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GENOTOXIC EFFECTS OF OVATIFOLIN ACETATE, A SESQUITERPENE LACTONE ISOLATED FROM *Podanthus ovatifolius* Lag. (Compositae)

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ABSTRACT

Ovatifolin acetate (OA), a sesquiterpenic lactone has been isolated from *Podanthus ovatifolius* Lag. Compositae. When tested in standard tests in KB cells of human epidermoid nasopharingeal carcinoma, it showed antineoplastic activity. Since these drugs are well known to induce genetic damage the genotoxic activity of OA was evaluated using the micronucleus test in mice with male Balb/c mice. OA showed a cytotoxic effect by modifying cell proliferation kinetics of mouse bone marrow erythrocytes and also increased micronucleated cells.

RESUMEN

Se ha aislado acetato de ovatifolina (AO), una lactona sesquiterpénica de *Podanthus ovatifolius* Lag. Compositae, planta chilena; ella muestra propiedades antineoplásicas en la prueba estándar de células KB, epidermoides de carcinoma nasofaríngeo humano. Siendo los antineoplásicos inductores potenciales de un amplio daño genético, se valora la acción genotóxica del acetato de ovatifolina mediante la prueba de micronúcleos, en médula ósea de ratones machos *Mus musculus*, Balb/c. AO se muestra como un poderoso agente citotóxico capaz de alterar la cinética de proliferación o maduración de los eritrocitos en médula ósea de ratón, que así mismo produce un incremento de células micronucleadas.

INTRODUCTION

More and more drugs for cancer therapy have been developed during the last years, but an undesirable genotoxic effect on normal cells is however frequently present (Bhakuni *et al.* 1976, Jenssen and Ramel 1980, Mac Gregor *et al.* 1987, Cea *et al.* 1990). For this reason chemotherapeutic treatment would only be recommended when the risk-benefit evaluation makes it appropriate.

Genotoxic agents are capable of causing a variety of cell nuclear changes, such as micronuclei in mouse bone-marrow polychromatic erythrocytes (PCE) (Wild 1978, Hart *et al.* 1983, Hayashei *et al.* 1984, Alarcón *et al.* 1986, Mirkova 1987). They are generally accepted to reflect clastogenicity.

OA, a sesquiterpene lactone isolated from natural extracts of *Podanthus ovatifolius*, exhibits antineoplastic properties as tested in standard KB human epidermoid carcinoma of nasopharynx and PS (P 338 lymphocytic leukemia) assay procedures.

A program for screening of antitumoral effects of chemicals isolated from chilean plants, has been going on for the last decades (Gnecco *et al.* 1973, Bhakuni *et al.* 1976, Cea *et al.* 1990).

This agent (OA) and others were tested in our laboratory for their genotoxic activity (Alarcón *et al.* 1986, Cea *et al.* 1990). Based on this study it is necessary to make a clinical determination to see whether this compound is convenient as an antitumoral drug.

MATERIALS AND METHODS

OA was supplied by the Phytochemistry Lab. University of Concepción. The cytotoxicity test for KB cells and *in vivo* PS test were carried out at the National Cancer Institute (NCI), USA.

Two month old male Balb/c mice (Molecular Biology Department Biotherium, University of Concepción) weighing ca. 20 g were intraperitoneally injected with a single OA dose (volume 0.2 ml) diluted in dioxan-distilled water (1:10).

Four doses were selected (1.25, 2.5, 5.0 and 10.0 mg/kg) on the basis of the data with KB cells LD 50 (2.50 mg/l of TC medium). Doxorubicine (Adriamycine, Farmitalia) at 10.00 mg/kg was used as positive control and a mixture of dioxane distilled water as negative control. Dioxan-water was selected as a solvent, since the OA is insoluble in water and other solvents tested. The dilution was 1:10 because in this way dioxan was less toxic and it was the lowest dilution which disolved the highest dose of OA.

Four mice per dose were sacrified 30 h after injection and femurs were removed. Femurs were prepared for the bone-marrow micronucleus test as proposed by Schmid (1975) but modified according to Oliver and Goldstein (1978) and Das and Kar (1980). Slides were stained with May-Grünwald and Giemsa solutions according to the schedule outlined by Cole *et al.* (1979) which maximized the differenciation between polychromatic (PCE) and normochromatic (NCE) erythrocytes. An average of 5000-9000 PCE and NCE per animal per dose were scored from coded slides and micronucleated polichromatic (MPCE) and normochromatic (MNCE) erythrocytes were recorded.

The Mann-Whitney U test was employed for statistical analysis.

TABLE I. AVERAGE VALUES AND STANDARD DEVIATIONS OF QUANTITATIVE ANALYSIS OF BONE-MARROW ERYTHROCYTES FROM MICE TREATED WITH OVATIFOLIN ACETATE

DOSES mg/Kg	*MPCE/1000 PCE*	*MNCE/1000 NCE*	*PCE/NCE*
DOXO 10.00	27.57±4.33	5.78±1.45	0.58±0.06
D-W 1:10 ;	6.94±0.82	3.25±0.92	1.02±0.05
OA 1.25	11.77±2.97	3.72±0.33	0.71±0.11
OA 2.50	16.99±2.06	7.34±1.32	0.43±0.007
OA 5.00	6.06±0.96	3.09±0.34	0.37±0.02
OA 10.00	1.88±0.38	4.16±0.83	0.42±0.02

PCE = polychromatic erythrocytes
NCE = normochromatic erythrocytes
MPCE = micronucleated polychromatic erythrocytes
MNCE = micronucleated normochromatic erythrocytes
DOXO = doxorubicine
D—W = dioxan-water
OA =ovatifolin acetate

TABLE II. MANN-WHITNEY U TEST RESULTS OF BONE-MARROW ERYTHROCYTES FROM MICE TREATED WITH OVATIFOLIN ACETATE

	MPCE/1000PCE					*MNCE/1000NCE*					*PCE/NCE*				
D—W 1:10	U=0 p=0.014 ∧					U=0 p=0.014 ∧					U=0 p=0.014 >				
1.25	U=0 p=0.014 ∧	U=1 p=0.029 >				U=0 p=0.014 ∧	U=5 p=0.243				U=6 p=0.343	U=0 p=0.014 ∧			
2.50	U=0 p=0.014 ∧	U=0 p=0.014 >	U=0 p=0.014 ∧			U=0 p=0.014 >	U=0 p=0.014 >	U=0 p=0.014 >			U=3 p=0.100	U=0 p=0.014 ∧	U=0 p=0.014 ∧		
5.00	U=0 p=0.014 ∧	U=8 p=0.557	U=0 p=0.014 >	U=0 p=0.014 ∧		U=0 p=0.014 ∧	U=7 p=0.443	U=0 p=0.014 ∧	U=0 p=0.014 >		U=0 p=0.014 ∧	U=0 p=0.014 ∧	U=0 p=0.014 ∧	U=3 p=0.100	
10.00	U=0 p=0.014 ∧	U=0 p=0.014 ∧	U=0 p=0.014 >	U=0 p=0.014 ∧	U=0 p=0.014 ∧	U=1 p=0.029 ∧	U=3 p=0.100	U=6 p=0.343	U=0 p=0.014 >	U=5 p=0.243	U=3 p=0.100	U=0 p=0.014 ∧	U=0 p=0.014 ∧	U=8 p=0.557	U=1 p=0.029 >
DOSIS	DOXO 10.00	D—W 1 : 10 ;	1.25	2.50	5.00	DOXO 10.00	D—W 1 : 10 ;	1.25	2.50	5.00	DOXO 10.00	D—W 1 : 10 ;	1.25	2.50	5.00

The > indicates that the effect of the left-column dose is greater than the correspondent effect of bottom-column dose
The ∧ indicates that the effect of bottom-column dose is greater than the correspondent effect of left-column dose
MPCE = micronucleated polychromatic erythrocytes
MNCE = micronucleated normochromatic erythrocytes
PCE = polichromatic erythrocytes
NCE = normochromatic erythrocytes
DOXO = doxorubicine
D—W = dioxan-water

RESULTS AND DISCUSSION

The data of the mouse bone-marrow study are presented in Table I. Table II shows the "U" and asocciated p values as compared one to one with the effects of different doses by the Mann-Whitney "U" test.

OA significantly increases the MPCE incidence at dosages of 1.25 and 2.50 mg/kg, but at 5.00 and 10.00 mg/kg the incidences of MPCE decreased significatively below the frequency induced by dioxan-water mixture (negative control). On the other hand, the MNCE frequency increased significantly at 2.50 mg/kg and then decreases at higher OA doses.

The PCE/NCE ratio decreased at 2.50 mg/kg and reached the lowest values at 5.00 mg/kg coincidently with the MPCE and MNCE frequencies peaks.

The negative control showed unexpectedly high MPCE frequency. Since negative controls are usually water or saline solutions, we suspect that our results are due to dioxan itself.

The micronuclei observed were both round and oval-shaped with sizes ranging between 1/5 - 1/8 of the cell diameter, which according to Heddle and Carrano (1977) and Yamamoto and Kikushi (1980) confirms the clastogenic origin of the MN. On the other hand, the bone-marrow data show a remarkable depression and it is evident that it became flooded with peripheral blood at all OA doses used.

The induction of micronucleated cells provides a sensitive measure of chromosome breakage although not all types of chromosome aberrations become micronuclei (Hayashei *et al.* 1984). Short term *in vivo* assays such as the micronucleus test do reflect the complex pharmacokinetics involved in the uptake, metabolism and distribution of genotoxic agents (Jenssen and Ramel 1980). Nevertheless, a closer approach to evaluate the genetic-risk of the organism would be to analyze not only the micronuclei formation in bone marrow but also other nuclear damage in the tissue of actual interest, because the tissue-response to clastogenic injury could be tissue-specific (Goldberg *et al.* 1983, Proudlock and Allen 1986).

The *in vitro* LD_{50} of OA for KB cell LD_{50} is 2.50 mg/l TC medium (National Cancer Institute USA). Considering this as a reference, at an empirically equivalent dose of 2.50 mg/kg OA induced the formation

of typical micronuclei. At 1.25 and 2.50 mg/kg OA shows a high clastogenic activity in mouse bone-marrow cells. At higher dosages this effect disappears due to the increased cell lethality observed by both the histological analysis and an anatomical inspection of the bone-marrow. The PCE/NCE ratio decreased below 0.5 in mice treated with 2.5-10.0 mg/kg OA indicating impaired proerythroblast-polychromatic erythrocyte fluxe or polychromatic-normochromatic erythrocyte transition.

These results showed that OA is a cytotoxic agent with ability to modify the proliferation of bone-marrow cells and is a clastogenic agent capable of inducing micronuclei in mouse bone-marrow polychromatic erythrocytes.

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