Trabajo Original | Toxicología Experimental

Efficiency of different rat lines as biomodel in the chromosomal aberrations assays bone marrow cells.

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Abstract

The aimed of this research was to compare the efficiency in terms of sensibility of different rat lines for chromosomal aberration assays. Rats of different lines (Sprague Dawley, Lewis and Wistar rats) and both sexes were used in this study. The spontaneous and induced indexes (cyclophosphamide) were evaluated according to chromosomal aberration assay of bone marrow cell. Sprague Dawley rat showed the lower spontaneous indexes and high induced indexes to the mutagen used; that allowing detecting with a narrow error margin those substances that are classified of very low genotoxicity. These results suggesting the biggest use of this line of rat on *in vivo* genotoxicity assay.

Key-words: Rats, genotoxicity, chromosomal aberrations.

Resumen

Eficiencia de diferentes líneas de ratas como biomodelo en el ensayo de aberraciones cromosómicas de células de la médula ósea.

El objetivo de esta investigación fue comparar la eficiencia en términos de sensibilidad de diferentes líneas de ratas en el ensayo de aberraciones cromosómicas. Ratas de diferentes líneas genéticas (Sprague Dawley, Lewis y Wistar), de ambos sexos fueron utilizadas. Los índices espontáneos e inducidos con ciclofosfamida fueron determinados teniendo en cuenta las variables analizadas en el ensayo de de aberraciones cromosómicas en células de la médula ósea. En la línea de ratas Sprague Dawley se observaron los índices espontáneos más bajos y los inducidos altos al mutágeno utilizado. Lo cual permite detectar en un menor margen de error aquellas sustancias que son clasificadas como de poco o nulo efecto genotóxico. Estos resultados sugieren el mayor uso de esta línea de rata en los ensayos *in vivo* de genotoxicidad.

Palabras claves: Ratas, genotoxicidad, aberraciones cromosómicas.

Introduction

The main problem in genotoxicity assay is that the researchers use the different rat lines for convenience, but in generality of the cases this decision is far from a theoretical-practical basement that justifies the selection.

At the present time only those products that are very safe or very genotoxic are probably truly classified while those capable ones of causing small damages could be not classified.

When determining the line with the smallest number of chromosomic aberrations and sensitive to the used mutagen will allow bigger robustness to this technique, also the toxicologist will have a potent tool, endorsed statistically, when selecting the genetic line of rats to perform the mutagenesis and carcinogenesis studies.

The aim of this research was to perform a comparative study of the spontaneous and induced frequency of chromosomic aberrations in bone marrow cells among Sprague Dawley (SD), Lewis and Wistar rats of both sexes.

Materials and methods

Animals and experimental conditions. Rats of different genetic lines were used (SD, Lewis and Wistar). Young adult animals of both sexes (6-8 weeks) were used; the corporal weight was oscillated among 180-210g. Each experimental group was formed by 5 animals/sex/lines for a total of 10 animals in two replicas [1,2].

Experimental groups. Two experimental groups were evaluated.

Group 1: Animals not tried as negative control. Was performed the intraperitoneally (i.p) technique, the administration was twice 48 and 24 hours before the euthanasia programmed.

Group 2: Animals treated with cyclophosphamide (CF) as positive control, in dose of 50 mg/kg, via i.p, (Ledoxina®, Lemery, CORP), which was diluted in saline solution (NaCl) to 0.9 %. The solution was administered immediately after being prepared, 48 and 24 hours before the euthanasia programmed to reason of 10 ml/kg [1].

Chromosomic aberration assay in bone marrow cells. The chromosomic aberration assay in bone marrow cells were performed according to the standardized protocols and adjusted by Arencibia y col., 2009 [2]. The cellular division in metaphase stopped using colchicine (4 mg/kg, via i.p), in the next day schedule (4 hours before the euthanasia). One femur was extracted and the medullar cavity was washed with 3 mL of foetal bovine serum (FBS). The cellular suspension was centrifuged, being eliminated the liquid suspension. After the hypotonic treatment of the cells of the button with KCL (0.075 M), it was performed a second centrifugation [3]. The cellular button was fixed in mixture methanol-glacial acetic acid (3:1 proportion) during 15 minutes. Three fixations with successive centrifugations were performed, and extended in humid sheets with previous cooling. The sheets dried off to the air and they were tinted with solution from Giemsa to 10% during 30-35 min. 100 metaphases was counted by animal, being determined the number of cells with aberrations (ruptures and chromosomes exchanges, ruptures and chromatids exchanges) and gaps frequency [3]. Also the mitotic index MI% was calculated (metaphases percentage in 1 000 readable cells) and the number of polyploidy cells in 1 000 readable cells. All the determinations were ready by two observers, and then an average among both was established [2].

Analysed variables. The analysed variables were the total of cells with structural aberrations in the chromosomes and mitotic index (cells number in metaphase) [2].

Euthanasia methods and statistical analysis. The method of euthanasia selected was the cervical dislocation with previous ether atmosphere. All the results were compared against negative control group and compared between lines of rats for the same group and sex. The continue variables were analysed by ANOVA test ($p \leq 0,05$) and the categorical variables by Chi Squared ($p \leq 0,01$) [2]. All the analyses were performed using the Statsoft for Windows. StatSoft, Inc. (2003). STATISTICA (data analysis, software system), version 6.

Results

In the comparison among lines of rats were obtained as results that the both sexes of SD rats differed with the other evaluated lines in the spontaneous and induced frequency of damage (numeric and structural aberrations) (Table 1). There were not significant differences among sexes of the animals that belonged to the same experimental group and rat lines. This comparison was been worth for all the variables evaluated in the assay.

Discussion

The results of the chromosomic aberrations assay demonstrated that found in other genotoxicity assays in the same cellular line evaluated; since the SD rats demonstrated to have the lowest values in chromosomal damage [4,5]. The analysed variables such as aberrations of structural type, mitotic index, number of cell with polyploidy and gaps aberrations, differed among SD rats in both sexes when being compared with Lewis and Wistar rats. But the biggest effect in the animals treated with CF it was obtained in Lewis rats.

In other studies, they were not observed differences among sexes of the animals that belonged to the same experimental group and rat lines [6, 7]. In performed study using the micronucleus assay to determine the spontaneous indexes among different species of rats, it was also differences the SD lines against Lewis and Wistar rats [7].

In a comparative study evaluated in the alkaline comet assay of individual cells it was observed smaller number of the basal DNA damage in SD rats (both sexes) [8-10]. The induction obtained with the CF did not differ among lines, result that it was manifest in both sexes.

In other study the CF differed in the three lines of rats evaluated in the micronucleus assay. The smallest basal result of cytotoxicity was obtained in SD rats [4,11]. The response of SD rats to the CF was high, but smaller to the clastogenic results obtained in Lewis and Wistar rats in both sexes. On the other hand the CF induced a

considerable number of micronucleus in SD rats but the biggest clastogenic effect it was in Wistar rats (male sex) [11].

The male SD rats overcame the other evaluated lines in the number of normal basal sperms. Of the other two evaluated lines, was obtained bigger results of basal anomalous sperms in Lewis rats. In three evaluated rat line were obtained a genotoxic ambient in the germinal cells with the use of the CF, being obtained bigger results of induction of anomalous sperms in Lewis rats [12].

The different response of CF among rat lines, it is for consequence that existing different expression levels of cytochrome P-4501A1 genes, important enzyme in the liver. Maybe the founded that the CF induced biggest effect in Lewis rats, this in the structure of the P450 enzymes, because they have been obtained bigger metabolism rates in Lewis, when administering genotoxic substances [13].

Conclusion

It is possible conclude that the Sprague Dawley rat is an excellent biomodel, being the lower spontaneous indexes and high induced indexes to the mutagen used; allowing detecting in a narrow error margin those substances that are classified of very low genotoxicity.

Table 1. Results of the comparison of the spontaneous and induced frequency of chromosomic aberrations in bone marrow between Sprague Dawley, Lewis and Wistar rats of both sexes.

Groups	Sex	MI (%) [a]	Cells with Poliploidy [b]	Gaps [b]	Number of cells with aberrations [b]
Sprague Dawley Rats (both sexes)					
Negative Control	F	4,81 ± 0,10**c**	1**c**	5**c**	18**c**
	M	4,93 ± 0,09**c**	2**c**	6**c**	17**c**
Cyclophospham ide (50 mg/kg, i.p)	F	3,40 ± 0,26*__c__	28**__c__	69**__c__	246**__c__
	M	3,58 ± 0,43*__c__	23**__c__	62**__c__	220**__c__
Lewis Rats (both sexes)					
Negative Control	F	4,38 ± 0,11	7	16	50
	M	4,41 ± 0,10	10	17	52
Cyclophospham ide (50 mg/kg, i.p)	F	2,99 ± 0,11*	42**	90**	342**
	M	3,10 ± 0,13*	40**	96**	360**
Wistar Rats (both sexes)					
Negative Control	F	4,32 ± 0,13	9	20	55
	M	4,37 ± 0,11	16	22	51
Cyclophospham ide (50 mg/kg, i.p)	F	2,93 ± 0,09*	48**	99**	339**
	M	3,02 ± 0,10*	45**	100**	354**

[a]**X ± E.D**, 10 000 total cells/group/serie for a total of 20 000 evaluated cells, *p≤0,05; ANOVA Test. [b]**p≤0,01; Chi Squared χ2 non parametric test. Comparison against negative control for both tests in the same species. **c=**p<0.05 (It differs when comparing among line of rats keeping in mind the same variable in the same experimental group, using the same statistic test).

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