non-muscle cells.

Actin is a globular protein which is polymerized into filamentous actin (F-actin), an essential component of the cytoskeleton in non-muscle cells. Non-muscle cells show a variety of motile behaviour, such as cell division, locomotion and secretion, in which actin polymerization plays a crucial role (Stossel 1993; Hennessey et al 1993; Mitchison & Cramer 1996). Actin polymerization is also known to be important in changing the cell morphology (Gumbiner 1996). The state of actin polymerization changes in response to extracellular stimuli, and the rearrangement of F-actin correlates with quantitative changes in the motile behaviour of a cell (Howard & Oresajo 1985; Ha & Exton 1993; Ridley & Hall 1994).

Goniodomin A is an antifungal polyether macrolide isolated from the dinoflagellate *Goniodoma pseudogoniaulax* (Murakami et al 1988). We previously reported that goniodomin A inhibited cell division in fertilized sea-urchin eggs (Murakami et al 1988) and induced modulation of rabbit skeletal actomyosin ATPase activity through a conformational change of actin (Furukawa et al 1993).

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There are at least six actin isoforms in warm-blooded vertebrates: two non-muscle actins, two smooth-muscle actins and two sarcomeric actins from cardiac and skeletal muscles (Firtel 1981). In this paper we describe the possibility that goniodomin A affects non-muscle actin in the same manner as skeletal actin.

Materials and Methods

Materials

Goniodomin A (Figure 1) was purified from the dinoflagellate *Goniodoma pseudogoniaulax* (Murakami et al 1988). *N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) (NBD)-phallacidin and rhodamine-phalloidin were obtained from Funakoshi (Tokyo, Japan). Foetal calf serum was from JRH Biosciences (Lanexa, KS). Other reagents were of reagent grade or the highest quality available.

Cell culture

1321N1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 5% foetal calf serum, 50 units mL^{-1} penicillin, and 50 $\mu g\,mL^{-1}$ streptomycin at 37 °C in an atmosphere of 5% CO_2 in air.

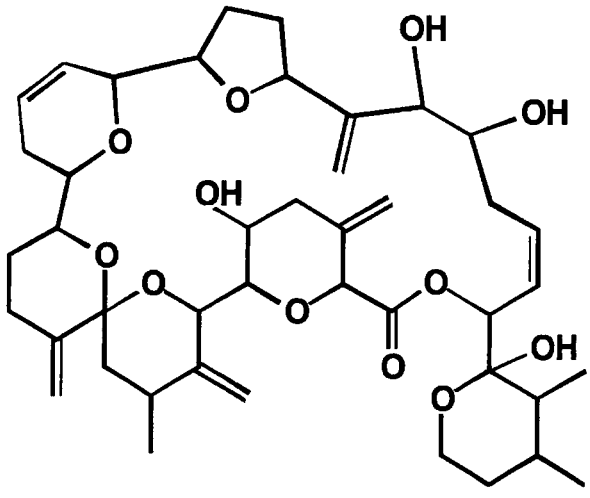

Figure 1. Chemical structure of goniodomin A.

Scanning electron microscopy

1321N1 cells cultured on cover glasses were washed three times with Eagle's minimal essential medium containing 10 mM HEPES (pH 7·35), pre-incubated in the same medium for 10 min, and then treated with the drugs. The treated cells were fixed with 1% glutaraldehyde in phosphate-buffered saline (PBS) for 30 min at 37 °C. The fixed cells were treated with 0·5% osmium tetraoxide in PBS, dehydrated in graded concentrations of ethanol, dried with an Hitachi (Tokyo, Japan) HCP-2 critical-point drying apparatus, and finally sputter-coated with platinum and palladium by means of an Hitachi E-102 coating apparatus. These specimens were observed by scanning electron microscopy (S-800; Hitachi) operated at a 3-kV accelerating voltage.

Quantification of F-actin

1321N1 cells on 12-well plates were washed three times with Eagle's minimal essential medium containing 10 mM HEPES (pH 7·35), pre-incubated in the same medium for 10 min, and then treated with various drugs. F-actin content was measured by a modification of the procedure developed by Howard & Oresajo (1985). The drug-treated cells were fixed with 1% glutaraldehyde in PBS for 30 min at 37 °C. After being made permeable with 0·2% Triton X-100 in PBS for 30 min, the cells were stained with 10 units mL^{-1} NBD-phallacidin in PBS for 1 h, washed three times with PBS, and then the bound NBD-phallacidin was extracted with methanol for 1 h on ice. The fluorescence intensity was measured by spectrofluorimetry, (F-2000; Hitachi) with an excitation wavelength of 465 nm and an emission wavelength of 530 nm. The results were expressed as the relative fluorescence intensity calculated from the ratio of the fluorescence intensity of stimulated cells to that of unstimulated cells.

Distribution of F-actin

1321N1 cells cultured on cover glasses were washed three times with Eagle's minimal essential medium containing 10 mM HEPES (pH 7·35), pre-incubated in the same medium for 10 min, and then treated with various drugs. The drug-treated cells were fixed with 1% glutaraldehyde in PBS for 30 min at 37 °C. After being made permeable with 0·2% Triton X-100 in PBS for 10 min, the cells were stained with 10 units mL^{-1} rhodamine-phalloidin in PBS for 40 min at room temperature. The cells were viewed on the Bio-Rad MRC-1024 confocal microscope.

Results

As shown in Figure 2, goniodomin A induced morphological changes in 1321N1 cells. The 1321N1 cell surface was smooth at time 0 (Figure 2A). In cells treated with 1 μM goniodomin A for 1 min (Figure 2B), the shape became contractile from adhesive and long needles grew from the cell body. In cells treated with 1 μM goniodomin A for

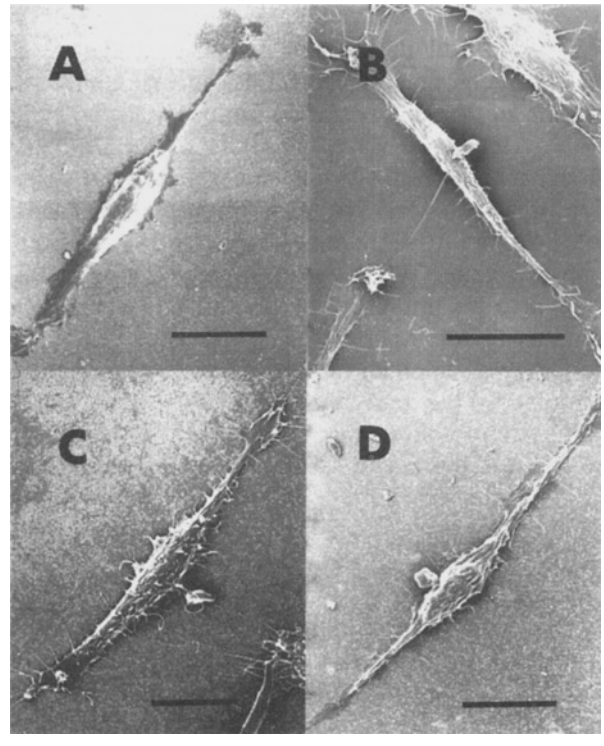

Figure 2. Morphological change in 1321N1 cells treated with goniodomin A. 1321N1 cells cultured on cover glasses were incubated with 1 μM goniodomin A for 0 min (A), 1 min (B), 3 min (C) and 10 min (D). The treated cells were prepared for observation by electron scanning microscopy. Bar represents 20 μm.

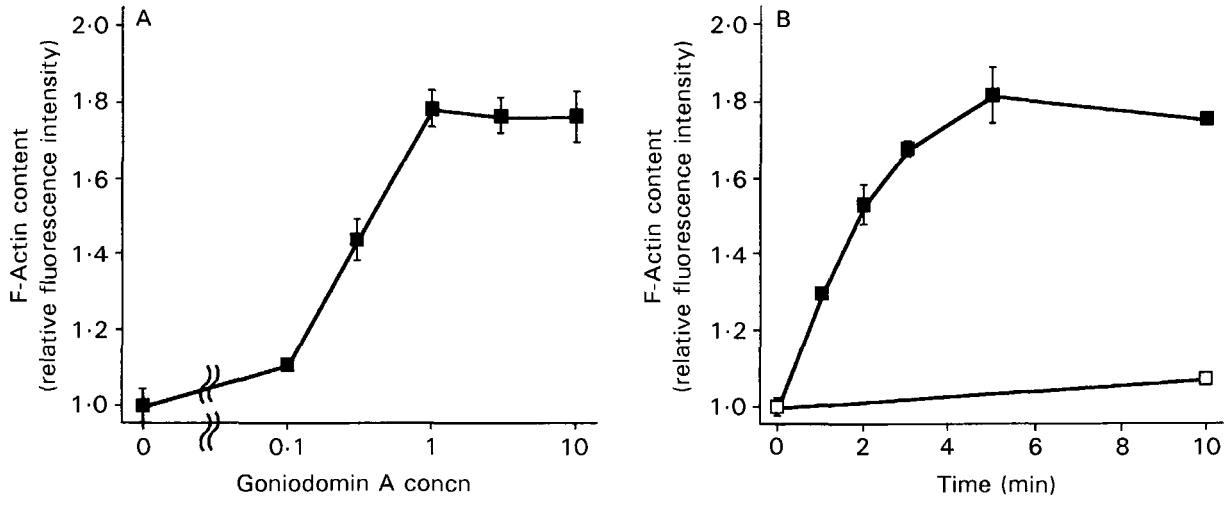

Figure 3. Effect of goniodomin A on the F-actin content of 1321N1 cells. 1321N1 cells were incubated with the indicated concentrations of goniodomin A for 10 min (A), and with vehicle (□) or 1 μM goniodomin A (■) for the indicated time (B). The F-actin level was measured with NBD-phallacidin. Data are means ± s.e. of results from three independent experiments.

3 min (Figure 2C), axial spikes and long needles grew from the cell surface. In cells treated with 1 μM goniodomin A for 10 min (Figure 2D), the axial spikes apparent after 3-min treatment had disappeared and the cell surface had become wrinkled.

Goniodomin A increased cellular F-actin content in a concentration- and time-dependent fashion (Figure 3). The goniodomin A-induced increase in F-actin was observed in the concentration range 0·3–10 μM with the maximum at 1 μM. Goniodomin A (1 μM) induced an approximately two-fold increase in F-actin content. The goniodomin A-induced increase in F-actin content reached a maximum at 5 min; this was sustained for at least 1 h (data not shown).

A few thin actin fibres were apparent in 1321N1 cells that had not been treated with goniodomin A (Figure 4A). As shown in Figures 4B and 4C, the intensity of rhodamine-phalloidin fluorescence was clearly increased by treatment with 1 μM goniodomin A and F-actin extension was observed. In cells treated with 1 μM goniodomin A for 10 min (Figure 2D), the filamentous constructs disappeared but the cells were still stained by rhodamine-phalloidin as dots.

Discussion

This study demonstrated that goniodomin A caused a morphological change in 1321N1 cells (Figure 2). In non-muscle cells cell-shape is critically dependent on the state of the cytoskeleton (Gumbiner 1996). Therefore, it is important to compare cell shape and F-actin content. To elucidate the underlying mechanism of goniodomin A-induced morphological change, we measured intracellular F-actin by use of NBD-phallacidin. In 1321N1 cells goniodomin A increased the quantity of F-actin in a time- and concentration-dependent fashion (Figure 3). Moreover, the extension of F-actin and accompanying morphological change was observed in goniodomin A-treated 1321N1 cells (Figure 4). These results suggest that goniodomin A might

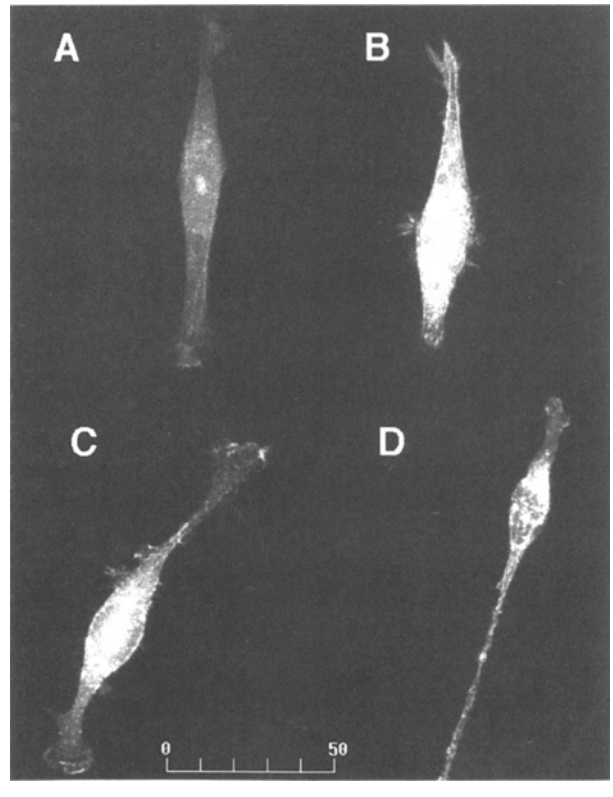

Figure 4. F-Actin distribution in 1321N1 cells. 1321N1 cells cultured on cover glasses were incubated with 1 μM goniodomin A for 0 min (A), 1 min (B), 3 min (C) and 10 min (D) and examined by confocal microscopy. Bar represents 50 μm.

cause morphological change by increasing the F-actin content of 1321N1 cells.

Our previous study reported that goniodomin A modulated rabbit skeletal actomyosin ATPase activity as a result of a change of the conformation of actin (Furukawa et al 1993). Goniodomin A also affected the state of actin in 1321N1 cells (Figures 3 and 4) and changed cell shape to rounded (Figure 2). It is assumed that this morphological change results from the stimulation of actomyosin ATPase by goniodomin A. Thus it seems that goniodomin A modulates non-muscle actin in addition to skeletal actin. Actin is a highly conserved structural protein present in eucaryotic cells. Although the difference in the amino-terminal end of the actin might influence actin polymerization, it is not certain that the difference has any functional consequence (Firtel 1981). Goniodomin A might become a useful tool for the investigation of the relationship between the structure and function of actin.

Acknowledgements

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