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Clastogenic Effect of Hippeastidine (HIPP) (1,2,3,4,4a,6 hexahydro-10,hydroxy-3,8,9,trimethoxy-5, 10b, ethanophenanthridine)*

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In a screening of chilean plants for anticancer activity, a number of alkaloids have been isolated from Hippeastrum añañuca Phil.(Amaryllidaceae).HIPP is the one that has been shown to exhibit the major antineoplastic activity as tested in KB cells (a human transformed nasopharingeal cell line) showing an ED50 = 0.270 µg/ml dosage required to inhibit by 50% the growth of a cell population (Bhakuni et al.1976 and Pacheco et al.1978).

Treatments of human malignancies have increased in efficiency during the past 2 decades due to development of improved drug therapy (Blacklock et al. 1981). However a number of patients have been shown to exhibit secondary malignant effects as a result of chemotherapy due to the drug treatments themselves. These produce genetic damage which gives rise to inheritable gene mutations, chromosomal structural changes and secondary cancers (Weisburger et al. 1975; Harris 1976; Dickins et al. 1985; Sorsa et al. 1985 and Meistrich et al. 1985).

In the present study the micronucleus test was carried out to determine whether HIPP induce in vivo chromosomal changes, an important fact for its eventual use as citostatic drug.

MATERIALS AND METHODS

The micronucleus test is based on the following facts: In anaphase, chromosome and acentric chromatids fragments lag behind when the centric elements move towards the spindle poles. After telophase the undamaged chromosomes as well as the centric fragments, give rise to regular daugther nuclei. The lagging elements are included in the daugther cells too, but a considerable proportion of them is transformed into one or several secondary nuclei which are, as a rule, much smaller than the principal nucleus and are therefore called micronuclei. In bone marrow smears from mammals treated with chromosome-breaking agents, micronuclei are found in

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numerous cell types, always provided that their cell have completed, under the influence of the mutagen, one or a few mitoses. Very young erythrocytes bear micronuclei, remmants of last erythroblasto' mitoses. These originate when the cells fail to expel them and are easily recognizable because these cells stain differently from older forms (polychromatic cells).

The micronucleus test was carried out as proposed by Schmid (1977) but modified according to Das and Kar (1980) in normal 2 month old Balb/c male mice bone marrow smears (20 g b.wt). Eight animals per dosage were treated intraperitoneally with 0.2 ml of distilled water (negative control) or with HIPP at 1.083, 0540 or 0.270 µg/g b.wt, or adriamycin (ADR) at 10 µg/g b.wt (positive control). Chemicals were dissolved in 0.2 ml distilled water to desired concentration. The HIPP concentrations were selected on the basis of the KB cells ED50. Mice were sacrificed 30 h after the injection. Bone marrow from femur was collected in 1% sodium citrate, resuspended and centrifuged for 10 min at 224 x g. Smears were prepared by extending a drop of concentrated cell suspension over to slide. Cells were stained with May Grünwald-Giemsa solution. About 3000 polychromatic erythrocytes per animal per dose were scored blind and those with micronuclei were recorded. A Mann- Whitney U-test was employed for statistical analysis. The significance was tested at $p<0.05$ level.

RESULTS AND DISCUSSION

The results are summarized in Table 1 and 2. When compared with negative control, the highest HIPP doses used (1.083 and 0.540 µg/g b.wt) induced a significantly increase in micronuclei frequency, whereas the effect of the lowest dose was not significantly differents. ADR induced a micronuclei frequency which is significantly higher than the effect of all HIPP doses used.

The micronuclei most frequently induced by HIPP were single round-shaped or oval-shaped, both with 1/5-1/7 of the cell diameter. Minor abundant irregular-shaped or larger micronuclei were found. According to Yamamoto and Kikushi (1980) the rounded or ovoid micronuclei are produced by clastogenic chemicals, and irregular shaped and of size larger than 1/5 of cell diameter, by mitotic-spindle-poison chemicals. Both effects can be imputed to HIPP being the clastogenic effect the most important at two highest doses used.

Maritidine (MAT) (1,2,3,4,4a,6 hexahydro-3, hydroxy-8,9 dimethoxy-5, 10b ethanophenanthridine) other alkaloid isolated from H. añañuca Phil. has also shown to exhibit antineoplastic activity as tested in KB cells (ED50 = 0.510 µg/ml). MAT induces 10.09± SD 0.72 micronuclei per 1000 cells (MN/EPC) in Balb/c male mice using a dose equivalent to its KB cells ED50 (unpublished data obtained in our laboratory). HIPP at dose equivalent to its KB cells ED50 (0.270 µg/g b.wt) induces 8.3± SD 1.2 MN/EPC, but at 0.540 µg/g b.wt induces 9.9± SD 1.20 MN/EPC which is not significantly different at the MN/EPC induced by MAT at close dose. Both alkaloids

Table 1. Induction of micronuclei in bone marrow cells by hippeastidine (mean± SD; n = 8).

Dose of hippeastidine (µg/g b.wt)	N° of polychromatic erithrocytes scored (EPC)	N° of micronuclei scored (MN)	Micronuclei per 1000 cells (MN/EPC)
0 (distilled water: negative control)	3067.3±50.82	20.7±2.08	6.7±0.60
0 (adriamycin 10 µg/g b.wt positive control)	2460.0±15.39	33.0±2.50	13.4±1.04
0.270	2988.6±28.54	24.7±2.52	8.3±1.20
0.540	2846.0±51.64	28.3±3.06	9.9±1.20
1.083	3060.0±58.23	32.7±1.53	10.6±0.30

Table 2. Values of U (Mann-Whitney U test) for all paired comparisons of the values of micronuclei per 1000 cells (MN/EPC) given in Table 1 for different doses of hippeastidine (µg/g b.wt) and control conditions.

	Negative control	Positive control	0.270	0.540	1.083
Negative control	-				
Positive control	0*	-			
0.270	1	0*	-		
0.540	0*	0*	3	-	
1.083	0*	0*	0*	3	-

* $p<0.05$

showed to be clastogenic agents less potent as compared with ADR.

The chemical structures of HIPP, MAT and ADR are outlined in Figure 1. ADR is known to be a DNA intercalation agent (Zunoni et al. 1972), but the mechanisms of citotoxicity of HIPP and MAT are un-

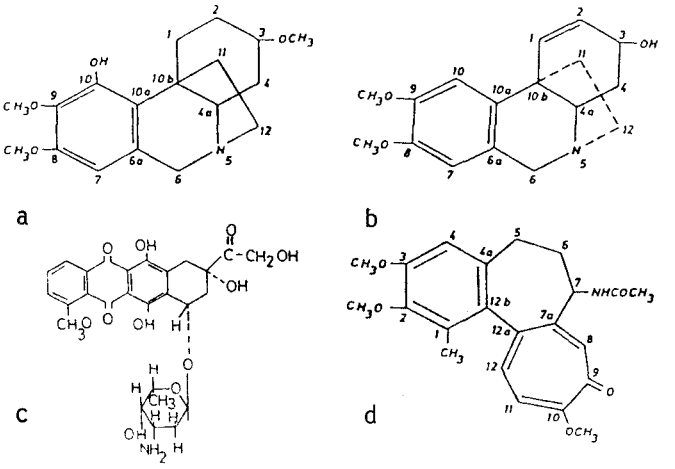

Figure 1.- a.- Hippeastidine. b.- Maritidine.
c.- Adriamycin. d.- Colchicine.

known. It is possible that the interaction of 5, 10b, C-C bridge spatial disposition and positions of OH and/or methoxy groups plus the aromatic ring 3 resonance could be the explication of the clastogenic action of both alkaloids. Fig. 1 also shows the structural formula of colchicine, a known mitotic spindle poison. HIPP and MAT both exhibit action on the mitotic spindle that could be related to the similitude of their aromatic ring 3 and the colchicine ring 1.

1) Since MAT KB cell ED50 is about twice as that exhibited by HIPP in the same system, 2) since the clastogenic effect of HIPP is significantly smaller than of MAT effect at its respective equivalents ED50, 3) HIPP effects are smaller than ADR effect, and 4) without including other clinical aspects, we are lead to conclude that HIPP might be a very convenient citostatic drug.

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