Effect of Colchicine Treatment on Cytoplasmic and Nuclear Perimeters of Cultured Placental Cells

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ABSTRACT

Eucaryotic cells maintain distinct shapes and internal organization by complex networks of protein filaments that constitute their cytoskeleton. Microtubules are one important group of cytoskeletal filaments, which are specifically disassembled by the drug colchicine. This work tested a hypothesis that cytoplasmic but not nuclear perimeters change in flattened cells in monolayer culture following microtubular disassembly via colchicine treatment. A mixed population of cells cultured from first trimester human placentas was treated with 0.5 µg/ml colchicine for 30 minutes. The two-dimensional perimeters of cytoplasm and nuclei of colchicine-treated and control (untreated) cells were measured by means of electronic planimetry. A mean cytoplasmic perimeter of drug-treated cells of 151.10 (SD 46.09) µm was statistically smaller ($P<0.0001$) than 239.40 (SD 91.35) µm of control cells. Unexpectedly, the mean nuclear perimeter of drug-treated cells (54.45; SD 10.08 µm) was also significantly smaller ($P<0.0001$) compared with that of control cells (65.52; SD 21.52 µm). It is inferred from the results that both the cytoplasm and nuclei of cells in monolayer culture are under microtubular traction, to the extent that they exhibit diametric changes after microtubules have been disassembled by colchicine.

Keywords: Colchicine, Cytoplasm, Nucleus, Perimeter.

INTRODUCTION

The action of colchicine on cells is mediated primarily by its effect on microtubules [1]. Microtubules are one of three types of protein filaments in the cytoplasm of eucaryotic cells. Together with intermediate and (actin) micro-filaments, microtubules constitute the cyto-skeleton of eucaryotic cells. Composed of globular proteins, microtubules can assemble and disassemble rapidly in the cell [2]. By these properties, microtubules (and the other cytoskeletal filaments) enable eucaryotic cells to maintain distinct shapes and internal organi-zation, segregate chromosomes during mitosis, transport organelles and vesicular structures within the cell, and promote movement in whole or in part, as well as alter cellular shape [2,3].

Colchicine and its analogs are popular antimitotic agents, but they have also been used to study reorganization of mitochondria in cultured placental cells [4] and effects on trophoblast differentiation [5]. In recent years, taxol a drug that stabilizes microtubules, as well as vinblastine and vincristine that inhibit micro-tubular assembly have been used in cancer chemotherapy since they prevent progress through M-phase of the cell cycle and thereby stall growth of fast-dividing cancer cells [6]. It is well known that in M-phase of the cell cycle, cells undergo changes in their cytoskeleton that

cause them to "round up" [7]. This phenomenon suggests a change in cytoplasmic diameter (and by extension perimeter) of a cell in mitosis concomitant with the altered shape when it "rounds up", but whether there occurs corresponding alteration in any nuclear dimension has apparently not been explored. A similar "rounding up" is observed when placental cells in monolayer culture are treated with colchicine [4]. However, it seems that the effect of mitoclasic agents such as colchicine on nuclear structure and dimensions has not received enough research attention and offers a good area for investigation. The present study tested the hypothesis that cytoplasmic but not nuclear perimeters change when microtubules are disassembled with colchicine in flattened placental cells in monolayer culture.

MATERIALS AND METHODS

Placentas and cultures:

First trimester placentas (8-10 weeks gestation) were collected from Leicester Royal Infirmary and General Hospital after elective terminations of pregnancy for social reasons. The procedure for isolating and culturing cells from the placentas has been described [4]. Briefly, chorionic villi were dissected under aseptic conditions and mechanically minced before dissociation of cells using a trypsin-EDTA solution. Dissociated cells were washed in growth medium and inoculated onto glass coverslips (round, 13mm diameter) placed in a multiwell plastic culture dish. The growth medium consisted of Ham's F-12K medium supplemented with 1% L-glutamine, 30% fetal calf serum, and 3% Pen-Strep (antibiotic) solution. Cells were grown in a humidified incubator at 37ºC and equilibrated with 5% CO_2 /95% air. Results shown in this paper were pooled from cells cultured from four placentas.

Treatment of cells with colchicine and microscopic examinatiom:

Colchine (Sigma) was dissolved in double distilled water to a stock solution of 10 μg/ml. A further dilution was made in growth medium to a concentration of 0.5 μg/ml in which experimental cells were incubated for 30 minutes. The working concentration of 0.5 μg/ml and the duration of treatment were determined from pilot studies in which serial dilutions of colchicine were applied to cells to find the concentration/duration at which microtubules were disassembled without apparent cytotoxicity. Control cells from cohort cultures of the same placentas were incubated for 30 minutes in colchicine-free growth medium to which an equal volume of double distilled water as was used in preparing stock colchine solution was added. Both experimental and control cells were manipulated simultaneously but kept in separate multiwell dishes to avoid mixing. At the end of drug treatment, coverslips (on which cells were grown) were inverted onto drug-free growth medium to create a live cell observation chamber. The chamber was limited by drawing a circular ring of silicon grease on a standard microscope glass slide. The cells were then examined under phase contrast optics fitted to a Zeiss photomicroscope using x100 (oil immersion) objective lens. Microscopic fields of view that showed cells whose peripheral outlines were clearly seen were photographed using Ilford film (400 ASA). Five coverslip cultures were used from each of the four placentas, and at least five fields of view were photographed per coverslip.

Electronic planimetry:

Monochromatic prints (12 x 8cm) of micrographs of cells were used. The two-dimensional image was used as basis for measurement of perimeter in the cells. A set of data processing instruments employed consisted of a MOP (Kontron) electronic planimeter pen and graphics tablet, APPLE II microcomputer, a Hitachi monitor and a printer. The photomicrographs were placed on the MOP graphics tablet and each cell outline was individually traced with the planimeter pen to obtain its cytoplasmic perimeter. The perimeter

of a particular cell's nucleus was similarly traced. The planimeter pen left an ink trace indicating which part of the cell or its nucleus had already been measured. The length measured was recorded by the microcomputer in units that were converted to micrometers by calibration using the image of a microscope stage graticule that had been photographed at the same magnification and printed to the same size as the micrographs. Data collected were cytoplasmic (Cyto) perimeter, nuclear (Nu) perimeter, and nuclear/ cytoplasm ratio (Nu/Cyto). (Ninety-one colchicine-treated and eight-six control cells and their nuclei were measured and the data obtained statistically analyzed using Graphpad Prism software on a Gateway personal computer. Only cells whose entire cytoplasmic and nuclear outlines were unequivocally discernible were used in this study.

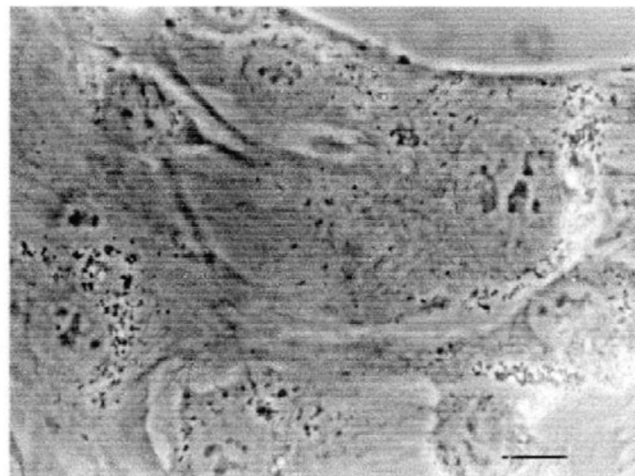

Fig. 1: Control (untreated) cultured human placental cells in monolayer culture showing flattened cells whose cytoplasmic and nuclear outlines are discernible. Bar represents 15 μm.

RESULTS

The placental cells in monolayer culture were flattened (Fig. 1) and the entire cytoplasmic and nuclear outlines in most of them could be discerned. Following colchicine treatment, the cytoplasm of cells appeared to have shrunk (Fig. 2). A histogram of the frequency distribution of cytoplasmic perimeters in drug-treated and control cells revealed that the latter cells tended to have larger values than the former cells (Fig. 3). Similarly, nuclear perimeters of control cells tended to be larger than colchicine-treated cells (Fig. 4), and more control cells had smaller nuclear/cytoplasm ratios than drug-treated cells (Fig. 5).

The graphical trends of cellular variables were confirmed by the mean values of cytoplasmic and nuclear perimeters and nuclear

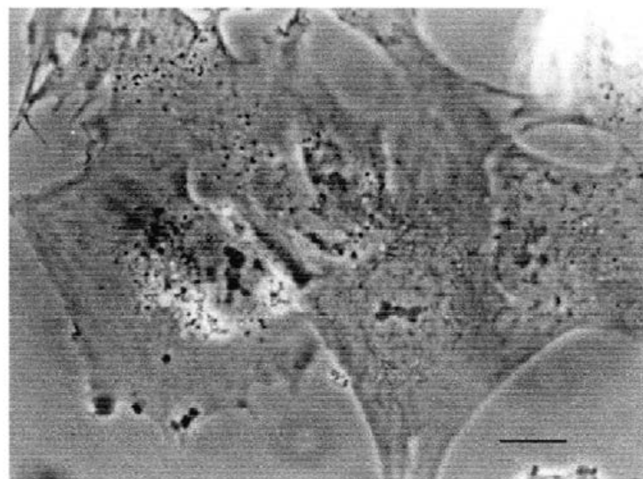

Fig. 2: Colchicine treated (0.5 μg/ml for 30 minutes) cultured human first trimester placental cells. Note that the peripheral regions of cells appear to have somewhat retracted, and not as " spread out" as in Fig. 1.

Table 1: A summary of planimetric measurements of the perimeters of cytoplasm (Cyto) and nuclei (Nu), as well as ratios of nucleus/cytoplasm (Nu/Cyto) obtained from living first trimester human placental cells in monolayer culture.

	Colchicine Treated Cell			Control (Untreated) cells		
Parameters	**Cyto**	**Nu**	**Nu/Cyto**	**Cyto**	**Nu**	**Nu/Cyto**
Sample size	91	91	91	86	86	86
Mean (µm)	151.10	54.45	0.38	239.40	65.52	0.29
SD	46.09	10.08	0.08	91.35	21.52	0.08
SEM	4.83	1.14	0.01	9.85	2.32	0.01
95% C. I.	141.50 to 160.70	52.19 to 56.70	0.36 to 0.39	219.80 to 259.00	60.91 to 70.14	0.27 to 0.31
Minimum (µm)	68.68	31.16	0.19	73.19	28.34	0.16
Maximum (µm)	337.60	76.47	0.59	461.30	174.40	0.59

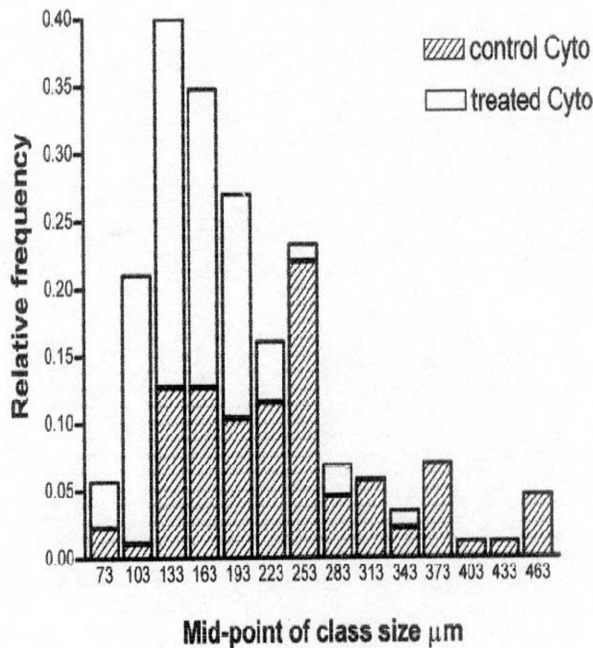

Fig. 3: Frequency distribution of cytoplasmic (Cyto) perimeters measured in 91 colchicine –treated and 86 control cells cultured from first trimester human placentas.

to cytoplasm ratios obtained on drug-treated and control cells presented in Table 1. The mean cytoplasmic perimeter of colchicine-treated cells was 151.10 µm (SD 46.09) whilst that for control cells was 239.40 µm (SD 91.35) {Table 1}. It is also shown in table 1 that the mean nuclear perimeter of colchicine-treated cells was 54.45 µm (SD 10.08) and 65.52 µm (SD 21.52) for control cells. Furthermore, the nuclear /cytoplasm ratio of drug-treated cells was 0.38 (SD 0.08) and 0.29 (SD 0.08) for control cells (Table 1). An unpaired t-test (with Welch's correction) comparison of cytoplasmic perimeter of colchicine-treated with control cells gave a significant t-value of 8.05 ($P<0.0001$) with 124 degrees of freedom (d.f). A similar statistical comparison of nuclear perimeter of the two groups of cells yielded a significant t-value of 4.29 ($P<0.0001$, d.f. 123). A significant t-value (6.78; $P<0.0001$; d.f. 174) was obtained when the ratio of nuclear/cytoplasm in the two groups of cells was compared by similar statistic.

To ascertain whether the differences between colchicine-treated and control cells obtained were not fortuitous, data for each variable was divided into two arbitrary sub-groups for statistical comparisons. The drug-treated cells were divided into sub-group A containing the first 48 cells measured, and sub-group B containing the remaining 43 cells. Similarly, control cells were split into sub-group A containing the first 43 cells measured, and sub-group B containing the remaining 43 cells.

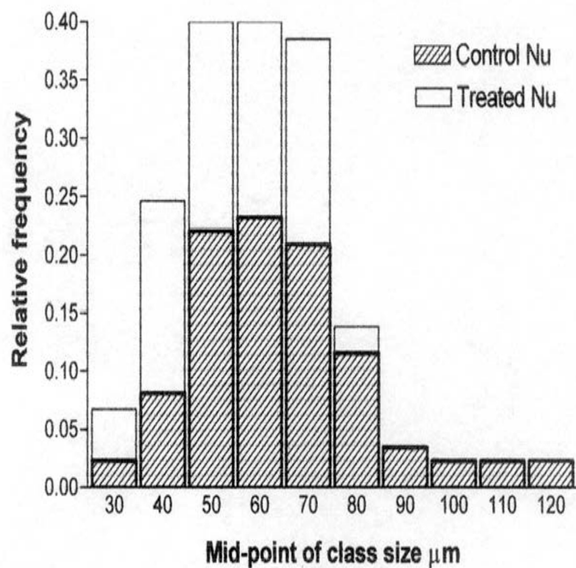

Fig. 4: Frequency distribution of perimeters on nuclei (Nu) in 91 colchicine-treated and 86 control cells cultured from first trimester human placentas.

Preliminary to testing whether there were significant differences between the sub-groups of drug-treated cells or the sub-groups of control cells, all four sub-groups were compared by a one-way ANOVA. This was done to verify that there were statistical differences among the four groups (i.e., the two drug-treated and the two control groups of cells). The F-values obtained for cytoplasmic perimeter (22.4), nuclear perimeter (6.45), and ratio of nuclear/cytoplasm (15.47) had respective probabilities of $P<0.0001$, $P<0.0004$, and $P<0.0001$.

However, when the mean cytoplasmic perimeter of colchicine-treated cells in subgroup A were compared with that of sub-group B by Welch's unpaired t-test, a t-value of 0.98 (d.f. 86) was obtained that was not significant ($P<0.05$). A similar comparison of cytoplasmic perimeter of control cells in sub-group A with sub-group B produced a t-value of 0.74 (d.f. 82), which was not significant. For nuclear perimeter, using Welch's unpaired t-test, a non-significant t-value (0.56; d.f. 88) was obtained by comparing colchicine-treated cells in sub-group A with sub-group B. Likewise, the t-value (0.47; d.f. 80) produced by comparing nuclear perimeter in control sub-group A and sub-group B was not significant. The respective t-values of 0.85 (d.f. 88) and 0.16 (d.f. 73) were obtained by similar statistical comparisons of nuclear/cytoplasm ratios of drug-treated and control sub-groups. These t-values were not significant.
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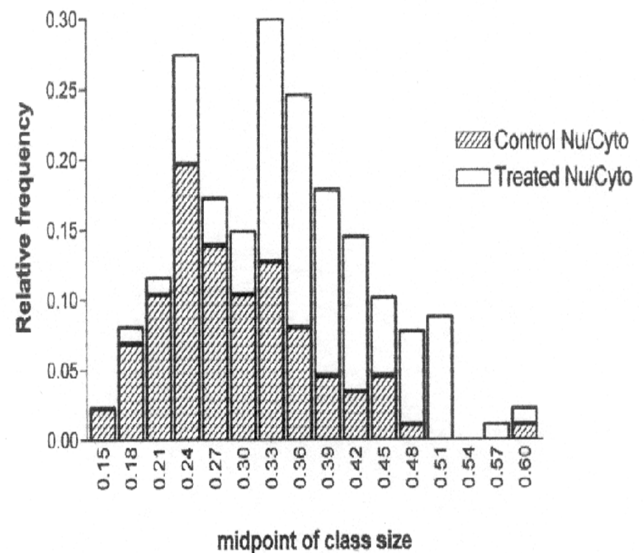

Fig. 5: Frequency distribution of the ratios of nucleus to cytoplasm (Nu/Cyto) in 91 colchicine-treated and 86 control cells cultured from first trimester human placentas.

DISCUSSION

The differences in cytoplasmic and nuclear perimeters obtained between drug-treated and control cells in this study were confirmed by the

significant ANOVA for the three variables (studied) when two sub-groups of colchicine-treated and two-sub-groups of control cells were compared. The fact that the differences were not accidental was borne out by the non-significant differences obtained when data on each pair of sub-group within the drug-treated and control cells were separately compared. The smaller than control cytoplasmic perimeters of colchicine-treated cells is expected on account of their observed smaller shapes following disassembly of microtubules. However, the finding that nuclear perimeters in drug-treated cells were smaller than in control cells is unexpected and intriguing. One interpretation of this is that both the cytoplasm and nuclei of cells in monolayer culture are under microtubular traction. Cytoplasmic microtubules are organized from perinuclear microtubular organizing centers (MTOCs) [3,8]. Dustin [1] included in the centers grouped under MTOCs the centrioles, the polar regions of the mitotic apparatus, the basal bodies, the Kinetochores, and the pores of the nuclear membrane. The structure of nuclear pores is conferred by a set of protein granule subunits arranged in octagonal pattern [9], and where they serve as MTOC it is proposed that association with cytoplasmic microtubules extending to the periphery of the cell may impose stretching forces on the nucleus. This may account for the reduction in perimeter of the nucleus when cytoplasmic microtubules were disassembled with colchicine.

Another possible target in the nucleus that could be disrupted by colchicine on account of its filamentous structure, to cause reduced nuclear perimeter or shrinkage, is the nuclear lamina. As a protein meshwork lining the inner surface of the nuclear envelope in interphase cells, the nuclear lamina provides connections between the inner nuclear membrane and perinuclear chromatin and it is built from intermediate filaments 10 nm in diameter [9]. These filaments are composed of three structurally similar extrinsic membrane protein (lamins A,B,and C), which have a rod-like structure of about 52nm in length. If the organization of lamins into filaments is a dynamic process, comparable to the assembly and disassembly of cytoplasmic microtubules [2], then it is attractive to speculate that colchicine treatment may have interfered with their filamentous formation in a fashion similar to its effect on cytoplasmic filaments. Paradoxically, an increased number of similar sized filaments (10 nm in diameter) were seen in the cytoplasm of many cells when their microtubules were disassembled by colchicine [1]. Whether or not the cytoplasmic fibrils seen after disruption of microtubules are similar to the nuclear lamina filaments must await future research.

It is worthy of note that the mean nuclear/ cytoplasm ratio of drug-treated cells in this work was bigger than for control cells, suggesting that colchicine caused relatively greater shrinkage in cytoplasm than in nuclei. It is proposed that the matrix of the nucleus permitted only a limited shrinkage in volume following disassembly of cytoplasmic microtubules. It is pertinent that when nuclei are subjected to extraction in high-ionic-strength, neutral-detergent-containing buffers to remove most of the internal components, a fibrous network of proteins (the nuclear matrix) remains intact and roughly maintains the outward appearance of the nucleus [9]. The latter reference also indicates that although the nature of the individual nuclear matrix components remains to be determined, it is known from electron microscopic studies that they comprise mainly fibrillar elements associated with the nuclear pore complex.

On the other hand, the big decrease in cytoplasmic perimeter after disruption of microtubules suggests that the organization of other cytoskeletal filaments were affected, because the distribution of microfilaments and intermediate filaments bear a striking resemblance to the shape of most cultured cells. [10,11]. Moreover, actin-binding proteins known as spectrins are known to link micofilaments to microtubules in neurones [3].

More research on cytoskeletal elements in general is also becoming increasingly called for,

in view of their conjectured role in cell growth, survival, and apoptosis. For instance the implication of cell adhesion molecules (CAMs) such as integrins in granulosa cell survival and death is proposed to be through their interactions with extracellular matrix and regulation of re-organization of their cyto-skeleton [12]. Similarly, cadherins (that mediate calcium-dependent cell-cell adhesion) bind catenins to the cytoskeleton, offering a possible explanation for their ability to maintain cell viability.Further research is also required to explain why the anti-microtubular effect of colchicine is not useful in cancer treatment, whilst similar action by vinblastine and vincristine as well as promotion of microtubular assembly by taxol have cancer chemo-therapeutic usefulness [6].

It is useful to state that although there is strong evidence to suggest that the observed changes in cytoplasmic and nuclear perimeters are mediated by the effect of colchicine on microtubules [1], it is possible that some of the other effects the drug has on cells [4] may have contributed to the observations being reported.

In conclusion, colchicine treatment of placental cells in monolayer culture indicates that both the cytoplasm and nuclei changed in perimeter compared to control cells. It is inferred that both the cytoplasm and nuclei were under microtubular traction. It is also speculated that colchicine affected nuclear matrix fibrillar elements and/or nuclear lamina filaments to cause changes in nuclear perimeter in treated cells.

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