

A multivariate approach to the association pattern of reciprocal translocations induced by chemicals and ionizing radiation in mouse germ cells

J.C. De Luca¹, E. Ortiz Jaureguizar² and F.N. Dulout¹

Abstract

The degree of similarity between chemical and physical agents in their capacity to induce reciprocal translocations was analyzed by means of multivariate analysis techniques. The effect of three different doses of gamma rays, four doses of X-rays and different doses of adriamycin, mitomycin C, thio-tepa and bleomycin was analyzed. Data were arranged in a basic matrix by two methods: cluster analysis and ordination. Two main groups were found, one including doses of 9 and 10 Gy and the other including the remaining lower doses of ionizing radiation and the other chemicals. Various subgroups were found within the second group. Accordingly, using presence/absence data there was not a specific pattern of chromosomal damage induction for physical and chemical agents. The increase in the frequencies of reciprocal translocation observed with 9 and 10 Gy was due to an increase in the kind of multivalent configurations. This variability could be dose dependent. Likewise, the similarity observed in the second group between the chemicals and the lower doses of ionizing radiation could also be dose dependent.

INTRODUCTION

Cells are constantly exposed to exogenous and endogenous agents which may damage the genetic material, being the DNA molecule the main target. Physical and chemical agents can interact with this molecule in different ways through the induction of DNA double- and single-strand break, cross-links and base damage. In all cases the primary lesions must be amplified to be detected as chromosomal aberrations. On the other hand, ionizing radiations can interact with macromolecules or cellular structures in two ways: first, when the damage is directly induced in the macromolecules by ionized particles, and second, when the damage is induced by free radicals produced by the ionization of cellular water. In a similar way, some chemicals like adriamycin (ADR), mitomycin C (MMC) and bleomycin (BLM) can interact with DNA in different ways such as intercalation, DNA adduct formation and free radical induction, respectively (Pigram *et al.*, 1972; Povirk and Austin, 1991; Sanderson and Shield 1996). Information obtained from analysis of reciprocal translocations induced by physical and chemical agents in mammalian germ cells includes multivalent configuration types and frequencies. The data obtained provide information about the effects of the agent tested as well as dose-response relationships. However, analysis of the frequencies of multivalent configurations induced by different treatments helped to develop two questions: 1) Is there a specific pattern of chromosomal damage induction for each agent? 2) Is it possible to compare the effects of dif-

ferent agents in order to establish similarities and differences? Attempts to answer this last question have included comparing the effect of chemicals and ionizing radiation (e.g., van Buul and Gouzdward, 1980) from a subjective point of view, without any statistical or mathematical evaluation. In order to accurately determine the degree of similarity or difference between the effects of chemicals and ionizing radiations in relation to the induction of reciprocal translocations in germ cells, multivariate analysis methods were used. Originally developed for systematic biology (Rohlf, 1963; Sneath, 1964; Crovello, 1968; Crisci and López Armengol, 1983), these methods have been recently employed to solve similar problems in other disciplines (e.g., Sneath, 1966; Williams *et al.*, 1966; Gillie and Peto, 1969; Wishart and Leach, 1970; Winkler and Sokal, 1987; Pascual and Ortiz Jaureguizar, 1990).

MATERIAL AND METHODS

Operational taxonomic unit (OTU) selection

Relationships between three gamma ray (GR) doses (1, 5, and 9 Gy) as well as different doses of the chemicals ADR, MMC, BLM and thio-tepa (TT) were analyzed. Data were obtained from experiments carried out in male BALB/c mice (De Luca *et al.*, 1988, 1990a,b). In addition, data obtained from van Buul and Léonard (1980) about the effect of 0.25, 0.50, 0.75, 1, and 10 Gy of X-rays (XR) were included in the analysis. Table I summarizes the different treatments considered. Some of the 14 treatments

¹Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, Avenida 60 y 118, s/n, CC 296, 1900 La Plata, Argentina. Send correspondence to J.C.L. E-mail: jdeluca@fcv.medvet.unlp.edu.ar

²Laboratorio de Sistemática y Biología Evolutiva (LASBE), Facultad de Ciencias Naturales y Museo, Paseo del Bosque s/n, 1900 La Plata, Argentina. E-mail: eortiz@museo.fcny.unlp.edu.ar

induced the same types of reciprocal translocations (i.e., ADR5-MMC-0.75-1 Gy; TT-BLM20-BLM40; 5 Gy-BLM60; see Tables III and IV), although frequencies were different (van Buul and Léonard, 1980; De Luca *et al.*, 1988, 1990a,b). Data were not clustered since this procedure did not modify the results obtained.

Data accumulation

The data considered include 10 “characters”, i.e., reciprocal translocations, induced in stem cell spermatogonia and scored as multivalent configurations in diakinesis-metaphase I. All characters were qualitative and scored as presence/absence. Table II summarizes the different configurations considered and their corresponding codifications.

Table I - Experimental protocol employed to compare reciprocal translocation type induced by the different treatments.

Agent	Dose	Number of mice studied
ADR	5 mg/kg	11
ADR	10 mg/kg	4
MMC	2 mg/kg	8
BLM	20 mg/kg	6
BLM	40 mg/kg	6
BLM	60 mg/kg	6
TT	0.2 mg/kg	7
GR	1 Gy	4
GR	5 Gy	10
GR	9 Gy	11
XR*	0.25 Gy	10
XR*	0.50 Gy	18
XR*	0.75 Gy	10
XR*	10 Gy	9

ADR: Adriamycin; MMC: mitomycin C; BLM: bleomycin; TT: thio-tepa; GR: gamma rays; XR: X-rays. *Data from van Buul and Léonard (1980).

Table II - Codification of the multivalent configurations employed as “characters”.

Multivalent configuration (“characters”)	Codification
18 II + 1 CIV	Present (1) : Absent (0)
18 II + 1 RIV	Present (1) : Absent (0)
18 II + CIII + 1 I*	Present (1) : Absent (0)
17 II + 1 CVI	Present (1) : Absent (0)
16 II + 1 CVIII	Present (1) : Absent (0)
16 II + 1 CIV + 1 RIV	Present (1) : Absent (0)
16 II + 2 CIV	Present (1) : Absent (0)
16 II + 2 RIV	Present (1) : Absent (0)
15 II + 1 CIV + 1 CVI	Present (1) : Absent (0)
15 II + 1 CIV + 1 RVI	Present (1) : Absent (0)

I: Univalent; II: bivalent configuration; III: trivalent configuration; IV: tetravalent configuration; VI: hexavalent configuration; VIII: octovalent configuration; C: multivalent chain; R: multivalent ring. *Data from van Buul and Léonard (1980).

Data processing

Data were arranged in a basic data matrix (BDM) (Table III) of 14 OTUs by 10 reciprocal translocation types. BDM was analyzed by means of two methods: cluster analysis and ordination. Computer analysis was done on a Pentium-II™ IBM-compatible PC at the LASBE (Laboratorio de Sistemática y Biología Evolutiva, Museo de La Plata) by means of the NTSYS-pc 2.0 library of numerical taxonomical programs developed by Rohlf (1997). Details about methods and computational procedures can be found in Sneath and Sokal (1973) and Rohlf (1997).

Cluster analysis

BDM was transformed into a similarity association matrix (SAM) among OTUs using the Jaccard coefficient (Sneath and Sokal, 1973). SAM served as input in the calculation of a phenogram by the unweighted pair-group method using arithmetic averages (UPGMA) (Sokal and Michener, 1958). Cophenetic correlations between the phenogram and SAM input were computed using the cophenetic correlation coefficient (CCC) (Sokal and Rohlf, 1962). If more than one phenogram was obtained, a consensus tree was performed to summarize the results. A consensus tree represents the consensus topology (subset of relationship) of two or more trees, and was obtained by the majority rule consensus method (Margus and McMorris, 1981). The consensus index C_{ic} (Rohlf, 1982) was employed as a measure of the similarity of the original trees.

Ordination

The OTU x OTU SAM obtained in 3.1. was transformed into a scalar product form so that its eigenvalues and eigenvectors could be computed, resulting in a principal coordinate analysis (Gower, 1966). The results of the principal coordinate analysis were used as an initial configuration matrix to perform a nonmetric multidimensional scaling analysis (Kruskal, 1964a,b). This technique represents dissimilarities among any objects or variables by any point in K-dimensional space so that the interpoint distances in the K-dimensional space correspond as much as possible to the observed distances between the objects. Distances were fitted in K-dimensional space to a monotone function of the original distances with a coefficient called “stress” (Kruskal, 1964a,b). This author suggests verbal evaluations for various levels of stress:

Stress	Goodness of fit
0.40	Poor
0.20	Fair
0.10	Good
0.05	Excellent
0.00	“Perfect”

Table III - Basic matrix data employed for the multivariate analysis (BMD).

OTU	Multivalent configurations ("character")									
	18 II+ 1 CIV	18 II+ 1 RIV	15 II+ 1 CIV+1 CVI	15 II+ 1 CIV+1 RVI	17 II+ 1 CVI	16 II+ 2 CIV	16 II+ 1 CVIII	16 II+ 2 RIV	18 II+ CIII + 1 I	17 II+ 1 RVI
ADR5	1	1	0	0	0	0	0	0	0	0
MMC	1	1	0	0	0	0	0	0	0	0
0.75 Gy	1	1	0	0	0	0	0	0	0	0
1 Gy	1	1	0	0	0	0	0	0	0	0
ADR10	1	1	0	0	1	0	0	0	0	0
5 Gy	1	1	0	0	0	0	1	0	0	0
BLM60	1	1	0	0	0	0	1	0	0	0
9 Gy	1	1	1	1	1	1	0	1	0	0
0.25 Gy	1	1	0	0	0	0	0	0	1	0
0.50 Gy	1	1	0	0	0	0	0	0	1	0
10 Gy	1	1	0	0	1	0	0	1	1	1
TT	1	0	0	0	0	0	0	0	0	0
BLM20	1	0	0	0	0	0	0	0	0	0
BLM40	1	0	0	0	0	0	0	0	0	0

OTU, Operational taxonomic units. For other abbreviations see Tables I and II.

RESULTS

Cluster analysis

Using UPGMA, we obtained four phenograms ($CCC = 0.93885$) of association among OTUs (Figure 1A-D). The consensus tree (Figure 2) showed seven common groups: a) formed by ADR5, MMC, 1 Gy, 0.75 Gy, ADR10, 5 Gy, BLM60, 0.25 Gy, 0.50 Gy, TT, BLM40 and BLM20; b) formed by ADR5, MMC, 1 Gy, 0.75 Gy, ADR10, 5 Gy, BLM60, 0.25 Gy and 0.50 Gy; c) formed by ADR5, MMC, 1 Gy and 0.75 Gy; d) formed by 5 Gy and BLM60, e) formed by 0.25 Gy and 0.50 Gy; f) formed by TT, BLM40 and BLM20, and g) formed by 9 Gy and 10 Gy. The C_{IC} was 0.58333. Differences between the four phenograms consisted of minor variations in the relationships (transpositions) between members of group b.

Ordination

In the bidimensional graphic (Figure 3) three treatment groups can be recognized (56.88% of the variance): a) formed by TT, BLM20, BLM40; b) formed by BLM60, 5 Gy, ADR5, MMC, 1 Gy, 0.75 Gy, 0.25 Gy, 0.50 Gy and ADR10, and c) formed by 9 Gy and 10 Gy. The final "stress" was 0.03893.

DISCUSSION

The results obtained showed two main groups: one including treatments with 9 Gy of GR and 10 Gy of XR, and the other including all the chemical compounds and the remainder doses of GR and XR (Figures 1, 2 and 3). Accordingly, using presence/absence data there was not a specific pattern of chromosomal damage induction for

physical and chemical agents. In effect, both kinds of agents were grouped together, with the only exception of the greatest doses of XR and GR, that comprise a separate group not only in comparison with the chemicals but also with the lower doses of GR and XR.

The association of 9 Gy of GR and 10 Gy of XR is expected since GR and XR induce similar kinds of reciprocal translocations. This agrees with the fact that these doses correspond to the descending part of the dose-response curve for induction of reciprocal translocations by single doses of ionizing radiation (Léonard and Deknadt, 1969). Table III shows that 9 Gy of GR and 10 Gy of XR induced a similar kind of reciprocal translocations and a higher variability of reciprocal translocations than drugs and the remaining doses of radiation. On the other hand, Table IV shows that these doses increased the frequency of reciprocal translocations (the frequencies of 5 and 9 Gy of GR could be overestimated by the lower number of cells scored).

Some increase in the frequency of reciprocal translocations at high doses of ionizing radiation was observed in the guinea pig (Lyon and Cox, 1975), the mouse (van Buul and Goudzwaard, 1986) and the rhesus monkey (van Buul, 1991). On the other hand, all results so far reported on high exposure indicate some induction of translocation (van Buul, 1991).

Leenhouts and Chadwick (1981) and van Buul and Seelen (1991) have postulated three main parameters of the stem cell spermatogonial population that affect the level of induced translocations: 1) the proportion of sensitive and resistant cells; 2) the ratio of the probabilities that a basic lesion in the DNA leads to cell killing or translocation formation, and 3) the proliferation-differentiation pattern of surviving spermatogonial stem cells after irradiation. Significant increase of intra- as well as inter-in-

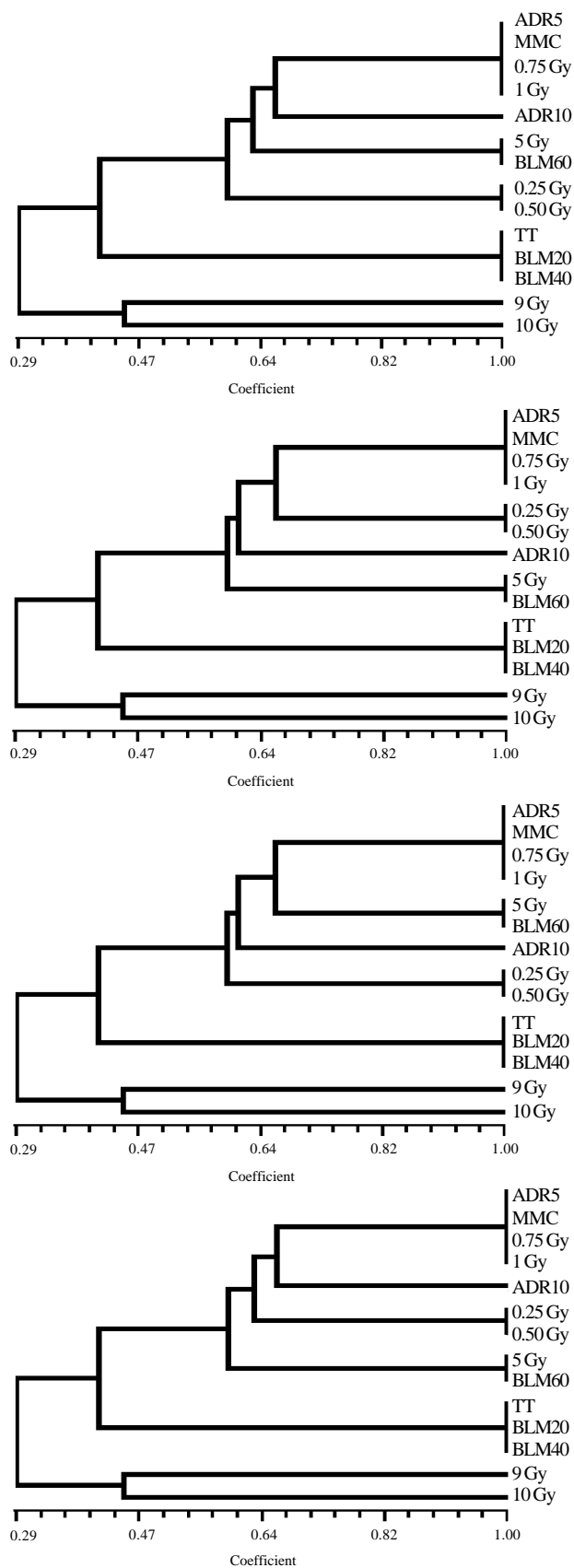


Figure 1 - A-D - Phenogram (UPGMA) of association (Jaccard coefficient) obtained from the 14 treatments. CCC was 0.93885. See references on Material and Methods, and Table I.

dividual variability is produced by high degrees of cell killing due to the increase in the effect of clonal proliferation of the relatively few surviving stem cells (Léonard and Deknadt, 1969; van Buul and Léonard, 1984).

From our data (see Tables III and IV) the increase in the frequencies of reciprocal translocation observed in 9 and 10 Gy is due to an increase in the kind of multivalent configurations. This variability could be dose dependent because the higher doses of ionizing radiations produced six or seven different kinds of multivalent configurations, while the lower ones, two or three (see Table III).

The second group showed an association between all the chemical compounds and the lower doses of ionizing radiation. ADR5, MMC, 0.75 and 1 Gy are clustered because they all induce the same kind of reciprocal translocations (see Table III). Results obtained from these treatments showed that the two chemicals interacting with DNA through different mechanisms, mainly intercalation (ADR) (Waring, 1970) and alkylation (MMC) (Iyer and Szybalsky, 1963; Schwartz *et al.*, 1963), respectively, induce similar types of chromosomal aberrations as those induced by ionizing radiations. Although the number of cells scored was different (Table IV), the number of "rare multivalent configurations" was similar. This could indicate that presence or absence of these multivalent configurations could be independent of the agent as well as of the doses employed (Table IV). Several explanations have been suggested to explain the mode of action of these compounds. Like other anthracyclines, ADR can intercalate in DNA molecule (Pigram *et al.*, 1972). This feature seems to be an important factor for its genotoxic effect.

Cellular enzymes can convert ADR into free-radical metabolites (Bachur *et al.*, 1979, 1982; Pan and Bachur, 1980), which can bind to DNA or react further to yield DNA-damaging oxygen radicals (Lown and Sim, 1977; Goodman

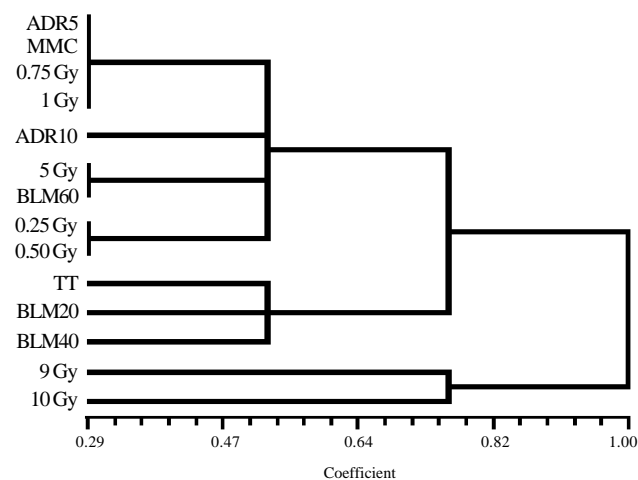


Figure 2 - Consensus dendrogram (majority rule) obtained from the four phenograms presented in Figure 1. The CCC was 0.58333. See references on Material and Methods, and Table I.

and Hochstein, 1977; Demopoulos *et al.*, 1980). Other anthracycline actions are membrane disruption and generation of activated oxygen species as well as formation of iron-anthracycline complexes (Hoey *et al.*, 1988).

On the other hand, participation of the hydroxyl radical on the induction of DNA damage by MMC has been reported (Hamana *et al.*, 1985). Furthermore, Dusre *et al.* (1990) reported that MMC and two analogues produced both free radicals and interstrand cross-links. They suggested that this property may be positively correlated with cytotoxicity, since free radical and interstrand cross-link formation fit cytotoxicity curves for MMC. These results suggest that MMC can act as a radiomimetic compound.

Results obtained with ADR (5 and 10 mg/kg) showed a different degree of association, because 5 mg/kg presented higher affinity with the lowest doses of ionizing radiations

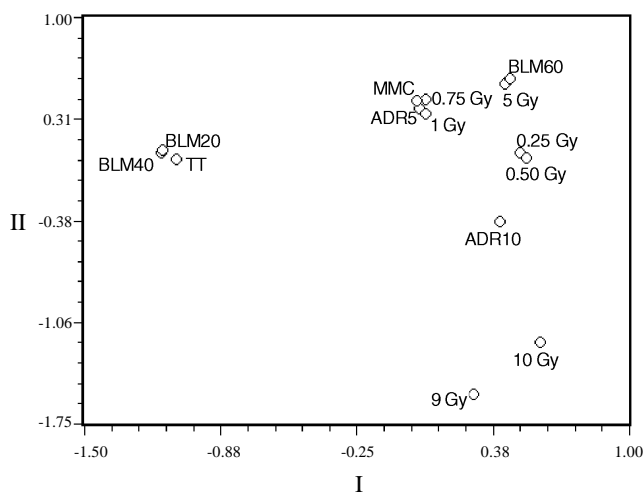


Figure 3 - Multidimensional scaling graph, showing the location of the 14 treatments in a bidimensional graph (axis I-II, 56.88% of variance). The final “stress” was 0.03893. See references on Material and Methods, and Table I.

than that observed for the 10 mg/kg dose of ADR (Figures 2 and 3). This fact suggests a dose-dependent radiomimetic effect of ADR. When male mice were irradiated with 9 Gy of GR 24 h after intraperitoneal treatment with two different doses of ADR, only the lower dose (5 mg/kg) presented a potentiating effect, similar to that induced by 1 Gy plus 9 Gy of XR separated by the same lapse (van Buul and Léonard, 1980). The finding of a dose-dependent radiomimetic effect of ADR agrees with results obtained by De Luca *et al.* (1990a).

BLM is a radiomimetic compound that can induce free radicals and DNA strand breaks (Lown and Sim, 1977; Povirk and Austin, 1991). Then, the association of BLM with different doses of ionizing radiation could be expected. BLM can induce reciprocal translocations in germ cells of mice, and although the frequency is lower than that obtained by ionizing radiations, the kind of multivalent configurations is similar in both cases (van Buul and Gouzdward, 1980). Likewise, whereas doses of 60 mg/kg BLM can be associated with 5 Gy of GR, lower doses (20 and 40 mg/kg) are not related (Figures 2 and 3). This fact could indicate that the radiomimetic effect of this drug is dose dependent. Supporting this inference, when a dose of 60 mg/kg BLM was combined with 9 Gy of GR, a potentiating effect was observed (De Luca *et al.*, 1988), and the frequency of reciprocal translocation obtained was similar to that induced by the combined treatment of 1 Gy plus 9 Gy of XR (van Buul and Léonard, 1980).

It has been proposed that results obtained with combined treatments could be explained by assuming that depletion of any kind of spermatogonia is sufficient to modify the chromosomal response of stem cells (van Buul, 1984). This means that the first dose of radiation, after fractionation experiments or chemical treatments 24 h before irradiation, induces selective cell killing of proliferating spermatogonial cells, triggering the surviving stem cells to enter and active cycle to repopulate the germinal epithelium

Table IV - Multivalent configurations scored for the different treatments.

OTU	18 II+ 1 CIV	18 II+ 1 RIV	15 II+ 1 CIV+1 CVI	15 II+ 1 CIV+1 RVI	17 II+ 1 CVI	16 II+ 2 CIV	16 II+ 1 CVIII	16 II+ 2 RIV	18 II+ CIII+1 I	17 II+ 1 RVI	Total cells scored
ADR5	1	1	0	0	0	0	0	0	0	0	1,100
MMC	5	3	0	0	0	0	0	0	0	0	1,069
0.75 Gy	1	1	0	0	0	0	0	0	0	0	1,900
1 Gy	1	1	0	0	0	0	0	0	0	0	2,000
ADR10	1	1	0	0	1	0	0	0	0	0	1,200
5 Gy	1	1	0	0	0	0	1	0	0	0	995
BLM60	1	1	0	0	0	0	1	0	0	0	458
9 Gy	1	1	1	1	1	1	0	1	0	0	1,088
0.25 Gy	1	1	0	0	0	0	0	0	1	0	2,000
0.50 Gy	1	1	0	0	0	0	0	0	1	0	2,500
10 Gy	1	1	0	0	1	0	0	1	1	1	2,000
TT	1	0	0	0	0	0	0	0	0	0	981
BLM20	1	0	0	0	0	0	0	0	0	0	528
BLM40	1	0	0	0	0	0	0	0	0	0	560

OUT, Operational taxonomic unit. For other abbreviations see Tables I and II.

(Cattanach *et al.*, 1976; Cattanach and Crocker, 1980; van Buul, 1983). Accordingly, additive and potentiating effects observed after combined treatments of chemicals and ionizing radiation doses do not depend on the amount of chromosomal damage induced by the chemicals, but rather on the ability of the compound to induce depletion of differentiating or differentiated spermatogonia. Then, two types of effects could be expected: additive, when combined treatments induce a considerable amount of chromosomal damage without killing a great number of cells, and potentiation, when the amount of chromosomal damage induced is so great that the repair mechanisms triggered are not enough to reduce cell mortality. Combined treatments induce extensive cell death and only few surviving cells with a high yield of chromosomal damage can be scored.

However, the subadditive effect observed after combined treatment of MMC plus 4 Gy (Deknudt and Léonard, 1979), MMC plus 5 Gy (De Luca *et al.*, 1990b) or ADR 5 mg/kg (De Luca *et al.*, 1990a) cannot be explained by this assumption. A subadditive effect could be explained assuming involvement of the DNA repair mechanism (De Luca *et al.*, 1988, 1990a; van Buul, 1984). These repair mechanisms can reduce cell mortality in such a way that a higher frequency of more radioresistant cells survive. With a higher dose (ADR 10), the amount of chromosomal damage induced is so great that the repair mechanisms triggered are not enough to reduce cell mortality.

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RESUMO

O grau de similaridade entre agentes químicos e físicos quanto a sua capacidade de induzir translocações recíprocas foi analisado por meio de técnicas de análise multivariada. Analisou-se o efeito de três diferentes doses de raios gama, quatro doses de raios-X e diferentes doses de adriamicina, mitomicina C, tio-tepa e bleomicina. Os dados foram arranjados em uma matriz básica por 2 métodos: análise de cluster e ordenação. Dois grupos principais foram encontrados, um incluindo doses de 9 e 10 Gy e o outro incluindo as outras doses de radiação ionizante e as substâncias químicas. Vários subgrupos foram encontrados dentro do segundo grupo. Usando dados de presença/ausência, não se encontrou um padrão específico de indução de danos cromossômicos para os agentes físicos e químicos. O aumento na frequência de translocação recíproca observado com 9 e 10 Gy

deveu-se a um aumento no tipo de configurações multivalentes. Esta variabilidade pode ser dependente da dose. Igualmente, a similaridade observada no segundo grupo entre as substâncias químicas e as doses menores de radiação ionizante também pode ser dependente da dose.

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