The Influence of Certain Colchicine Derivatives on the Growth Characteristics of L Cells

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Abstract. Colchicine and its derivatives (demecolcine, 2-demethyldemecolcine and acetylated 2-demethyldemecolcine) blocked the mitotic activity of L cells and inhibited proliferation and DNA synthesis. This effect depended on the length of the exposure and the concentration of the substances tested (from 0.05 μg/ml up to 2.6 μg/ml). The greatest values of plating efficiency, cell proliferation, mitotic index and inhibition of DNA synthesis, respectively, were found after the application of colchiciline (0.2 μg/ml). These effects were reversible, since L cells underwent regeneration of the mitotic spindle and renewal of their function after the removal of colchiciline. Reversible effects were much less with other colchicine derivatives tested. The different effects of colchicine and its derivatives were due to their chemical structure, which determines the interactions with respective L cell receptors and thus modifies the mechanism of action on the mitotic apparatus of L cells.

Key words: Colchicine derivatives — Cell proliferation — DNA-synthesis

Introduction

The isolation and study of the chemical structure of some colchicine derivatives from plant material has in recent years brought certain original and interesting results (for a review, see Šantavý 1979). Since the effects of colchicine on the mitotic apparatus of mammalian and plant cells (Taylor 1965; Mizel and Wilson 1972; Wilson et al. 1974; Kopnin 1981; Durnam and Jones 1982) and the initiation of their DNA synthesis (Otto et al. 1981; Otto 1982) are already known, the question arises whether certain newly isolated derivatives of colchicine have similar effects. The study on this problem is aimed at obtaining new information on drugs with a possible cytostatic effect. For this reason we studied the effects of three derivatives of colchicine: colchiciline, 2-demethyl-demecolcine and its acety-

		R^1	R^2	R^3	R^4	R^5	C^6
1	COLCHICINE	CH_3	CH_3	H	$COCH_3$	CH_3	H_2
2	COLCHICILINE	CH_3	CH_3	H	$COCH_3$	CH_3	HOH
3	2-DEMETHYL-DEMECOLCINE	H	CH_3	H	CH_3	CH_3	H_2
4	ACETYLATED 2-DEMETHYL-DEMECOLCINE	$COCH_3$	CH_3	$COCH_3$	CH_3	CH_3	H_2
5	DEMECOLCINE (COLCEMID)	CH_3	CH_3	H	CH_3	CH_3	H_2

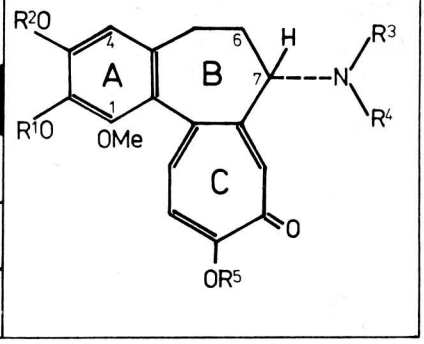

Fig. 1. Chemical structure of colchicine and its derivatives.

lated derivative, on the mitotic activity, growth characteristics and the initiation of DNA synthesis in L cells, and compared the results with the effects of colchicine and demecolcine (Colcemid).

Materials and Methods

Colchicine derivatives. The alkaloids colchicine, colchiciline and 2-demethyl-demecolcine, isolated from *Colchicum latifolium S. S.* (Potěšilová et al. 1977) were used. Another substance used was an acetylated derivative of 2-demethyl-demecolcine, prepared by acytelation with anhydride of acetic acid and anaqueous potassium acetate. Colchicine and demecolcine (Colcemid Ciba) were used as reference drugs (Fig. 1). Sterile stock solutions were prepared from the above substances by filtration through a Millipore filter at a concentration of 100 μg/ml phosphate buffer saline (PBS). The sterile stock solutions were diluted with PBS to make a final concentration of 0.02—2.6 μg/ml, and were used for experiments.

Cell culture. L cells were cultured on a monolayer in minimum Eagle's medium with 10 % inactivated bovine serum and antibiotics (200,000 μ/l penicillin and 0,5 g/l streptomycin) (MEM).

Growth characteristics. Plating efficiency (P.E.) was studied after the seeding of 200 or 500 L cells in plastic Petri dishes ("Koh-i-noor") and their cultivation in MEM medium containing colchicine or its derivatives in concentrations between 0.2 and 2.6 μg/ml; demecolcine was added in a concentration of 0.02 μg/ml. P. E. was determined after ten days of cultivation. L cells cultured in pure MEM medium with no substances added were used as controls, and the values of their P. E. were taken for 100 %. The colonies were fixed with 70 % methyl alcohol and stained with a 10 % Giemsa-Romanowski solution ("Lachema").

In order to obtain growth curves, 10^5 L cells were seeded in Müller flasks (50 ml) and cultured in MEM medium containing respective concentrations of colchicine or its derivatives until saturation density of cells was reached (6—10 days). The number of cells in each bottle was assayed in a Bürker chamber after trypsinization (0.25 % trypsin Difco). Five to eight flasks were assayed from each sample.

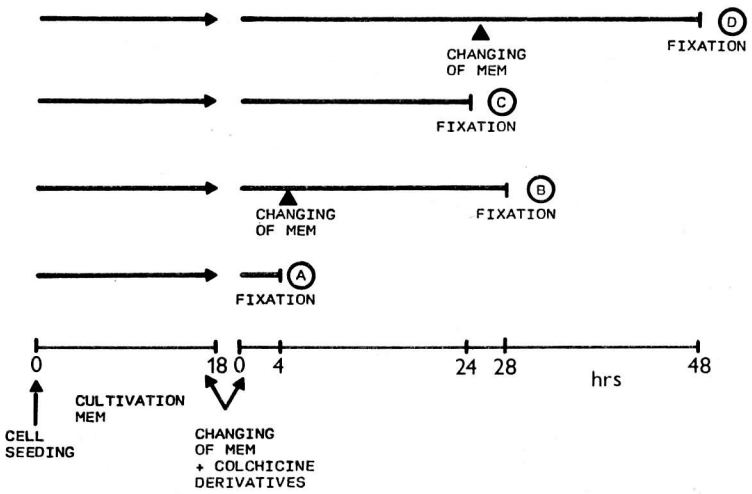

Fig. 2. Experimental schedule.

Mitotic activity of L cells. 10^5 L cells were seeded on cover slips (16 × 16 mm) placed in "Pyrex" Petri dishes (5.5 cm). The cells were cultured in MEM medium for 18 h; then, the medium was changed and a new medium containing colchicine or its derivatives was added. The cells were cultured for additional 4 hours (Group A) or 24 hours (group C) and fixed. In another two groups, the MEM medium was substituted after 4 or 24 h of cultivation with colchicine or its derivatives for a medium without colchicine of derivatives, and the cells were cultured for additional 24 h (group B: after precultivation for 4 h and group D: after precultivation for 24 h with colchicine and its derivatives, see Fig. 2).

The cells were fixed with acetic acid and methanol (1:3), 70 % ethanol, and after rinsing in distilled water, they were stained with methylgreen-pyronine. One thousand cells were evaluated from each sample.

DNA synthesis. In order to determine the number of cells in the S phase, the cultures were divided in the same manner as when evaluating mitotic activity (Fig. 2). Prior to each sampling, the medium was replaced by another one containing ^{3}H-thymidine (18.5 kBq/ml, specific activity 6.03×10^{11} Bq/mmol, Praha-Řež) and the cells were pulse labelled for 30 minutes. They were then fixed and the acidosoluble material was removed at 4 °C for 20 minutes in 1 % perchloric acid, rinsed in distilled water and exposed to a solution of inactive thymidine (25μg/ml) for 60 minutes. The preparations were processed autoradiographically and stained with methylgreen-pyronine (Kodak K2 emulsion, exposure 2—5 days).

Results

Effect of colchicine and its derivatives on cell growth

The cell growth in a medium containing colchicine or its derivatives was evaluated by means of both the plating efficiency (P.E.) (Fig. 3) and the growth curves (Fig. 4).

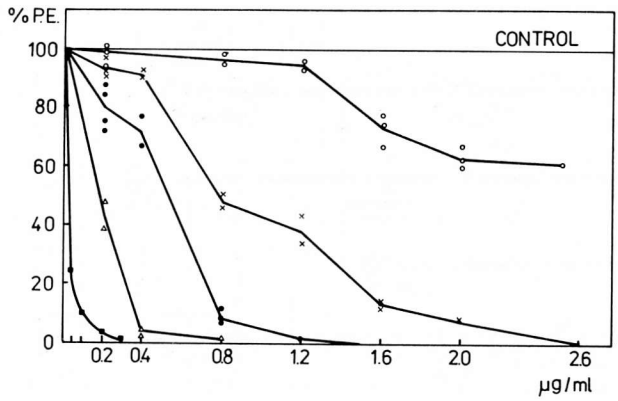

Fig. 3. The effect of the concentration of colchicine and its derivatives on plating efficiency (P.E.) of L cells. △ colchicine, ○ colchiciline, × acetylated 2-demethyl- demecolcine, ● 2-demethyl-demecolcine, □ demecolcine (Colcemid). Controls: Absolute P.E. of 88 % was related to 100 %.

Colchicine and 2-demethyl-demecolcine at a concentration of 1.2 μg/ml medium inhibited P.E. of L cells totally; the acetylated derivative of 2-demethyl-demecolcine decreased the capacity to form colonies by about 40 %, while colchiciline in this concentration was almost without effect (90 % P.E.). The P.E. of cells following the application of demecolcine was the lowest, even at a concentration of 0.05 μg/ml medium (Fig. 3).

The influence of the concentration of colchicine or its derivatives on the growth capacity of L cells was also studied as the time required to reach saturation density of the population. The respective growth curves of L cells showed that the growth of cells depends on the concentration of the test substance (0.2—2.6 μg/ml of colchicine or its derivatives) (Fig. 4). A partial inhibition of cell growth occurred even at a concentration of 0.2 μg/ml medium, with the exception of colchiciline; this inhibition was particularly apparent in the presence of colchicine and demecolcine. Cells cultured in a medium containing colchiciline at a concentration of 2.6 μg/ml medium were growing slower than the controls and also reached a lower saturation density, but their growth was not entirely inhibited. Demecolcine, colchicine, 2-demethylcolchiciline and its acetylated derivative at concentrations higher than 0.8 μg/ml medium greatly or entirely inhibited the growth of L cells.

The reversibility of the inhibitory effects of colchicine and its derivatives on the growth of L cells was studied during long-term cultivation of L cells in a medium containing these substances; L cells were cultured for 4 h, 24 h or 6 days in a medium containing colchicine or its derivatives (in a concentration of 0.2 μg/ml, or 0.05 μg/ml in the case of demecolcine), trypsinated and seeded in MEM medium without additives. The values of relative P.E. of the cultures tested were lower than those of the control population (P.E. of cells cultured in MEM

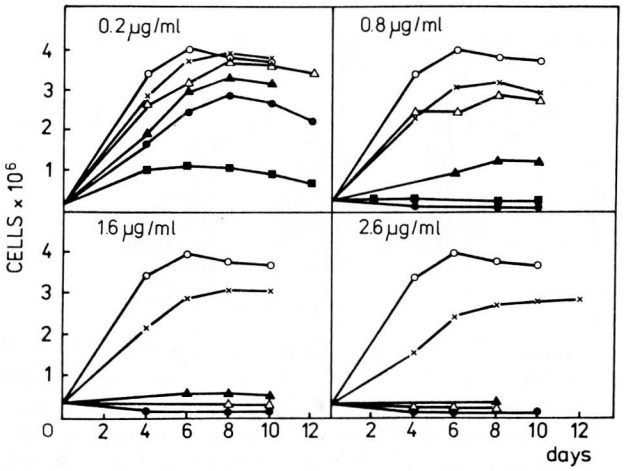

Fig. 4. The growth curves of L cells cultured with various concentrations of colchicine and its derivatives. ○ control in MEM, ● colchicine, × colchiciline, △ acetylated 2-demethyl-demecolcine, ▲ 2-demethyl-demecolcine, ■ demecolcine (Colcemid). 2×10^5 L cells were seeded in Müller Flasks (50 ml) and after seeding they were assayed in the closen time-intervals. The values are the means of parallel assays.

alone = 100 %) and fell with the increasing length of cultivation in a medium with colchicine or its derivatives. The greatest relative P.E. in each series of experiments was that after the application of colchiciline; this means that the P.E. of L cells was diminished 24 hours after colchiciline application by only 8 %, and by 48 % after six days. After the same time of a continuous application of acetylated 2-demethyl-demecolcine, the P.E. of L cells decreased by as much as 72 %, and after the application of colchicine up to 86 % (Fig. 5).

Mitotic index. After four hour exposure of cells (group A, see Fig. 2) in a medium containing colchicine or its derivatives (0.2 µg/ml colchicine or its derivatives, or 0.05 µg/ml demecolcine) the mitotic index of cells increased; the highest values of mitotic index were obtained on the application of colchicine, demecolcine and particularly of colchiciline (Fig. 6). After a continuous 24-hour application (group C) a mitotic index of up to 42 % was obtained in a medium with 0.2 µg/ml colchiciline, and up to 38 % in a medium with 0.05 µg/ml demecolcine. The blocking of cell mitoses was abolished if the cells were cultured after 4 or 24-h application of colchicine or its derivatives for additional 24 hours in a medium without these substances (group B and D). In these intervals, the differences in the mitotic index of the samples studied were insignificant.

DNA synthesis. The ability of cells to pass from mitosis into interphase and to enter

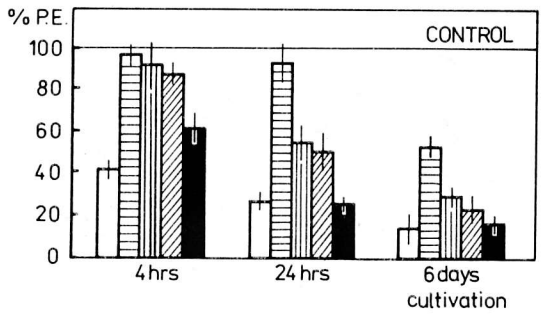

Fig. 5. The effect of the application length of colchicine and its derivatives on the plating efficiency (P.E.) of L cells. Before seeding, cells were cultured for 4 hours, 24 hours or 6 days in MEM containing 0.2 μg/ml colchicine □, colchiciline ▤, acetylated 2-demethyl-demecolcine ▥ 2-demethyl-demecolcine ▨, or 0,05 μg/ml demecolcine (Colcemid) ■. The cells were subsequently trypsinated and seeded in MEM medium without colchicine or its derivatives. P.E. was evaluated after 10 days of cultivation. Absolute P.E. of L cells in MEM 88 % was related to 100 %.

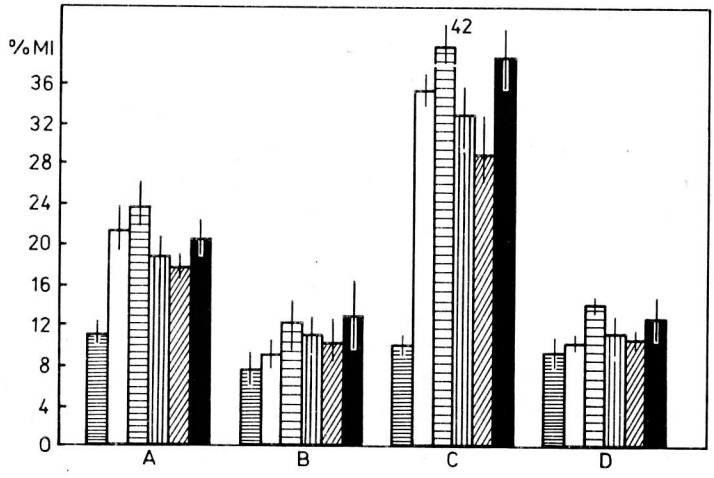

Fig. 6. The mitotic index of L cells after the application of colchicine or its derivatives. The concentration used was 0.2 μg/ml (demecolcine 0.05 μg/ml) medium. The mitotic index was determined in cells cultured for four hours (A) or24 hours (C) in MEM with the respective substances; after this time, the medium was replaced by a fresh one and the cells were cultured without colchicine or its derivatives for additional 24 hours (group B after precultivation for 4 hours and group D after precultivation for 24 hrs with colchicine or its derivatives, see Fig. 2). ▤ control in MEM, □ colchicine, ▤ colchiciline, ▥ acetylated 2-demethyl-demecolcine. ▨ 2-demethyl-demecolcine, ■ demecolcine (Colcemid). 1000 cells were evaluated from each sample.

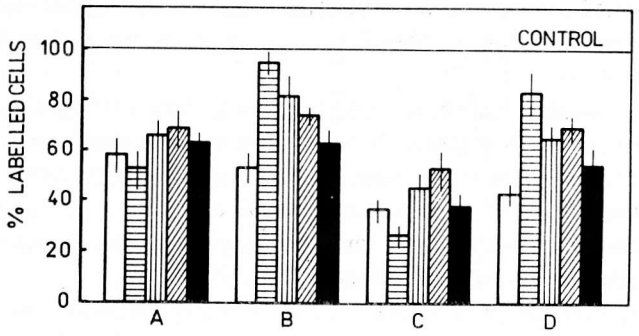

Fig. 7. The number of L cells in the S phase (%) after the application of colchicine or its derivatives (concentration 0.2 μg/ml, demecolcine 0.05 μg/ml). A, B, C and D group L cells (see fig. 6) were labelled 30 minutes prior to fixation with ^{3}H-thymidine at 37 kBq/ml. 1000 cells were evaluated in the autoradiogram of each sample. Control: % cells in S phase, cultured in MEM without colchicine or its derivatives related to 100 %. □ colchicine, ▤ colchiciline, ▥ acetylated 2-demethyl-demecolcine, ▨ 2-demethyl-demecolcine, ■ demecolcine (Colcemid).

the S-phase was studied autoradiographically in L cells exposed to colchicine or its derivatives for four hours (group A) or 24 hours (group C) following a 24 hour cultivation without additives (groups B or D, see Fig. 2). While only 25 % of cells subjected to the action of colchiciline for 24 h were capable of synthesizing DNA and entering the S-phase, as compared to controls (group C), after subsequent 24-h cultivation in a medium without colchiciline, 80 % of cells synthesized DNA (group D). When colchiciline was applied for 4 h only, followed by 24-h cultivation without it (group B), almost as many cells entered the S-phase as in the controls (Fig. 7).

Discussion

The aim of our work was to compare the effect of colchicine on the proliferation and initiation of DNA synthesis in L cells with that of some other derivatives. The colchicine derivatives used were demecolcine (Colcemid), 2-demecolchicine, acetylated 2-demethyl-demecolchicine and colchiciline, isolated by one of the authors (F.Š.).

All the substances tested in a concentration of 0.2 μg/ml blocked cell mitoses and inhibited the proliferation of L cells. Both the number of mitotic cells and cell proliferation were dependent on the lenght of the exposure to a medium containing colchicine or its derivatives, and on the concentration of the substances.

While demecolcine inhibited L-cell growth at a concentration of only 0.05 μg-/ml, proliferation of cells at 0.8 μg/ml colchiciline was only partially inhibited. At a concentration of 0.2 μg/ml colchiciline also stimulated the blocking of the cell

mitotic activity. After 4 hours of application, the mitotic index was 24 %. The strongest inhibition of DNA synthesis in L cells was also seen after 4 or 24 hours action of colchiciline.

The effect of colchiciline on the mitotic activity, DNA synthesis and proliferation of L cells was reversible, since the cells underwent regeneration of the mitotic spindle and a renewal of their function. After a long-term application (24 hours or 6 days) following the cell seeding in a medium without colchiciline, L cells continued proliferating and they entered the S-phase. This indicated reversible binding of colchiciline to tubulin (Wilson et al. 1974).

The inhibition of the growth of L cells after the application of colchicine or its derivatives at a concentration higher than 0.2 μg/ml (or higher than 0.05 μg/ml for demecolcine) may be brought about by blocking their mitosis, or by partial toxic effect induced by the modification of certain important physiological cell functions.

In the first case, after the application of the test substances at concentrations lower than 0.2 μg/ml colchicine and its derivatives or 0.8 μg/ml colchiciline, the growth of L cells is reversibly inhibited; after the removal of the substances, the cells may again be capable of continuing in their generation cycle and entering, though with a delay, into the S-phase. This indicates a reversible binding of colchicine and its derivatives to tubulin. In this case, it is possible to assume regeneration of tubulin similarly as reported by Brinkley et al. (1975) after the application of Colcemid. This effect might be further increased in the presence of certain growth factors, since it has already been shown that there is a synergic effect of colchicine and similar substances with growth factors (Otto et al. 1981; Otto 1982).

Colchicine, like other substances, such as podophylotoxine, vinblastine or szeganacine, interferes with the division of plant and mammalian normal and neoplastic cells (Baker 1976; Schrek and Stefani 1976; Garland 1976; Cortese et al. 1977; Nunez et al. 1979). At lower concentrations, colchicine has a reversible and direct effect on the microtubules of the mitotic spindle. A damage to the microtubules may also affect the mechanism regulating the initiation of DNA synthesis.

The second mechanism involves an irreversible effect of the compounds studied, which has not yet been fully clarified. These irreversible changes may occur during the interphase, when there may be a decrease in the permeability of the cell membrane under the influence of colchicine, changes in the transport of nucleotides and the degradation of various proteins, as has been found on the cellular and molecular levels (Šantavý et al. 1950; Černoch et al. 1954; Ling et al. 1979; Cabral et al. 1982; Crie et al. 1983). At the same time, changes in the morphology of cells occur, particularly in the nucleus; multinucleate homokaryons are formed, even before the cell enters mitosis. A decreased growth capacity of cells after the application of colchiciline or its derivatives may be due to the cells'

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