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INVESTIGATION OF THE EFFECT OF HYDROGEN PEROXIDE ON THE CHROMOSOMES OF YOUNG AND ELDERLY INDIVIDUALS

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SUMMARY

Active oxygen species have been considered to be responsible for the aging process and for the induction and initiation of neoplastic processes.

The effect of hydrogen peroxide, an active oxygen species, was investigated in the chromosomes of three young women (20—21 years of age) and of three elderly women (73—79 years of age) in a culture medium favorable to the appearance of folate-sensitive fragile sites.

Hydrogen peroxide at a final concentration of 5×10^{-6} during the final hours of culture caused a significant increase in hypodiploidy and structural aberrations, chromatid gaps in particular, only in the cultures from the three elderly women, suggesting that the chromosomes of older women are more sensitive to this agent than those of younger women.

The preferential chromosome loss in both treated and untreated cultures from the elderly women involved chromosome X. The preferential sites for structural aberrations were 9p12, a constitutive heterochromatin site and 6q21, where the gene of mitochondrial superoxide dismutase, an enzyme involved in antioxidant processes in the cell, is located.

Hydrogen peroxide significantly intensified the effect naturally occurring in the cells of elderly persons, such as hypodiploidy and increased structural aberrations, thus acting at the chromosome level in a manner similar to that of the natural aging process of the organism.

Key words: Free radicals; Ageing; Cytogenetics; Chromosomes; Oxidants agents

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INTRODUCTION

The nature of the aging process has been the subject of considerable speculation. Some time ago Harman [1] proposed a theory that attributed the greater vulnerability of the organism with time to the apperance of free radicals. Free radicals such as the superoxide anion, hydrogen peroxide and the hydroxyl radical originate from the mono and bivalent reduction of a small fraction of molecular oxygen and are common reactions in normal cell physiology. Normally, the concentration of these radicals in the cell is kept low by enzymatic and non-enzymatic protective mechanisms [1—3].

The deleterious effect of these radicals has been demonstrated in cells and specifically in DNA in a large number of pathological processes [2,4]. Over the last few years, there has been a strong tendency to hold these radicals responsible for the aging process and for the induction and initiation of neoplasias [2,5,6].

The induction of chromosome aberrations and of sister chromatid exchanges by oxidant systems of molecular oxygen has been detected by some investigators in human lymphocytes [7—9], in human fibroblast lines [10] and in the CHO line [11—15]. The predominant type of chromosome damage caused by different oxidant systems or by molecular oxygen was of the chromatid type [10—12]. Shabtai et al. [16] reported the induction of the fragile sites 16p13 and 16p23 by antioxidant agents. In contrast, the addition of antioxidant agents reduced the expression of the fragile site 16q22 induced by chromium chloride, possibly through the formation of free radicals [16,17].

Significantly elevated frequencies of endoreduplication and tetraploidy were observed in lymphocytes obtained from two individuals and submitted to elevated concentrations of molecular oxygen in 6- and 7-day cultures [8]. A greater incidence of structural chromosome aberrations, aneuploidies involving chromosome X in women and an increased frequency of spontaneous sister chromatid exchanges has been observed to occur with age [18—22].

The determination of folate-sensitive fragile sites under the effect of caffeine has shown a larger number of such sites in elderly people than in newborn infants [23]. An increased number of folate-sensitive fragile sites per cell has been observed to occur as a function of age [24].

The objective of the present study was to determine the action of hydrogen peroxide — a free radical — in the induction of chromosome aberrations in a culture medium favorable to the appearance of folate-sensitive sites both in chromosomes from young adults and in chromosomes from elderly subjects, a type of investigation which has not been reported previously in the literature.

MATERIALS AND METHODS

Lymphocyte cultures from six normal women, three of them young (20—21 years of age) and three older (73—79 years of age) were analyzed cytogenetically.

The culture medium used was TC 199 containing 7% fetal calf serum, which is appropriate for the appearance of folate-sensitive fragile sites [25]. The cultures lasted 92 h and hydrogen peroxide was added during the final 24 h of culture at the following final concentrations: 5×10^{-4} M, 5×10^{-5} M, 2.5×10^{-5} M, 1.25×10^{-5} M and 5×10^{-6} M.

The investigation of numerical and structural chromosome aberrations was carried out in a blind test using at least 100 cells per culture and considering only cells with 46 centromeres for analysis of structural aberrations. All cells with aberrations were initially analyzed by standard staining and then reexamined after G-banding [26] for identification of breakpoints. The structural aberrations were classified as chromatid and chromosome gaps and chromatid or chromosome breaks.

The degree of ploidy and the number of C-mitoses were determined by analyzing at least 1000 cells per culture.

Data were analyzed statistically by the χ^2 test or by the exact Fisher test for numerical alterations and by the Mann-Whitney test for structural aberrations [27].

RESULTS AND DISCUSSION

Only cultures submitted to the action of the oxidant at the 5×10^{-6} M concentration permitted apparently normal metaphase visualization and chromosome analysis, whereas the remaining hydrogen peroxide concentrations led to the appearance of degenerating metaphases which prevented chromosome analysis.

The results caused by the effect of the oxidant on the cultures from young and older women are shown in Tables I, II and III. Analysis of aneuploid (both hypo-

TABLE I

NUMBER OF HYPODIPLOID AND HYPERDIPLOID CELLS IN LYMPHOCYTE CULTURES FROM YOUNG AND OLDER WOMEN TREATED OR NOT WITH HYDROGEN PEROXIDE AT THE FINAL CONCENTRATION OF 5×10^{-6} M DURING THE LAST 24 h OF CULTURE

Individuals	*Untreated cultures*			*Total no. of cells*	*Treated cultures*			*Total no. of cells*
	No. of cells in terms of chrom. no.				*No. of cells in terms of chrom. no.*			
	45	*46*	*47*		*45*	*46*	*47*	
Young women								
1	9	102	1	112	10	101	0	111
2	4	106	2	112	7	102	0	109
3	5	102	1	108	11	102	0	113
Total	18	310	4	332	28	305	0	333
Older women								
1	18	104	3	125	32	104	4	140
2	11	104	1	116	29	100	0	129
3	22	103	7	132	26	102	7	135
Total	51	311	11	373	87	306	11	404

TABLE II

NUMBER OF POLYPLOID CELLS AND OF C-MITOSES IN LYMPHOCYTE CULTURES FROM YOUNG AND OLDER WOMEN TREATED OR NOT WITH HYDROGEN PEROXIDE AT A FINAL CONCENTRATION OF 5×10^{-6} DURING THE LAST 24 h OF CULTURE

Individuals	*Untreated cultures*		*Total no. of cells*	*Treated cultures*		*Total no. of cells*
	No. of C-mitoses	*No. of polyploid cells*		*No. of C-mitoses*	*No. of polyploid cells*	
Young women						
1	0	0	1012	5	1	1037
2	1	1	930	1	2	1800
3	0	3	1000	4	0	1097
Total	1	4	2942	10	3	3934
Older women						
1	3	8	1010	2	4	1035
2	5	10	1001	0	2	287
3	8	5	1005	2	2	1057
Total	16	23	3016	4	8	2379

TABLE III

NUMBER OF GAPS AND BREAKS IN CELLS FROM YOUNG AND OLDER WOMEN TREATED OR NOT WITH HYDROGEN PEROXIDE AT A FINAL CONCENTRATION OF 5×10^{-6} DURING THE LAST 24 h OF CULTURE (ONLY CELLS WITH 46 CENTROMERES WERE ANALYZED)

	Untreated cultures				*Total no. of gaps + breaks*	*Total no. of cells analyzed*	*Treated culture*				*Total no. of gaps + breaks*	*Total no. of cells analyzed*
	Gaps		*Breaks*				*Gaps*		*Breaks*			
	ct	*cs*	*ct*	*cs*			*ct*	*cs*	*ct*	*cs*		
Young women												
1	15	5	2	6	28	102	21	7	3	8	39	101
2	15	3	2	4	24	106	14	2	4	2	22	102
3	8	20	4	2	34	102	9	21	4	8	42	102
Total	38	28	8	12	86	310	44	30	11	18	103	305
Older women												
1	7	21	2	14	44	104	24	35	7	13	79	104
2	9	13	1	3	26	104	27	12	5	7	51	100
3	10	20	5	7	12	103	75	17	9	5	116	102
Total	26	54	8	24	112	311	126	64	21	35	246	306

ct: chromatid; cs: chromosomal.

and hyperdiploid) cell frequency (Table I) showed no significant difference as a function of treatment with the oxidant in the cultures from young individuals. In contrast, treated cultures from older women showed a significantly increased frequency of hypodiploid cells than untreated cultures ($\chi^2 = 8.19$, $P < 0.005$), suggesting that the chromosomes of these individuals are more susceptible to chromosomal loss and non-disjunction than those of young individuals under the action of treatment.

A higher frequency of hypodiploidy was observed in both treated ($\chi^2 = 23.88$, $P < 0.005$) and untreated ($\chi^2 = 13.54$, $p < 0.05$) cultures from older women when compared to cultures from young women, demonstrating that hypodiploidy is related to the aging process, as extensively documented in the literature [22].

Among the older women, the preferentially lost chromosome both in treated and untreated cultures was chromosome X, which was absent in 25.5% of untreated hypodiploid cells and in 43.7% of treated hypodiploid cells. These findings confirm the preferential loss or non-disjunction of chromosome X as a function of age (for a review, see Ref. 22). Thus, the oxidant significantly intensified the effect of hypodiploidy observed in untreated cultures from older women, producing a biological effect similar to that of aging *per se*.

The frequency of polyploid cells in untreated cultures from older women was significantly higher than in cultures from young women ($P = 0.00019$), results that, as in the case for hypodiploidy, may be related to effects on the mitotic spindle. These results have been observed during the final stages of culture in cultures aged *in vitro* [28]. A significant increase in C-mitotic cells was observed in parallel ($P = 0.00016$), a phenomenon that has been related to change in mitotic spindle that probably precede the formation of polyploid cells [29].

On the other hand, treatment of cultures from older women with hydrogen peroxide led to a reduced number of polyploid cells ($P = 0.028$) and of C-mitoses ($P = 0.023$), suggesting that the oxidant was selecting these cells *in vitro*. Studies in the literature in which the action of molecular oxygen or of oxidant systems was investigated did not include analysis of numerical chromosome changes.

Analysis of structural alterations in hydrogen peroxide-treated cultures from young women did not show significant differences from untreated cultures with respect to any type of structural alterations. Untreated cultures from older women showed an increase (which, however, was not significant) of chromosome gaps and breaks which may have been due to *in vivo* chromatid alterations. The literature suggests greater chromosome instability as a function of age [22—24]. Treated cultures from older women showed a significantly higher frequency of chromatid gaps ($z = 2.719$, $P < 0.05$, Mann-Whitney test). These data agree with reports in the literature showing an increase in the frequency of chromosome aberrations predominantly of the chromatid or SCE types induced by oxidant systems in lymphocyte cultures from one and two individuals [7—9] and in the CHO line treatments lasting 3 min to 68 h [12—15].

The location of preferential aberration sites under the action of hydrogen peroxide was investigated in a system favorable to the appearance of folate-sensitive fragile sites, as well as in untreated cultures from young and older women to evaluate the effect of age.

In untreated cultures from young women, the preferential locations of chromosomal aberrations were those of the fragile site 3p14, which was present in 3.5% of all cells and of the fragile site 6q26, which appeared in 3.3% of all cells, both locations being considered common fragile sites [25,30]. The frequency of structural aberrations in treated cultures from young women led to a discrete increase in structural aberrations which were distributed at random among the chromosomes.

In untreated cultures from older women, the preferential locations of structural abnormalities were those of the fragile site 3p14, which appeared in 3.2% of all cells and of the site 6p21, which appeared in 3.5% of all cells. In contrast, treatment with hydrogen peroxide caused a significant increase in structural alterations especially in terms of chromatid gaps, which were related to the following sites, present in cultures from all of the 3 older women: site 6q21, which appeared in 4.2% of treated cells and in no untreated cells and site 9q12, which appeared in 4.6% of treated cells and in no untreated cells. Site 17p12, a common fragile site, showed a marked increase under treatment (9.6—25.0%) in one older woman. The presence of the fragile site 17q21 ranged from 1.9% in untreated cells to 4.9% in treated cells in two of the three older women.

It is interesting to note that the sites preferentially induced by the oxidant in cultures from older women are not common fragile sites. They were sites 9q12, a site of constitutive heterochromatin, and site 6q21, where the gene for the enzyme mitochondrial superoxide dismutase is located [31]. Since this enzyme is involved in the antioxidant processes of the cell, we may speculate that the alteration at this site detected in cultures from older women may be related to the larger number of structural alterations in these individuals.

In conclusion, hydrogen peroxide markedly intensified the effect of aging *per se* by acting in a manner similar to this biological process at the chromosome level, thus indicating that it may be related to the development of the aging process.

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