POTENTIAL ANEUGENIC ACTION OF PHENANTHRIDINIC ALKALOIDS AND FLAVONOID DETERMINED TO BE MUTAGENIC THROUGH THE MICRONUCLEUS TEST. COMPARATIVE STUDY WITH THE ACTION OF COLCHICINE*

Accion Potencial Aneugenica de Alcaloides Fenantridinicos y Flavonoide Determinados como Agentes Mutagenos mediante el Test de Micronucleo. Estudio Comparativo con la accion de Colchicina

M. ALARCON**, S. DUK, M.A., GARCIA, W. VENEGAS AND G. WEIGERT***

RESUMEN

La determinación de las propiedades clastogénicas de compuestos fenantridínicos aislados de *Hippeastrum ananuca* y un flavonoide aislado de *Gutierrezia resinosa* se establecen mediante el test del Micronúcleo (MN). El tamaño de los MN mayores de 1/5 del diámetro de la célula de muchos de ellos presupone que tiene además un efecto aneugénico, lo que indica un mecanismo de veneno del huso tipo colchicina (COL).

De la comparación de las moléculas se sugiere un mecanismo de acción común con la COL en el bloqueo de la síntesis de tubulina en el huso.

Esto aporta conocimientos a la interpretación del mecanismo de acción de la COL en el bloqueo del huso y postula un mecanismo de acción de las cualidades aneugénicas de los compuestos fenantridínicos y flavonoide informados.

ABSTRACT

The determination of the clastogenic properties of phenanthridinic compounds isolated from *Hippeastrum ananuca* and one flavonoide compound isolated from *Gutierrezia resinosa*, was established through the Micronucleus Test (MN).

The size of many of the MN was larger than 1/5th of the cell diameter that suggested that besides being clastomutagenic these compounds were also aneugenic. We conclude that these compounds have a spindle poisoning activity, similar to that of colchicine (COL).

From the comparison of the molecules, a common action mechanism with COL is postulated, namely the blocking the spindle tubuline synthesis.

This sheds light on the interpretation of the mechanism of action of COL in blocking the spindle and a mechanism of action of the aneugenic properties of phenanthridinic and flavonoid compounds is proposed.

KEYWORDS: Genotoxicity. Aneugenesis. Phenanthridinics. Flavonoid. Colchicine. Micronucleus Test.

INTRODUCTION

The clastogenic action of the alkaloids isolated from *Hippeastrum ananuca*. Phil (Amarilidaceae) has been determined in the Cytogenetics Laboratory of the Molecular Biology Department of University of Concepcion. These compounds have shown a cytostatic activity, when tested in KB cell cultures;

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**e-mail: malarcon@ dpi.udec.cl Concepción, Chile.

*** Department of Molecular Biology. Faculty of Biological Sciences, University of Concepcion. P.O. Box 152-C. Concepción, Chile.

a line of transformed cells established from a nasopharyngeal cancer. (Pacheco, P. Silva, M. 1978).

The following phenanthridinic compounds have shown mutagenic activity with the bone marrow MN test (Alarcón *et al.* 1983, 1986. Cea, G. *et al.* 1986): Maritidine (MAT): 1, 2, 3, 4a, 6 hexahydro 8-9 dimethoxy-hidroy 5-10b etano phenanthridine. Hippeastidine (HIPP) 1, 2, 3, 4a, 6 hexahydro-10 hidroxy-3, 8, 9 trimethoxy-5 10b etano phenanthridine, Licorine (LIC): (Galatán-1, 2 diol-3, 12 didehydro-9, 10 [metilen bis (oxi)]). These compounds were isolated by Muñoz, O. *et al.* 1992. And the flavonoid THTMF: 5, 3', 4'-trihydroxy-3, 6, 7, 8-tetramethoxy flavone, isolated from *Gutierrezia resinosa* (H et A) Blake Compositae. (Cea, G. *et al.*, 1983). This compounds was isolated by Bittner, M. *et al.* 1982.

In the case of THTMF, its action could be due to a hydroxyl group in position 5 in the same aromatic ring; to a keto group in C-4 and to a double link between positions 2 and 3.

The presence of MN larger than 1/5 would confirm the aneugenic effects and would allow to suppose, according to other authors (Yamamoto and Kikushi, 1980, Parry and Sors 1993, Vanparys, Ph. *et al.* 1990) that the lagging chromosome would be due to an inhibitory action on the spindle.

The mutagenic action of the compounds has been determined by the bone marrow MN test, developed by Schmid in 1975 and modified by Das and Kar in 1980. This allows to detect clastogenic effects induced by chemical compounds. The test is based on the formation of smaller, secondary micronuclei, consisting of acentric chromosomal fragments produced by chromosomal fragments of complete chromosomes randomly delayed by a spindle alteration.

During erythropoyesis the main nucleus is expelled from the cell. Sometimes remains micronucleus in, the cytoblast, for reasons not yet clear. It could be thought that during cell replication cycle, repair mechanisms didn't get to act, allowing in this way, a normal base line for MN.

MATERIALS AND METHODS

The MN test was carried out with normal 2 month old Balb/c male mice (20 g b.wt.). Eight animals per dosage were treated intraperitoneally with 0.2 ml. of distilled water (negative control) the chemicals tested in KB cells (a human transformed nasopharingeal cell line) at doses selected on the basis of the ED50 KB cells.

Mice were sacrified 30 h after the injection. Bone marrow from the 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 the slide. Cell were stained with May Grünwald-Giemsa solution. About 3000 polychromatic erythrocytes (PCE) per animal per dose were scored from coded slides and PCE with micronuclei were recorded. A Mann-Whitney U-test was employed for statiscal analysis.

RESULTS

The action of clastogenic substances, as those mentioned, raises the rate of MN (see Table I)

TABLE I. Incidence of MN in mouse bone marrow by the phenanthridinic alcaloids and a flavonoid compound.

	DOSE	MN/1000 CELLS+SD
HIPPEASTIDINE		
(negative control)	0.0	6.7 ± 0.6
	0.27	8.3 ± 1.2*
	0.54	9.9 ± 1.2*
	1.083	10.6 ± 0.3*
MARITIDINE		
(negative control)	0.0	5.18 ± 1.72
	1.28	8.57 ± 0.2*
	2.55	10.25 ± 9.85*
	5.10	10.09 ± 9.72*
LICORINE		
(negative control)	0.0	4.68 ± 0.51
	4.55	5.23 ± 1.4
	9.10	8.01 ± 0.49*
	18.20	14.17 ± 9.17*
TETRAMETHOXYFLAVONE		
(negative control)	0.0	5.58 ± 0.96
	0.5	11.53 ± 2.47*
	1.0	15.26 ± 1.33*
	2.0	20.11 ± 0.97*

* Marked values are significantly different.

DISCUSSION

Alarcón, M. *et al.* in 1983, 1986 and Cea, G. in 1986 discussed the possibility of Hippeastidine, Maritidine, Licorine and Tetramethoxyflavone of having therapeutic value as antineoplastic agent, they also discussed clastogenic characteristics of these substances.

Through this analysis, it was determined that a certain amount of MN was bigger than 1/5 of the cell diameter. The difference in the shape and structure of MN produced by genotoxic agents is determined by the action site of the drug. Cells with rounded or oval MN with a size of 1/5-1/7 of the cell diameter can be due to a clastogenic action of the tested chemicals. When MN are bigger than 1/5 of the cell diameter, the genotoxic action is due to the action of the chemicals on the mitotic spindle (spindle poison) (Yamamoto, and Kikushi, 1980 Högstedt and Karlson, 1985).

Large sized MN are observed with HIPP, MAT and THTMF, but in all cases a higher clastogenic effects is observed, rather than the spindle poison effects.

The clastogenic effect of these alkaloids, as well as other molecules pointed out by other authors (Mc. Gregor and Jurd 1978 and Sahu *et al.* 1981) could be attributed to the presence of a hydroxyl group (HIPP in C-10, MAT in C-3 and LIC in C-1 and C-2) in all three phenanthridinic nuclei. The highest action of HIPP can be attributed to the fact that the hydroxyl group is in the same aromatic ring 3. The hydroxyl groups in MAT and LIC are not in the aromatic rings which resonance could also be an active factor of clastogenicity. The higher genotoxic activity of HIPP and MAT could also be due to the spatial action of C-C bridge between positions 5 and 10b. (Fig. 1).

In the case of THTMF, its action could be due to a hydroxyl group in position 5 in the same aromatic ring; to a keto group in C-4 and to a double link between position 2 and 3.

The presence of MN larger than 1/5 would suggest the aneugenic effects and would allow to suppose, according to other authors (Vanparys *et al.* 1990, Yamamoto and Kikushi 1980) that the lagging chromosome would be due to hibitory effect on the spindle.

The action of these compounds on the spindle could be explained if its action was compared with the action of colchicine (COL), a classic alkaloid poison for the spindle. It has been suggested that the COL action resides in the aromatic ring 1 of COL, with three methoxy groups, which is very similar to the aromatic ring 3 of the molecules HIPP and MAT and with ring A of THTMF. HIPP and MAT have a dimethoxy disposition in the aromatic ring and THTMF has a trimethoxy disposition. (See Figure 1). It is believed that the capacity of COL of blocking of tubuline polymerization to form the achromatic spindles is due to its chemical constitution (spindle poison and polyploids inductor). It would be possible to suppose that the molecules we have analyzed have the same effect due to their structural similarities. But it is evident though that the clastogenic effect predominates, LIC shows only clastogenic effects, and dimethoxy possition seems to have cycled, loosing its capacity to form MN larger than 1/5 of the cell diameter.

COL, being a blocker of the tubuline polymerization (Bergen and Borisy 1993; Sternlicht and Ringel 1979), fundamental for the microtubules formation, its inhibitory mechanism could be explained by analyzing the following: The basic tubuline subunit is a dimer (a and b tubuline) each monomer is cysteine rich and therefore rich in SH groups. The SH groups of cysteine from the tubuline are essencial for the polymerization process for microtubule formation. Units are a dimers, in which each unit is associated to two GTP molecules. Each dimer has an attachment site of high affinity to COL. If this dimer associated with COL is attached to the growing end of the microtubule, then no more dimeric agreggations could occur, thus blocking the polymerization. (Salmon *et al.* 1984; Bergen and Borisy 1983; Onfelt 1986; Kirschner and Mitchison 1986).

In this process sulphydril groups from the cisteine residues of the tubuline molecule are involved. It has been determined that the COL diminishes the number of SH-of the tubulines. This could mean that there is a relationship between the attachment sites of the drug to the tubuline, and the SH-groups, critical for the microtubules assambly (Ludueña and Roach 1981a, 1981b).

It has been shown that alkylating agents, among others, specificaly react with the SH-groups of tubuline and inhibit the dimer's assambly.

Experimental work has shown that COL has an important effect preventing the alkylation of the SH-groups of tubuline, where COL's methoxy groups can act similar to the methoxy groups of HIPP, MAT and THTMF. Through this effect COL inhibits the spindle formation ant it appears as a potential aneugenic compound. (Ludueña and Roach 1981b).

Due to simularities in chemical structures between the aromatic ring 1 of COL and the aromatic ring 3 of the HIPP and MAT molecules, and the A ring from THTMF, we assume a similarity in the genotoxication and the presence of MN larger than 1/5 of cell diameter confirm that the presence of aneugenic as well as clastogenic properties (Vanderkerken *et al.* 1988).

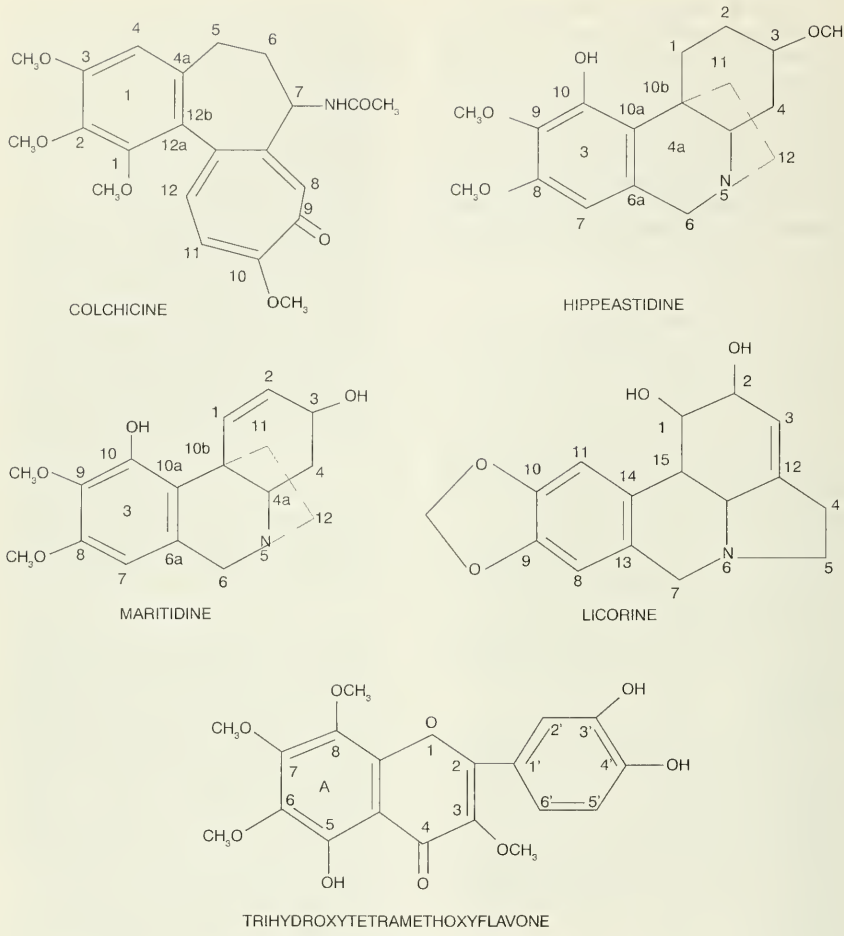

Figura 1. Phenantridinic alcaloids and trihydroxytetramethoxy flavona compound compared to colchine.

On the other hand, COL has shown no clastogenic effects with MN smaller than 1/5 of cell diameter, (Vanparys *et al.* 1989). This also agrees with the general principle of the need of free OH- groups on the molecule, to show this effect, among others. COL does not have OH-groups, this would be the reason of its selective effect on the spindle. The studied molecules, as apposed to COL present both conditions: free OH- groups and methoxy group in ortho position. This is why its clastogenic and aneugenic double effect can be asserted.

A clear distinction of both types of action on the chromosomes, will require new studies, using newer techniques such as fluorescence *in situ* hybridization (FISH). This would allow to distinguish clearly between chromosomal fragment and entire

chromosomes. (Mayne and Moyzis 1994). As it has been considered in various biphenols tested for aneuploidogenic potential compared with COL. (E. Pfeiffer *et al.* 1997).

Further studies will be also necessary to determine the conditions that this molecules require for its aneugenic action, and its similarity to COL.

CONCLUSIONS

From the genotoxic characteristics of the phenantridinic compounds and one flavonoid determined through the MN test, it was concluded that the clastogenic effect was clear, and the presence of MN larger than 1/5 of the cell diameter make the aneugenic effect evident.

By comparing the molecules, a common mechanism of action with COL is suggested; evidently aneugenic by blocking the tubuline synthesis at the mitotic spindle. This allows to assume a mechanism for the clastogenic and aneugenic properties of phenantridinic and flavonoid compounds mentioned here.

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