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EFFECT OF DEACETYLATION ON STATMHOKINETIC ACTIVITY OF COLCHICINE DERIVATIVES

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Screening of colchicine derivatives that was carried out with the tumor culture CaPa in a cytotoxicity test showed that deacetylation decreases the cytotoxicity and increases the mitotic index. Lower toxicity of the deacetylated colchicine derivatives was also noted.

Key words: alkaloids, colchicine, colchicine derivatives, cell culture, statmhokinetic and mitotic activity.

New colchicine derivatives are being prepared in order to reduce its toxicity and retain or increase the statmhokinetic activity, which is responsible for the cytotoxic and antimitotic properties that are required for potential antitumor preparations. The biological activity of colchicine on cells consists essentially of a specific effect on the aggregation and disaggregation of microtubule proteins, tubulins, which prevents formation of the mitotic spindle and separation of the chromosome. As a result, cell division is stopped in metaphase. The blocking of mitosis with time sharply increases the number of cells in metaphase [1].

We have previously investigated the mitotic index (MI) of new colchicine derivatives on intestinal glands of intact animals [2].

Our goal was to determine how deacetylation affects on the statmhokinetic activity of several new colchicine derivatives.

For this we investigated the cytotoxic and antimitotic properties of these compounds in CaPa cell culture and the toxicity for mice.

Several colchicine derivatives have been previously synthesized and characterized [2, 3]. The synthesis of 10-demethoxy-10-N-β-chloroethylamino-7-N-deacetylcolchicine is described in the Experimental.

We studied the effect of three pairs of compounds. Each pair contained amino derivatives on the tropolone ring of colchicine and their deacetylated analogs: 10-demethoxy-10-N-aminocolchicine (**1**), 10-demethoxy-10-N-amino-7-N-deacetylcolchicine (**2**), 10-demethoxy-10-N-β-chloroethylaminocolchicine (**3**), 10-demethoxy-10-N-β-chloroethylamino-7-N-deacetylcolchicine (**4**), 10-demethoxy-10-N-β-diethanolaminocolchicine (**5**), and 10-demethoxy-10-N-β-diethanolamino-7-N-deacetylcolchicine (**6**).

The cytotoxic and mitotic activities of the new colchicine derivatives were studied on human pancreatic cancer tumor culture (CaPa) [4, 5].

Table 1 presents the experimental results.

It can be seen that all derivatives possess distinct mitotic activity compared with colchicine (MI 88-120$^{o}/_{oo}$) whereas the MI of colchicine is 80$^{o}/_{oo}$. The deacetylated derivatives (**2**, **4**, **6**), both for tumor-cell culture and intestinal glands [2], typically have higher MI than their precursors with the acetyl group.

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TABLE 1. Cytotoxic and Mitotic Activity and Toxicity of Colchicine Analogs

Compound	CE_{50}, μg/mL	MI,* °/oo	LD_{50}, mg/kg
Colchicine	0.001±0.0001	80.0±2.1	4.0±0.3
1	0.1±0.01	110.0±3.7	105.0±3.5
2	0.1±0.008	115.0±3.8	309.0±5.2
3	0.1±0.01	88.0±2.3	50.0±0.9
4	1.0±0.17	102.0±2.9	210.0±3.7
5	0.1±0.01	106.0±3.4	35.0±0.2
6	1.0±0.05	120.0±3.9	200.0±3.2

*Mitotic index of CaPa cell line without colchicine (control) was 52°/oo.

The statmhokinetic properties of the compounds were confirmed on the CaPA cell line using the effect on DNA synthesis as measured with labelled ^{3}H-thymidine. Thus, the derivatives (**1**-**6**) actively suppressed inclusion of labelled DNA. The concentration causing a 50% cytotoxic effect, equal to 0.001 μg/mL for colchicine, was 0.1 μg/mL for substances **1**, **2**, **3**, and **5** and 1.0 μg/mL for **4** and **6**. This indicates that the cytotoxicity is three orders of magnitude lower compared with colchicine and an order of magnitude lower compared with the closest structural analogs and substances **1** and **2**. It is important to note the lower cytoxicity of substance **4** and especially of **6** with its very high MI in tumor culture.

The 100-fold reduction of cytotoxicity may be due to a sharp decrease in the toxicity of these compounds (LD_{50} values of substances **4** and **6** are 210 and 200 mg/kg, respectively). However, the LD_{50} value of substance **2** is 309 mg/kg whereas its cytotoxicity changed only 10-fold. Apparently this is a result not only of structural transformations of the molecule but also a higher solubility.

Thus, deacetylation of colchicine derivatives decreases the cytotoxicity and toxicity, preserves the statmhokinetic properties, and increases the mitotic activity for tumor cells.

EXPERIMENTAL

UV spectra were recorded on a SF-46 instrument in ethanol; IR spectra, on a UR-10 instrument in mineral oil and KBr pellets; PMR spectra, on a Varian XL-100 instrument in $CDCl_3$; mass spectra, in a MAT-301 instrument with a direct probe into the ion source. Elemental analyses corresponded to those calculated.

Synthesis of 10-Demethoxy-10-N-β-chloroethylamino-7-N-deacetylcolchicine (4). 10-Demethoxy-10-N-hydroxyethylamino-7-N-deacetylcolchicine hydrochloride (1 g) was dissolved in dry $CHCl_3$ (5 mL) and treated slowly (over 30 min) with thionyl chloride (4 mL). The reaction mixture was stirred for 1 h without heating and then for 7 h at 50-60°C, treated with water, and extracted with $CHCl_3$. The product was purified over an Al_2O_3 column with elution by $CHCl_3$ and then mixtures of $CHCl_3$ with alcohol in the ratios 10:1 and 5:1. A compound with R_f 59 (Silufol, $CHCl_3$—C_6H_6—$(CH_3)_2CO$—CH_3OH, 20:5:4:3) was precipitated from concentrated $CHCl_3$ solutions by dry ether. Yield 70%, mp 178-180°C, $[\alpha]_D^{20}$ -27° (*c* 0.5, $CHCl_3$). Compound **4** is yellowish-brown, very soluble in alcohols, $CHCl_3$, and acetone and poorly soluble in water and ether.

Found (%): N, 6.36. Calc. (%) for $C_{21}H_{25}N_2O_4Cl_2$: N, 5.85.

UV spectrum (I_{max}, nm): 210, 250, 380.

IR spectrum (cm^{-1}): weak, 3210-3340, NH_2; 1690, CO–NH; 1630, CO-tropolone; 1210, 1150, 1100, 1050, 1020, bands characteristic of vibrations of the colchicine tricyclic structure; 670, Cl.

Toxicological Studies. The acute toxicity of the compounds was determined for BALB/c male mice of average mass 20-25 g and age 2 months and for mongrel male mice of mass 20-25 g and age 2-4 months. Preparations were injected intraperitoneally. Animals were observed for 30 days.

The statistical treatment of the results followed a least-squares method [6].

Evaluation of Cytotoxic Effect of the Compounds in CaPa Cell Culture. The cytotoxic effect of the compounds on cells was estimated by determining the extent of DNA synthesis as measured by ^{3}H-thymidine incorporation [5]. For this, cells were dispersed in tubes with $1 \cdot 10^5$ cells/mL in 3 mL of RPMI-1640 nutrient medium with fetal calf serum (10%), glutamine (200 mM), and antibiotics and were cultured in a CO_2-incubator at 37°C. Compounds were added in 24 h after dispersion at concentrations from 0.0001 to 10.0 μg/mL. Cells were exposed to the compounds for 24 h. Then, ^{3}H-thymidine was added at a concentration of 10 μCi per tube for 1 h, after which labelled DNA precursor was removed and the culture was rinsed three times with Hanks solution. Then, versene solution (1 mL, 0.2%) was added for 3-5 min. The cells were suspended, transferred to GFC filters, fixed by TCA solution (5%), washed with water, dried, and counted in a β-counter.

The results were calculated in percent of the control. Next curves for the effect as a function of dose were constructed for each preparation to determine graphically the CE_{50} [7], i.e., that preparation concentration at which the index could decrease by half (50% cellular effect).

All experiments, including controls, were repeated three times. The controls were cells not treated with preparations.

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