COLCHICINE TREATMENT OF HeLa CELLS ALTERS THE G/F ACTIN RATIO

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1. Introduction

Several types of drugs and chemicals have been used in investigating the properties and functions of cellular filamentous structures. Some of these drugs exert a specific effect on the function of one of the fiber-systems. For example, alkaloids like colchicine and vinblastine cause a reversible disassembly of microtubules both in vivo and in vitro [1–4], while mushroom poisons like cytochalasins [5,6] and phalloidine [7] interfere with the organization of microfilaments.

Colchicine was originally described as a compound with antimitotic activity. Since then its interaction with tubulin subunits and effect on the stability of microtubules has been thoroughly documented [3,4, 8,9]. When cells are treated with colchicine, microtubules disappear <1 h as visualized by immunofluorescence microscopy [10] and the pool of free tubulin subunits increases [11]. The organization of the third filamentous structure in the cell, the 100 Å filaments, collapses and forms a large perinuclear aggregate indicating a functional interaction with microtubules. However, ruffled membranes are formed and motility of organelles in the cell perifery continues [12], but the development of polarized movements and cellular form are disturbed [1,2]. No interaction between colchicine and actin monomers or microfilaments has been reported.

In [13] we described an assay for rapid quantification of both unpolymerized and filamentous actin in cell homogenates. This type of measurement has been applied to several cellular systems undergoing changes in motility and function [14–17]. Here we report the effect of colchicine on the organization of actin. The results show that in HeLa cells treated with colchicine, the pool of filamentous actin increases by ~10% of the total actin. The effect does not seem to be dependent of the concentration of colchicine added. This result could be an indication of a direct organizational cooperativity between intact microtubules and microfilaments functioning in the cell.

2. Materials and methods

Stock cultures of HeLa cells (type S) were maintained at 37°C in closed plastic bottles (250 ml, Nunclon) in Eagle's minimal essential medium (Eagle's MEM) supplemented with 10% calf serum (Gibco), 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes, Sigma) (pH 7.3) and 5 μg/ml gentamycin sulfate (Sigma). Cells used for an experiment were removed from the substrate with a solution of 2.5 mg trypsin/ml, 5 mM EDTA dissolved in Eagle's MEM, pelleted, resuspended in Eagle's MEM with serum. The cells were then subcultured onto Nunclon plastic petri dishes (90 mm diam.) at either 5 or 10 × 10^6 cells/plate and incubated at 37°C in humidified 5% CO_2–95% air mixture for 1 or 2 days. At this stage the cells were near confluency.

For actin pool measurements [13] each plate was thoroughly rinsed twice with phosphate-buffered saline (PBS). Cells from one plate were directly scraped from the plate with a rubber policeman in 100 μl of the following lysis buffer (called MT-buffer): 100 mM piperazine-*N,N'*-bis(2-ethane–sulfonic acid) (Pipes, Sigma) (pH 6.75); 1 mM $MgCl_2$; 1 mM GTP; 1 mM ethylene–glycol–bis-(2-aminoethyl ether)*N,N'*-tetra-acetic acid (EGTA); 0.2 mM dithioerythritol (DTE); 1 M sucrose and 0.5% Triton X-100. An isotope dilution technique was applied for estimation of the total volume of the homogenates. The DNase I (DN-100, Sigma) used was purified and calibrated against purified rabbit skeletal muscle actin as in [13,14]. The specific inhibitor activity was estimated to 90 870 ± 890 (n = 14) inhibitor units/mg actin. Colchicine

(Sigma) was dissolved in Eagle's MEM as a 10^{-4} M stock solution and stored at +4°C before use.

3. Results and discussion

A long series of experiments on actin quantification in homogenates of HeLa cells have shown that Mg^{2+}-containing and isotonic lysis buffer in [13–15] was insufficient in keeping the pools of unpolymerized and filamentous actin stable after cell lysis. Therefore we have used here a buffer composition similar to what is used for stabilizing microtubules. A detailed description of measurements of actin pools in general in HeLa cell homogenates will appear in [18]. However, as can be seen in fig.1A, the buffer used here seems to keep a stable distribution between unpolymerized and filamentous actin in the homogenates for ⩾30 min. Homogenates from untreated cells usually contain 35–40% of the total actin in an unpolymerized form. With some batches of cells this number can be as high as 50%.

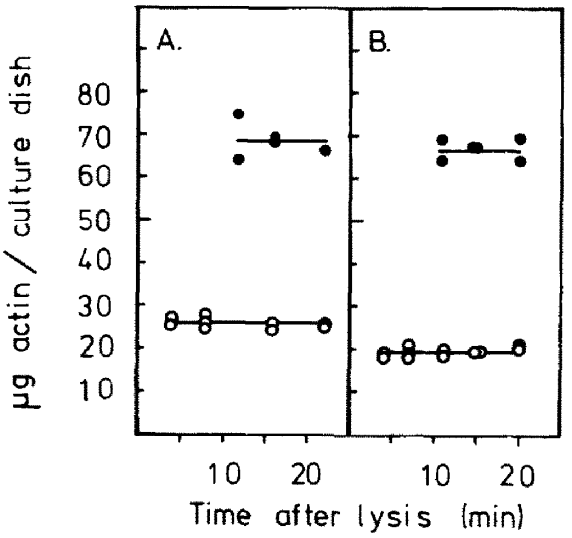

Fig.1. Determination of actin pools in homogenates of HeLa cells before and after colchicine treatment. Culture plates were either taken directly to actin measurements (A), or pretreated with 10^{-6} M colchicine for 3 h at 37°C (B) before actin measurements. Cells were then washed with PBS and lysed as in section 2 and DNase inhibitor activity in the homogenates was determined at different times after lysis (○). After lysis (2 min), 20 μl homogenate was diluted 5× with lysis buffer and treated with guanidine–HCl solution as in [13], before the total amount of actin was determined (●). The results are expressed as μg actin/plate, calculated from the estimated specific inhibitor activity (see section 2).

Table 1
Determination of actin pools in HeLa cells after colchicine treatment

Experiment	% Unpolymerized actin
1. Untreated	38.1 ± 0.7 (n = 9)
10^{-6} M colchicine	29.1 ± 0.4 (n = 11)
10^{-7} M colchicine	30.3 ± 0.5 (n = 11)
2. Untreated	49.8 ± 0.6 (n = 11)
10^{-7} M colchicine	36.6 ± 0.6 (n = 9)
10^{-6} M colchicine	39.8 ± 1.0 (n = 11)
3. Untreated	39.2 ± 0.5 (n = 8)
5×10^{-4} M colchicine	33.0 ± 0.9 (n = 8)
4. Untreated	43.3 ± 0.5 (n = 8)
5×10^{-4} M colchicine	33.1 ± 0.3 (n = 8)

Actin pool measurements were performed as in fig.1. The results represent mean values ±SEM, using for the calculation all time points from curves like in the figure. The experiments were performed over a time span of 6 months with different batches of cells

Colchicine was then added to the culture medium to give 10^{-6}–5×10^{-4} M final conc. The cells were incubated for 3 h in the presence of colchicine before actin measurements were performed. One example is shown in fig.1B and it can be seen that the unpolymerized pool of actin have decreased by ~20%. At all concentrations tested, a similar effect on the pools of actin was seen (table 1). The pool of filamentous actin increased 6–13% of the total amount of actin, with a concomitant decrease in the amount of unpolymerized actin. No significant change in the total amount of actin before and after colchicine treatment was observed.

The limitations with the assay have been detailed in [13,14]. However, we have no knowledge of events in the homogenates the first 2–4 min after lysis. Therefore, we can not definitely say if the observed change in the organization of actin represents an actual increase in the amount of filamentous actin in the cell or a stabilization of a fraction of filaments which is normally depolymerized before the first measurement. However, if the fraction of filaments really has increased, this change could be brought about in one of two ways:

(i) A stimulation of the formation of filaments;
(ii) A decrease in the rate of depolymerization of actin filaments in the cell.

The formation of actin filaments have been thought

of being a highly localized process confined to regions close to or even associated with membranes. Furthermore, microtubules usually seem to end at some distance from the plasma membrane. Therefore, we tend to favour the possibility that microtubules or some structure linked to microtubules regulates the depolymerization of actin filaments.

A direct linkage between microtubules and actin filaments has been proposed from in vitro interaction studies of MAPs and actin filaments [19]. Our results might indicate that microtubules or some associated structure is important for depolymerization of actin filaments in vivo, thus regulating one step of the turnover of actin between monomers and filaments supposed to occur in the cell. However, experiments on the time course and comparison of the effect of other microtubule-disrupting agents are needed to detail the interaction between microtubules and actin in the cell.

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