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Dolastatin 11, a Marine Depsipeptide, Arrests Cells at Cytokinesis and Induces Hyperpolymerization of Purified Actin

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ABSTRACT

The successful synthesis of dolastatin 11, a depsipeptide originally isolated from the mollusk *Dolabella auricularia*, permitted us to study its effects on cells. The compound arrested cells at cytokinesis by causing a rapid and massive rearrangement of the cellular actin filament network. In a dose-and time-dependent manner, F-actin was rearranged into aggregates, and subsequently the cells displayed dramatic cytoplasmic retraction. The effects of dolastatin 11 were most similar to those of the sponge-derived depsipeptide jasplakinolide, but dolastatin 11 was about 3-fold more cytotoxic than jasplakinolide in the cells studied. Like jasplakinolide, dolastatin 11 induced the hyperassembly of purified actin into filaments of apparently normal morphology. Dolastatin 11 was qualitatively more active than jasplakinolide and, in a quantitative assay we developed, dolastatin 11 was twice as active as jasplakinolide and 4-fold more active than phalloidin. However, in contrast to jasplakinolide and phalloidin, dolastatin 11 did not inhibit the binding of a fluorescent phalloidin derivative to actin polymer nor was it able to displace the phalloidin derivative from polymer. Thus, despite its structural similarity to other agents that induce actin assembly (all are peptides or depsipeptides), dolastatin 11 may interact with actin polymers at a distinct drug binding site.

The shell-less mollusk *Dolabella auricularia* has yielded a number of cytotoxic peptides and depsipeptides [for a review, see Pettit (1997)]. The most potent of these have been dolastatins 10 and 15 (Bai et al., 1990, 1992), both of which interact with tubulin and arrest cells in mitosis. Although dolastatin 11 (Pettit et al., 1989) (structure in Fig. 1) is not as potently cytotoxic, we observed in flow cytometric studies an accumulation of cells arrested at G_2/M. However, dolastatin 11 neither arrested cells in mitosis nor reacted with tubulin or microtubule protein. With some cell lines, treatment with dolastatin 11 caused a rapid shape change; ultimately, a large number of cells became binucleated. Thus, the cells seemed to be arrested at cytokinesis, suggesting actin or another component of the microfilament network was the target of dolastatin 11 [similar findings with other compounds described by Watabe et al. (1996) and Harrigan et al. (1998)].

A detailed investigation of this possibility required an adequate supply of dolastatin 11, and this was made possible by total synthesis of the depsipeptide (Bates et al., 1997). We found that dolastatin 11 caused rapid, concentration-dependent disruption of the intracellular microfilament network. These changes were similar to those observed with the sponge-derived depsipeptide jasplakinolide (Senderowicz et al., 1995; Spector et al., 1999; Bubb et al., 2000) and the myxobacterium-derived depsipeptides known as the chondramides (Sasse et al., 1998) (structures of jasplakinolide and chondramide D in Fig. 1). When we evaluated the interaction of dolastatin 11 with purified actin, we found that dolastatin 11, like jasplakinolide, the chondramides, and phalloidin (structure in Fig. 1) stimulated the assembly reaction. Furthermore, in a quantitative assay that we developed, dolastatin 11 was more potent than either jasplakinolide or phalloidin in stimulating actin assembly. Despite its efficient induction of actin assembly, however, dolastatin 11 differed from phalloidin, jasplakinolide, and chondramides in being unable to inhibit the binding of a fluorescently labeled phalloidin derivative to actin polymer.

Experimental Procedures

Materials. Actin and pyrenyl-labeled actin from rabbit muscle were obtained from Cytoskeleton (Denver, CO); jasplakinolide, phal-

ABBREVIATIONS: DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; AMB, actin monomer buffer, containing 5 mM Tris-HCl, pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP, and 5 mM dithiothreitol; PIB, polymerization inducing buffer, containing 2.5 M KCl, 100 mM $MgCl_2$, and 50 mM ATP.

loidin, and Antifade Mounting Solution were obtained from Molecular Probes (Eugene OR); latrunculin B was obtained from Calbiochem (San Diego, CA); PtK1 cells (normal kidney cells of the kangaroo rat *Potorous tridactylis*) were obtained from American Type Culture Collection (Manassas, VA); DAPI, FITC-conjugated phalloidin, and FITC-conjugated anti-β-actin monoclonal antibody were obtained from Sigma (St. Louis MO); and the Chambered Coverglass System was obtained from Nalge Nunc International (Naperville, IL). Natural and synthetic dolastatin 11 were prepared as described previously (Pettit et al., 1989; Bates et al., 1997). Majusculamide C was a generous gift of Dr. R. E. Moore, University of Hawaii.

Methods. PtK1 cells were maintained in culture as recommended by the supplier. Drug effects on the growth of the cells (increase in cell protein was the parameter measured) were evaluated as described previously (Bai et al., 1993).

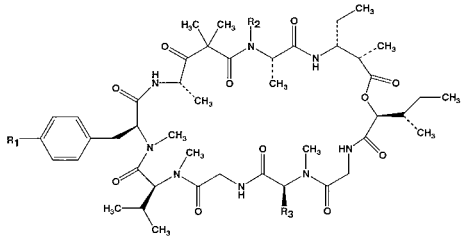
For immunofluorescence studies, PtK1 cells were grown to confluence, disrupted by trypsinization, and seeded at about 10% confluence into individual compartments of a Chambered Coverglass System. After 2 to 3 days of growth at 37° in a humidified 5% CO_2 atmosphere, drugs, as indicated, were added [final dimethyl sulfoxide concentration, 1% (v/v)], and cells were left for additional times at 37°, as indicated. Cells were washed twice with PBS, fixed with methanol at −20° for 10 min, and permeabilized with −20° acetone for 1 min. The coverglass was washed twice with PBS; DAPI at 1.0 μg/ml and the FITC-conjugated anti-β-actin monoclonal antibody, diluted 1:250 with PBS, were added in 150 μl to the coverglass, which was left for 1 h at 22° in the dark. The coverglass was washed twice with PBS, mounted on a slide with Antifade Mounting Solution, and examined with a Nikon Model Eclipse E800 microscope equipped with epifluorescence and appropriate filters. Images were captured with a Spot digital camera, model 2.3.0, using version 3.0.2 software (Diagnostic Instruments, Sterling Heights, MI). All images displayed here were obtained with the 40× oil objective (N.A. 1.30).

Actin polymerization was evaluated by either a fluorometric or a centrifugal assay. In the former method a fluorometer (Photon Technology International, Lawrenceville, NJ) was used, with FeliX for Windows software. Before performing assembly assays, actin and pyrenyl-labeled actin were diluted with AMB as a mixture to 12.5 and 1.0 μM, respectively, at 0°. In experiments in which assembly of pyrenyl-labeled actin only was examined, the protein preparation was diluted to 10 μM. After 1 h, the actin preparations were centrifuged at 45,000 rpm at 4° in a Beckman Instruments (Palo Alto, CA) Ti70 rotor. The supernatant was carefully removed and its protein content determined by the Lowry assay.

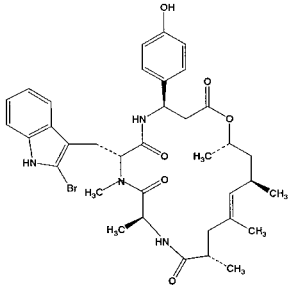
When the actin/pyrenyl-labeled actin mixture was used, it was diluted to 10 μM with AMB, and, for each assay, 100 μl of the final actin solution was transferred to a fluorescence cuvette at 22°. Without inducing salts, the fluorescence signal was referenced. Drug, if present, was added at this point in 1 μl of dimethyl sulfoxide, and fluorescence was monitored (excitation at 365 nm, emission at 407 nm). With inducing salts, drug in 1 μl of dimethyl sulfoxide was added, the fluorescence signal was referenced, 2 μl of PIB was added to the cuvette, and fluorescence emission was followed.

When the pyrenyl-labeled actin alone was used, it was diluted to 0.6 μM with AMB. PIB (2 μl) was added to 100 μl of the pyrenyl-labeled actin in the fluorescence cuvette at 22°. After about 4 min, drug in 5 μl of dimethyl sulfoxide was added. Fluorescence was continuously monitored after addition of the polymerizing salts.

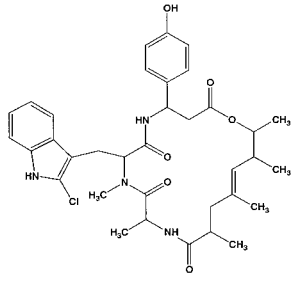
The centrifugal assay was developed (see below) as a means to obtain a quantitative comparison of the effects of drugs inducing actin assembly. The best data were obtained when the assembly inducing salts were not added to the reaction mixture, and it also proved unnecessary to perform the initial clarification centrifugation. In the presence of inducing salts and the absence of drug, most of the actin (average, 76%) was pelleted under the centrifugal conditions used here. In contrast, without inducing salts or drug, only 24% of the actin was pelleted. For quantitative evaluation of drug effects, actin was diluted to 25 μM with AMB, and the solution was left at 0° for 1 h. Various concentrations of drug in 5 μl of dimethyl sulfoxide were added to 95 μl aliquots of the actin solution. Reaction mixtures were incubated for 2 h at 22° and centrifuged for 30 min at 45,000 rpm in a Beckman TA-45 rotor in a TL100 micro-ultracentrifuge. The protein content of the supernatant was determined by the Lowry assay. The EC_{50} value for a drug was defined as the concen-



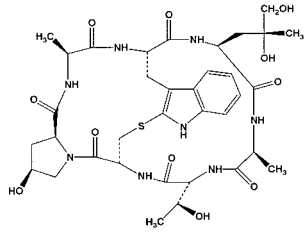
Dolastatin 11:	$R_1 = OCH_3$	$R_2 = H$	$R_3 = (CH_3)_2CHCH_2-$
Dolastatin 12:	$R_1 = H$	$R_2 = CH_3$	$R_3 = (CH_3)_2CHCH_2-$
Lyngbyastatin 1:	$R_1 = OCH_3$	$R_2 = CH_3$	$R_3 = (CH_3)_2CHCH_2-$
Majusculamide C:	$R_1 = OCH_3$	$R_2 = H$	$R_3 = (S)\text{-}CH_3CH_2(CH_3)CH-$



Jasplakinolide



Chondramide D



Phalloidin

Fig. 1. Structural formulas of dolastatin 11 and related structures, jasplakinolide, chondramide D, and phalloidin. The stereochemistry shown for phalloidin is derived from Kessler and Wein (1991).

tration required to reduce the supernatant actin concentration by 50% relative to control reaction mixtures without drug.

The inhibition of the binding of FITC-phalloidin to actin polymer was measured by removing polymer from the 100-μl reaction mixture by centrifugation, followed by measurement of fluorescence at 517 nm (excitation at 495 nm) of the supernatant. Several variations in order of addition of reaction components were examined. 1) Actin was diluted to 10 μM in AMB and incubated with or without potential inhibiting drugs at various concentrations for 1 h at 22°. FITC-phalloidin was added to 20 μM along with 2 μl of PIB (final dimethyl sulfoxide concentration, 6%). After an additional 1 h at 22°, reaction mixtures were centrifuged for 30 min at 40,000 rpm in a Beckman TA-45 rotor in a TL100 micro-ultracentrifuge. 2) Actin was incubated with or without potential inhibitors at various concentrations in the presence of 2 μl of PIB for 1 h at 22°. FITC-phalloidin (20 μM) was added. After an additional 1 h at 22°, reaction mixtures were centrifuged as in 1). 3) Actin was incubated with 20 μM FITC-phalloidin and 2 μl of PIB for 1 h at 22°. Various concentrations of the potential inhibitors were added to the reaction mixtures. After an additional 1 h at 22°, reaction mixtures were centrifuged as in 1).

For electron microscopy, 10-μl aliquots of reaction mixtures were applied to carbon-coated, Formavar-treated, 200-mesh copper grids. The sample droplet was immediately washed from the grid by 5 to 10 drops of 0.5% (w/v) uranyl acetate, and excess stain was wicked from the grid with torn filter paper. The grids were examined in a Zeiss model 10CA electron microscope. Reaction mixtures (50 μl) contained 10 μM actin in AMB, drug at 10 μM as indicated, 1 μl of PIB if indicated, and 5% dimethyl sulfoxide.

TABLE 1
Effects of dolastatin 11, jasplakinolide, and latrunculin B on the growth of PtK1 cells

Drug	IC_{50} ± S.D.
	nM
Dolastatin 11	47 ± 4
Jasplakinolide	150 ± 60
Latrunculin B	600 ± 400

Results

Initial Studies. Because of the precedents of dolastatins 10 and 15 (Bai et al., 1990, 1992), we initially assumed that dolastatin 11 would also inhibit mitosis through an interaction with tubulin. However, despite the accumulation of cells at G_2/M by flow cytometric analysis, there was no increase in the mitotic index, and the microtubules of cells treated with the depsipeptide were intact. Moreover, the compound had no effect on the polymerization of purified tubulin. When rat C_6 glial tumor cells were treated with dolastatin 11, within 15 to 30 min, virtually all cells in the culture underwent a dramatic shape change, characterized by apparent extensive retraction of the cytoplasm. Many hours later, many of the cells became binucleate (data not shown). Similar observations with other drugs interacting with actin have been described in fibroblastic and smooth muscle cell lines (Watabe et al., 1996; Harrigan et al., 1998). Finally, in a variety of experiments comparing natural and synthetic dolastatin 11, we observed no significant difference in the effects of the two preparations with either cells or purified actin. Therefore, unless indicated otherwise, the studies presented here were performed with the synthetic material.

Visualization of Intracellular Actin Filaments with a FITC-Labeled Anti-Actin Antibody. We evaluated the microfilament network of PtK1 cells with both FITC-phalloidin and a FITC-conjugated antibody directed against β-actin. The staining patterns obtained were identical, and only results with the antibody are presented here. We wished to compare cells at defined equitoxic concentrations of drug, and Table 1 summarizes IC_{50} values for growth of PtK1 cells obtained with dolastatin 11, jasplakinolide, and latrunculin B. We chose to study cells treated for varying time periods at these IC_{50} values and at 10 times these concentrations of the three drugs. Initial 24-h studies (data not shown) showed nearly complete disruption of stress fibers after latrunculin B

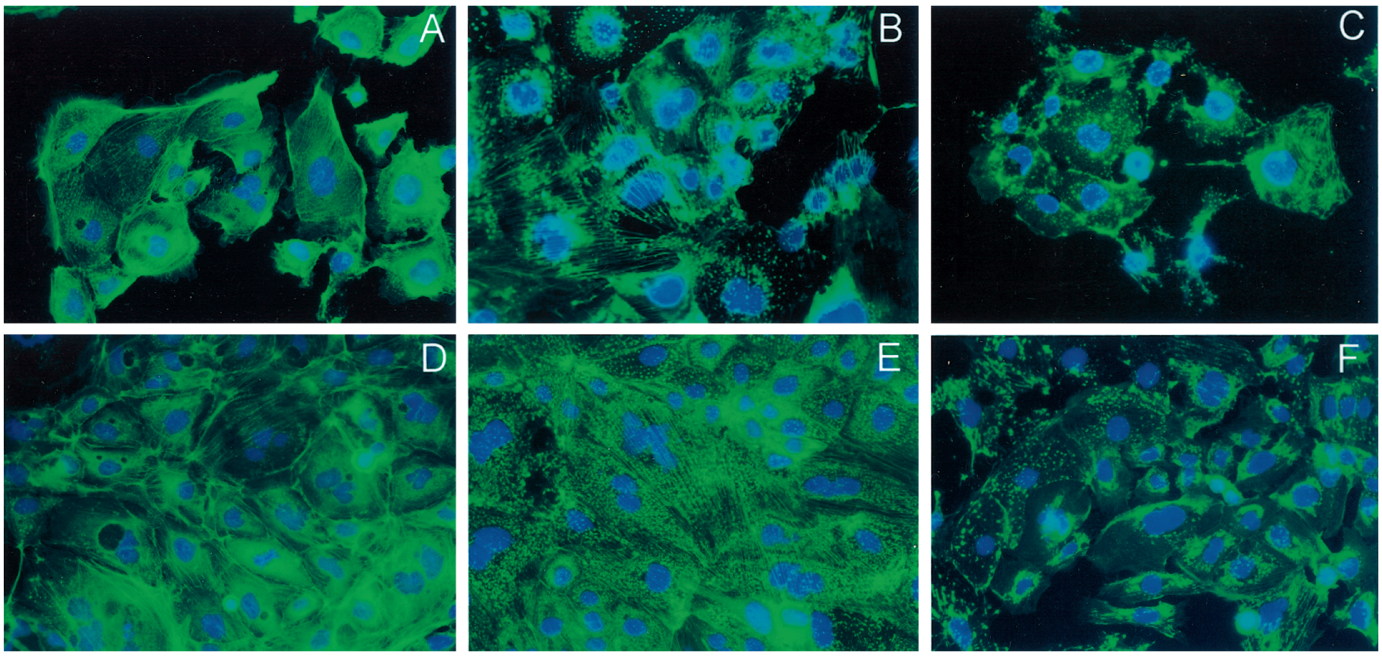


Fig. 2. Comparison of the effects of dolastatin 11 (A-C) with those of jasplakinolide (D-F) on PtK1 cells treated with the IC_{50} concentrations of each drug for varying time periods. Cells were grown for 30 min (A, D), 1 h (B, E), or 4 h (C, F) in the presence of drug, as described in the text. F-actin stained with the FITC-actin antibody is shown in green, nuclei stained with DAPI are shown in blue.

treatment, confirming the findings of Spector et al. (1999). In contrast, progressive and nearly indistinguishable aggregation of F-actin occurred with dolastatin 11 and jasplakinolide treatment. In addition, after 24 h with the latter two drugs, there was a much more extensive retraction of the cytoplasm of the PtK1 cells than occurred with latrunculin B.

In Figs. 2 and 3, we present cells treated at the lower and higher concentrations for shorter time periods with dolastatin 11 and jasplakinolide. Cells treated with the IC_{50} concentrations of the two drugs are shown in Fig. 2. Perhaps because these concentrations are difficult to define precisely, little difference was observed over time in the effects of dolastatin 11 compared with those of jasplakinolide. Minor dissolution of the microfilament network occurred with both drugs at 30 min, and this became extensive, although incomplete, by 60 min. There was perhaps somewhat greater clumping of F-actin structures with dolastatin 11 treatment (Fig. 2B) than with jasplakinolide treatment (Fig. 2E) at 60 min, but this was not striking when many fields of cells were compared. By 4 h with both drugs, only F-actin clumps were visible in the PtK1 cells. Cytoplasmic retraction was not prominent in cells treated at the IC_{50} concentrations of either dolastatin 11 or jasplakinolide.

In cells treated at 10 times the IC_{50} concentrations, there were noticeable differences between dolastatin 11 and jasplakinolide treatment as a function of time (Fig. 3). At 30 min, there were few stress fibers remaining in dolastatin 11-treated cells (Fig. 3A), but they were still relatively prominent in the jasplakinolide-treated cells (Fig. 3D). By 60 min, no actin filaments remained in the dolastatin 11-treated cells (Fig. 3B), whereas a few persisted with jasplakinolide treatment (Fig. 3E). By 4 h, however, cells treated with the two drugs were indistinguishable from each other (compare Figure 3, C and F) and identical in appearance to those treated for 24 h (not shown).

Effect of Dolastatin 11 on the Assembly of Purified Actin. We chose the coassembly of a tracer amount of pyrenyl-labeled actin with unmodified actin as the system to study drug effects on actin polymerization because of the readily measured enhancement of fluorescence that occurs when the labeled actin is incorporated into polymer (Cooper et al., 1983). Initially, we compared drugs at 10 μM concentrations, with actin at the same concentration of 10 μM, using a standard induction reaction condition (50 mM KCl/2 mM $MgCl_2$/1 mM ATP). At this actin concentration, well above the critical concentration for the protein (Cooper et al., 1983), we observed clear stimulatory effects with dolastatin 11 and jasplakinolide and modest stimulation with phalloidin (Fig. 4A). In contrast, latrunculin B completely inhibited actin assembly (data not shown; compare Coué et al., 1987). It seems unlikely, however, that the differences in fluorescence observed in the experiments of Fig. 4A are linearly related to extent of assembly, because pellets formed without drug and with 10 μM dolastatin 11 contained nearly identical amounts of actin. Moreover, electron micrographs of similar reaction mixtures showed dense and essentially identical networks of filaments, whether or not one of the three drugs was included in the reaction mixture (see below). This suggests the possibility that pyrenyl-labeled actin incorporated into drug-containing polymers may fluoresce with different intensities compared with control actin filaments.

We therefore examined drug effects on the assembly of pyrenyl-labeled actin (without unlabeled actin in the reaction mixture), measured by increase in fluorescence near the actin critical concentration (0.025 mg/ml, about 0.6 μM). As shown in Fig. 4B, there was little change in fluorescence in the absence of drug, and only a small increase in fluorescence upon addition of either 50 μM jasplakinolide or 50 μM phalloidin. The increase in fluorescence was more dramatic with 50 μM dolastatin 11. In additional experiments with a range

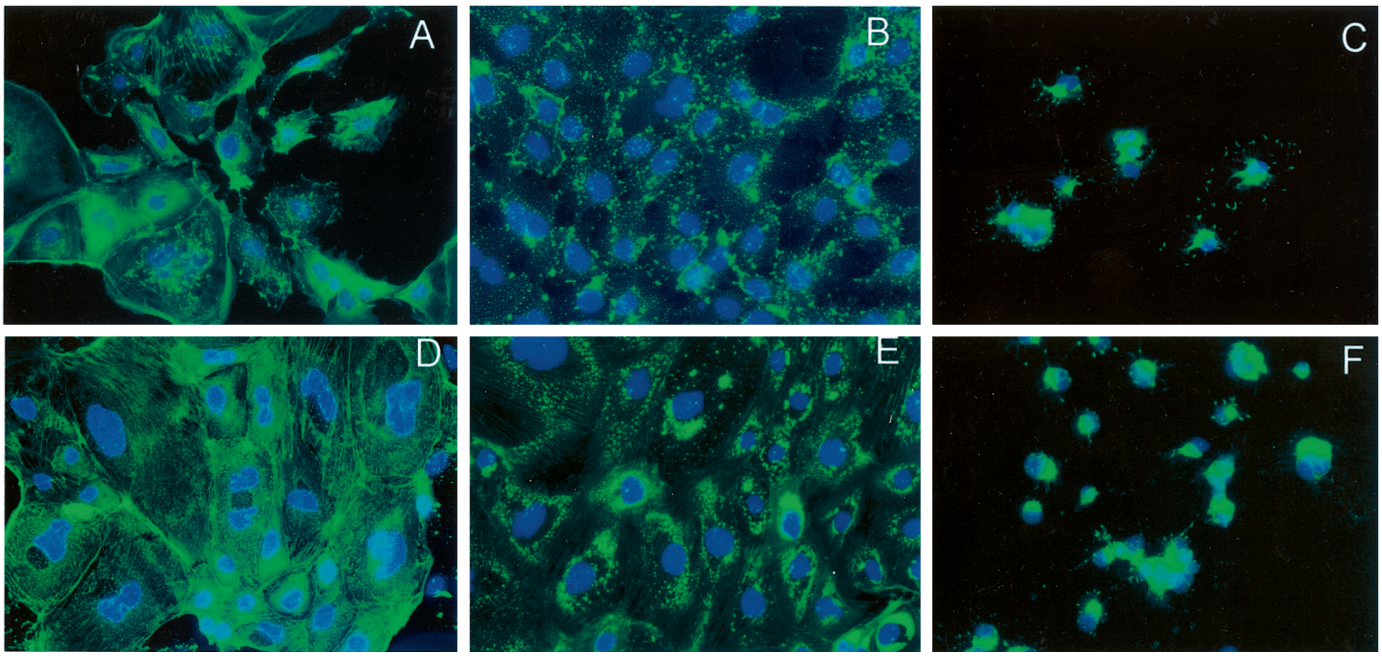


Fig. 3. Comparison of the effects of dolastatin 11 (panels A-C) with those of jasplakinolide (panels D-F) on PtK1 cells treated with 10 times the IC_{50} concentrations of each drug for varying time periods. Cells were grown for 30 min (A, D), 1 h (B, E), or 4 h (C, F) in the presence of drug, as described in the text. F-actin stained with the FITC-actin antibody is shown in green, nuclei stained with DAPI are shown in blue.

of jasplakinolide and dolastatin 11 concentrations, we observed progressive increase in fluorescence as increasing amounts of drug were added in the 2 to 50 μM range, which seems to exclude hypernucleation as an explanation for the relatively low fluorescence changes observed with low concentrations of pyrenyl-labeled actin.

Because we could only examine one specimen at a time with the fluorometer available to us, it seemed desirable to develop a centrifugation assay to more readily compare effects of different drug concentrations in enhancing actin assembly. We found that readily measurable differences required eliminating K^+ from the reaction mixtures. Moreover, the stimulatory effects of both jasplakinolide (Bubb et al., 1994) and chondramides (Sasse et al., 1998) were more readily demonstrated under "noninducing" reaction conditions. Figure 5 compares the fluorometrically followed reaction without drug to those with 10 μM dolastatin 11, jasplakinolide, or phalloidin. There was no apparent actin assembly in the absence of drug, and the relative activities of the three stimulatory drugs became apparent (dolastatin 11 > jasplakinolide > phalloidin).

The differences between the compounds were even more readily apparent when reaction mixtures were centrifuged. Figure 6 shows residual protein in the supernatant after assembly with varying concentrations of these three drugs. Average EC_{50} values obtained in at least three independent experiments with each drug are shown in Table 2. The relative values obtained with dolastatin 11 and jasplakinolide are consistent with their relative cytotoxicity toward the PtK1 cells.

Dolastatin 11, Unlike Jasplakinolide, Does Not Inhibit the Binding of FITC-Labeled Phalloidin to Polymer Formed from Purified Actin. Jasplakinolide was previously shown to inhibit binding, in a manner consistent with competitive inhibition, of fluorescently labeled phalloidin to actin polymer (Bubb et al., 1994), and a comparable result was obtained with chondramide A (Sasse et al., 1998). We performed a similar study with dolastatin 11 in comparison with jasplakinolide and phalloidin (Fig. 7), anticipating that

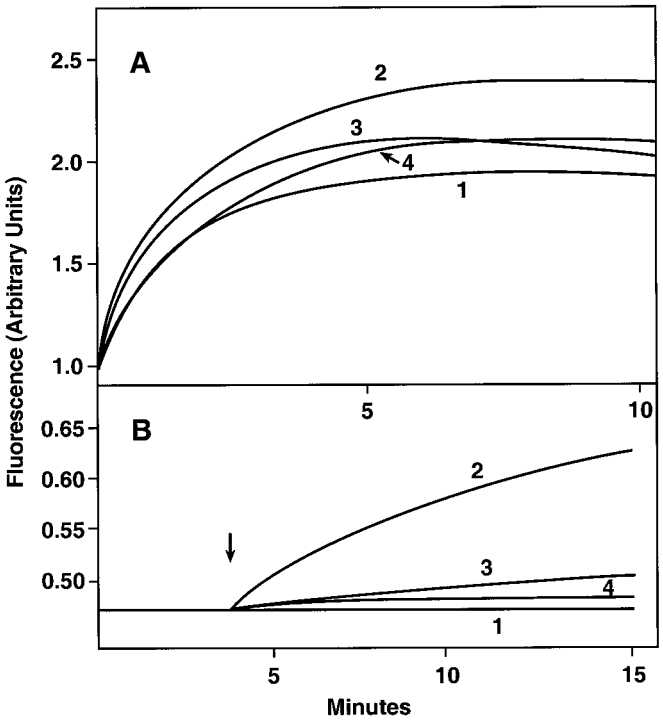


Fig. 4. Drug effects on actin polymerization. A, with inducing salts (50 mM KCl/2 mM $MgCl_2$/1 mM ATP), actin/pyrenyl-labeled actin at 10 μM, and drugs at 10 μM. Drug was added at zero time. B, with inducing salts, pyrenyl-labeled actin at 0.6 μM, and drugs at 50 μM. The PIB solution was added at zero time, and drug was added at the time indicated by the arrow. Reaction mixtures contained drug, as follows: curve 1, none; curve 2, dolastatin 11; curve 3, jasplakinolide; curve 4, phalloidin. See text for additional experimental details.

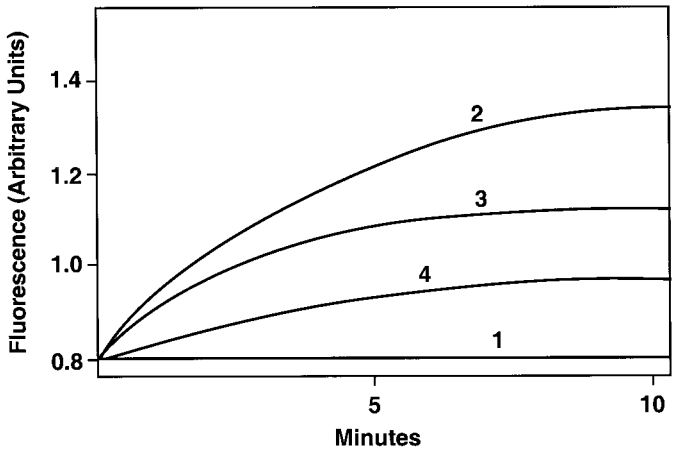


Fig. 5. Drug effects on actin polymerization in the absence of inducing salts (residual ATP from AMB). Drug was added at zero time. Reaction mixtures contained drug, as follows: curve 1, none; curve 2, 10 μM dolastatin 11; curve 3, 10 μM jasplakinolide; curve 4, 10 μM phalloidin. See text for additional experimental details.

TABLE 2

Relative drug effects on actin polymerization

The polymerization reaction was performed without polymerization inducing salts, as described in the text.

Drug	EC_{50} ± S.D.
	μM
Dolastatin 11 (natural)	9.5 ± 0.7
Dolastatin 11 (synthetic)	13 ± 3
Majusculamide C	19 ± 1
Jasplakinolide	24 ± 2
Phalloidin	42 ± 3

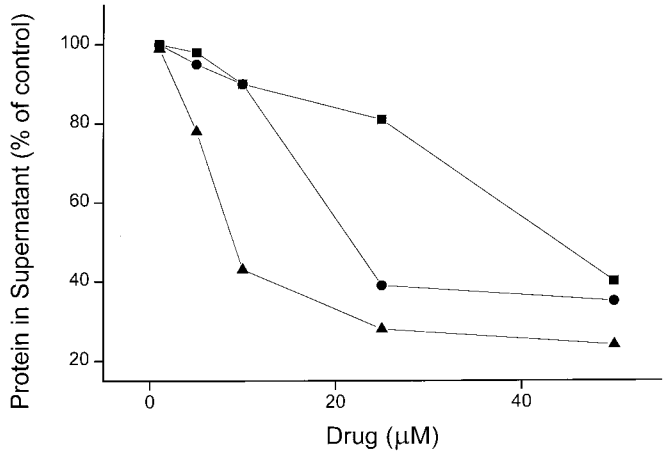


Fig. 6. Drug effects on actin polymerization without inducing salts. Reaction mixtures contained 24 μM actin in AMB, 5% dimethyl sulfoxide, and drugs, as indicated by the following symbols: ▲, dolastatin 11; ●, jasplakinolide; ■, phalloidin.

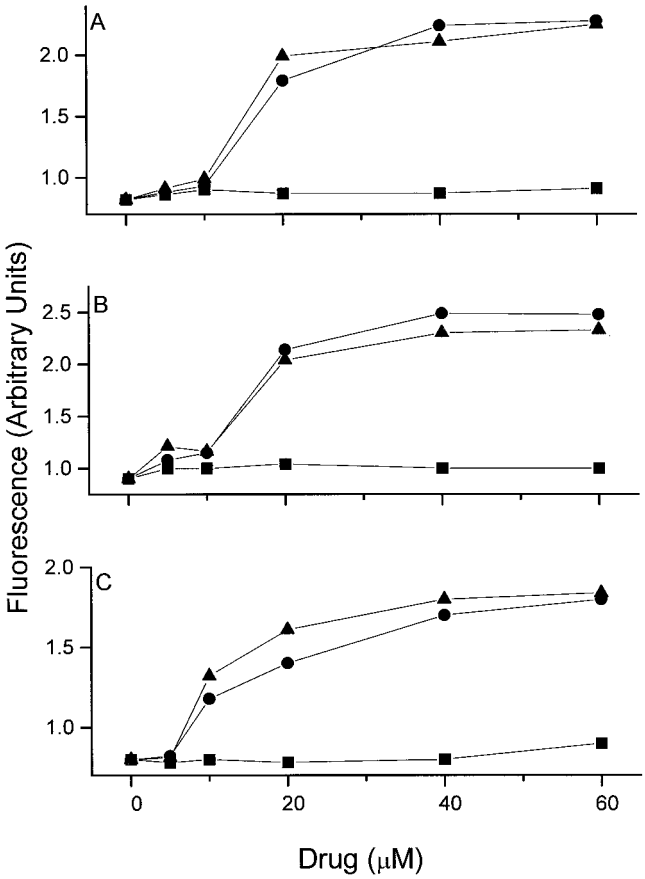


Fig. 7. Inhibition of binding of FITC-phalloidin to actin polymer. Reaction mixtures contained drugs symbolized as follows: ■, dolastatin 11; ●, jasplakinolide; ▲, phalloidin. Reaction conditions were described in detail in the text. A, actin and potential inhibiting drugs were preincubated before addition of FITC-phalloidin and PIB. B, actin, PIB, and potential inhibiting drugs were preincubated before addition of FITC-phalloidin. C, actin, FITC-phalloidin, and PIB were preincubated before addition of the potential inhibiting drugs.

dolastatin 11, too, would inhibit binding of FITC-phalloidin to actin fibers.

Quite unexpectedly, we could demonstrate no enhancement by dolastatin 11 of supernatant fluorescence after removal of actin polymer by centrifugation. Many variations in experimental design were attempted, both with and without polymerization-inducing salts, and three examples with the salts are shown in Fig. 7. In the experiment shown in Fig. 7A, actin and inhibitors were preincubated before induction of assembly in the presence of FITC-phalloidin. In the experiment shown in Fig. 7B, the preincubation of actin and potential inhibitors included the inducing salts with subsequent addition of FITC-phalloidin. In the experiment shown in Fig. 7C, FITC-phalloidin was preincubated with actin and inducing salts before addition of potential inhibitors. Both jasplakinolide and phalloidin had similar activity under all three reaction conditions. In addition, the results shown in Fig. 7C demonstrate that the binding of the FITC-phalloidin to actin filaments is readily reversible.

Morphological Studies. The inability of dolastatin 11 to inhibit FITC-phalloidin binding to actin polymer led us to ask whether the basic mechanism of action of dolastatin 11 differed from that of the other peptides. For example, dolastatin 11 could induce formation of a nonfilamentous polymer. The dolastatin 11-induced reaction would thus be comparable with drug-induced aberrant polymerization reactions that occur with tubulin [examples in Hamel et al. (1995) and Bai et al. (1996)]. We therefore performed initial evaluations of polymer morphology by negative stain electron microscopy of reaction mixtures with 1.5 or 10 μM actin with 10 or 50 μM dolastatin 11, jasplakinolide, or phalloidin with the polymerization inducing salts, and with 10 μM actin without the salts.

With the inducing salts at the higher actin concentration, the grids were covered by a thick mat of actin filaments under all conditions, including absence of drug. The number of filaments was much reduced at the lower actin concentra-

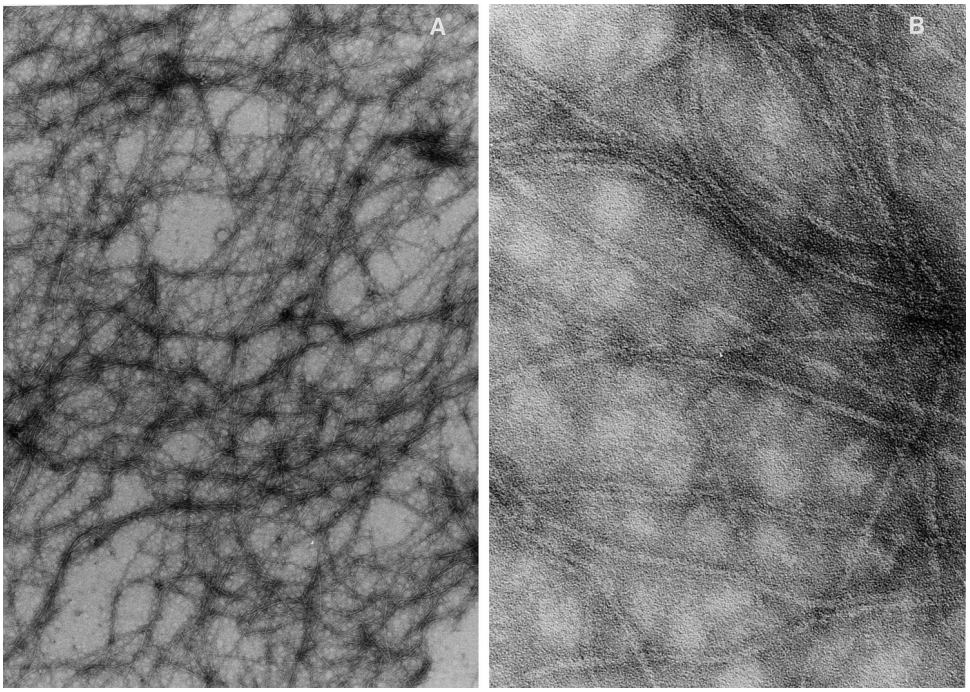


Fig. 8. Electron micrographs of actin polymer formed after addition of PIB in the presence of 10 μM dolastatin 11. Incubation was for 20 min at 22°. A, 24,000×. B, 185,000×.

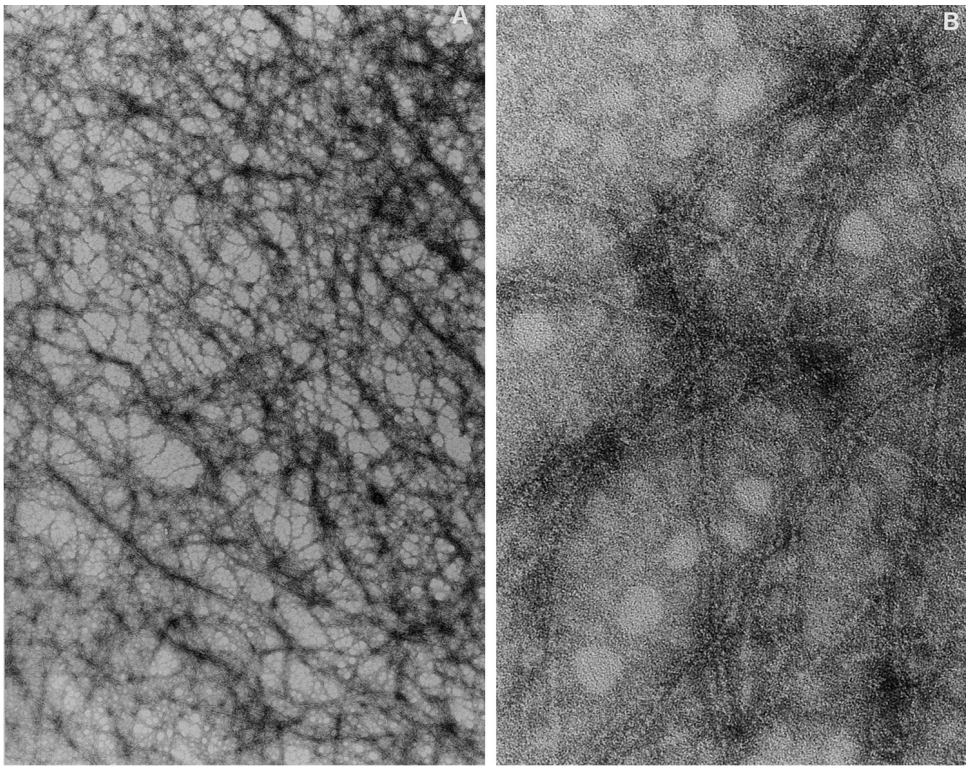


Fig. 9. Electron micrographs of actin polymer formed in the absence of polymerization-inducing salts in the presence of 10 μM dolastatin 11. Incubation was for 20 min at 22°. A, 24,000×. B, 185,000×.

tion. There were no significant morphological differences in the filaments formed under any reaction condition, and the width of the fibers observed was consistent with that of actin filaments. Typical images are shown in Fig. 8, which presents low and high magnification views of the polymer formed in the presence of 10 μM dolastatin 11.

Without the inducing salts, the actin filaments were much sparser on the grids, but they were identical in appearance to those observed with the inducing salts. Figure 9 presents low and high magnification views of the filaments formed with 10 μM dolastatin 11.

Discussion

Dolastatin 11 and related compounds represent the fourth peptide/depsipeptide family that induces the assembly of actin in vitro, after phalloidin, jasplakinolide, and the chondramides. Dolastatin 11 is probably the most potent of these compounds. With the exception of phalloidin, the anti-actin peptides are cytotoxic at nanomolar concentrations and cause cells to arrest at cytokinesis. In cells, however, the hyperassembly of actin is not associated with an apparent increase in actin filaments, but with the rapid appearance of clumped F-actin that stains both with FITC-phalloidin and with an FITC-labeled antibody to β-actin. Bubb et al. (2000) recently proposed that this phenomenon is caused by formation of multiple actin filament nuclei in jasplakinolide-treated cells, with fiber propagation limited by the resulting shortage of monomeric actin in the cells. Such a mechanism should also apply in the case of dolastatin 11, because we find it to be more potent than jasplakinolide as an inducer of assembly under the reaction conditions we have studied.

Harrigan et al. (1998) described dolastatin 12, lyngbyastatin 1, and majusculamide C. In a number of cell lines, these three compounds all had similar cytotoxic activity. Harrigan et al. (1998) also reported disruption of the actin filament network in a smooth muscle cell line treated with the dolastatin 12 and lyngbyastatin 1 preparations. The apparent interpretation in this study was that these agents were inhibitors of actin assembly. Although Harrigan et al. (1998) did not characterize the in vitro interaction of dolastatin 12, lyngbyastatin 1, or majusculamide C with actin, the published image of the lyngbyastatin 1-treated cells is similar to what we have described here with dolastatin 11. We therefore conclude that this entire group of compounds induces actin assembly in vitro (we have also directly demonstrated assembly induction with majusculamide C; see Table 2). Furthermore, dolastatin 11 has been 10 to 30 times more cytotoxic than dolastatin 12 in several cell lines (Pettit et al., 1989; R. Bai and E. Hamel, unpublished observations), indicating it is the most active member of the family yet isolated.

Dolastatin 11, despite its relative potency both against cells and as an inducer of actin assembly, had negligible ability to inhibit the binding of FITC-phalloidin to actin polymer. Dolastatin 11 was also unable to displace prebound FITC-phalloidin from polymer. Such inhibition or displacement was readily accomplished with jasplakinolide and phalloidin, confirming published reports of similar experiments for these compounds and for chondramide D (Bubb et al., 1994; Sasse et al., 1998). This finding is highly suggestive that dolastatin 11 may bind at a different site on actin polymer than the other peptides, but definitive proof of this conclusion will require preparation of radiolabeled dolastatin 11 or of a fluorescent active analog.

The possibility that dolastatin 11 and related compounds may bind to a distinct site on actin polymer could be regarded as a positive feature for development of this group of compounds as potential chemotherapeutic agents. Although no anti-actin compound has a role in cancer chemotherapy, jas-

plakinolide was under investigation at the National Cancer Institute as a candidate for clinical development. Jasplakinolide, however, was found to have significant pulmonary toxicity in both rats and dogs (Schindler-Horvat et al., 1998), involving edema and hemorrhage; consequently, the compound is no longer under active development. Whether pulmonary toxicity is universal for anti-actin compounds, restricted to those that interfere with phalloidin binding, or restricted to those that induce assembly is unknown at the present time. Although a distinct actin binding site for dolastatin 11 could be viewed as potentially avoiding the toxicity observed with jasplakinolide, the study of Harrigan et al. (1998) is not encouraging. They observed minimal antitumor activity with dolastatin 12 or lyngbyastatin 1, but toxic effects of the compounds included pulmonary hemorrhage.

Finally, it is perhaps worth pointing out a remarkable difference between drugs inducing actin assembly and those inducing tubulin assembly. Thus far only peptides and depsipeptides have had this activity with actin. With tubulin, an increasingly diverse group of molecules stabilize microtubules and induce hypernucleation of tubulin assembly. The newest members of the class are steroid derivatives (Mooberry et al., 2000; Verdier-Pinard et al., 2000).

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