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In Vitro Effect of Dapsone on Human Chromosomes [1, 2]

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It is presently well known that several chemical and physical agents to which human beings are exposed are able to induce chromosome aberrations that may play an important role in the pathogenesis of iatrogenic illnesses (2). Since the number of leprosy patients in the world is estimated to be more than ten million (1), it becomes important to investigate whether 4, 4′-diaminodiphenylsulfone (DDS), the most commonly used sulfone in leprosy therapy, is capable of damaging human chromosomes.

Taking into account that leprosy patients are under the effect of both an infection of *Mycobacterium leprae* and sulfone therapy, it is clear that, first of all, this investigation should be developed on *in vitro* dividing cells of healthy individuals. This was performed in the most readily accessible tissue for the study of chromosome aberrations, i.e., by culturing leukocytes of the peripheral blood.

MATERIALS AND METHODS

Chromosome analyses were made on metaphases obtained from leukocyte cultures of two samples of healthy Caucasoid individuals. Sample 1 was composed of ten individuals and Sample 2 of fifteen individuals. The plasma drawn from 10 ml of venous heparinized blood from each individual was distributed into two sterile 100 ml prescription bottles. In both samples, one bottle of each set was used as control and contained 6 ml of tissue culture medium (TCM) plus two drops of phytohemagglutinin (PHA), prepared in the authors' laboratory. In the Sample 1 sets, the second bottle contained 4, 4′-diaminodiphenylsulfone (DDS) at a final concentration of 0.4 μg per ml of TCM. The concentration of DDS in the sulfone treated cultures of Sample 2 was tenfold higher than that of Sample 1 (4 μg/ml). The TCM consisted of 60% Hanks balanced salt solution enriched with 0.5% lactalbumin hydrolysate, 20% fetal bovine serum, 20% ascitic fluid, 100 I.U./ml penicillin and 100 μg/ml streptomycin.

The cultures were incubated for 72 hours at 37°C after which colchicine (Houdé), at 3×10^{-6} final concentration, was added to each bottle in order to arrest the cell divisions in metaphase by inhibiting the spindle formation. Accumulation of metaphases was obtained by incubating the colchicine treated cultures for six hours. The contents of the bottles were then transferred to conical graduated tubes and centrifuged for five minutes at 800 rpm, after which 4 ml of distilled water, at 37°C, were added to the sediment which was suspended in 1 ml of the supernatant. This hypotonic treatment was finished by incubating the tubes at 37°C for 25 minutes and by adding 0.5 ml of freshly prepared fixative (methanol-acetic acid 3:1 v/v) before centrifuging for five minutes at 800 rpm.

Complete fixation of the cells was obtained by resuspending the sediment with a Pasteur pipette in 4 ml of fixative and by maintaining the tubes in the refrigerator overnight. The tubes were centrifuged again for five minutes at 800 rpm and the sediment resuspended in 0.5 ml of freshly prepared fixative for distribution on frozen and moistened slides, which were rapidly flamed shortly thereafter.

Hydrolysis in N HCl at 60°C for five minutes was interrupted by washing the slides in running tap water for ten minutes. Staining with Giemsa's reagent for ten minutes was followed by rinsing in running tap water. After being air-dried, the slides were mounted with balsam.

Chromosome analyses permitted evaluation of: 1) the proportion of accumulated metaphases by examining at least 600 cells

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TABLE 1. *Percentage of metaphases observed in DDS treated and untreated (control) leukocyte cultures from the individuals of Sample 1.*

Individual				Percent	
No.	Sex	Age	Control	DDS 0.4 μg/ml	Difference
1	M	21	13.60	13.49	0.11
2	M	20	10.82	7.20	3.62
3	F	25	8.74	10.61	–1.87
4	F	19	10.58	6.88	3.70
5	M	20	4.56	6.14	–1.58
6	M	24	9.10	9.20	–0.10
7	M	19	8.62	9.71	–1.09
8	M	16	8.82	13.60	–4.78
9	M	22	10.05	8.61	1.44
10	F	20	8.55	6.79	1.76
Mean		20.6	9.34	9.22	0.12

TABLE 2. *Percentage of metaphases observed in DDS treated and untreated (control) leukocyte cultures from the individuals of Sample 2.*

Individual				Percent	
No.	Sex	Age	Control	DDS 4 μg/ml	Difference
1	M	20	9.79	7.63	2.16
2	F	20	1.72	2.35	–0.63
3	M	29	7.69	7.63	0.06
4	F	20	2.94	1.89	1.05
5	M	21	0.86	1.48	–0.62
6	F	21	1.91	1.84	0.07
7	M	23	1.82	1.59	0.23
8	F	23	3.13	2.49	0.64
9	F	41	6.95	8.07	–1.12
10	F	27	7.50	6.63	0.87
11	F	20	5.10	4.59	0.51
12	M	21	9.40	7.79	1.61
13	M	27	3.27	3.47	–0.20
14	M	22	1.49	2.59	–1.10
15	F	20	8.22	4.47	3.75
Mean		23.7	4.78	4.30	0.48

of each culture; 2) the frequency of cells with numerical chromosome aberrations (aneuploidies) by analyzing the chromosomal counts of 20 metaphase figures of each culture; and, 3) the types and frequency of structural chromosome aberrations by analyzing ten metaphase figures of each culture, selected from those which did not show aneuploidies.

RESULTS

The proportion of metaphases observed in the control and DDS treated cultures of Samples 1 and 2 are shown in Tables 1 and 2. Since the data on these tables is paired, it is permissible to compare the effect of DDS on the rate of metaphases by testing the null hypothesis that the mean difference of the paired data is zero. This test was made by calculating $t = \bar{d}/s(\bar{d})$ with $n-1$ degrees of freedom (DF) where $\bar{d}$ is the mean difference, $s(\bar{d})$ is the standard error of $\bar{d}$, and n is the number of pairs.

From Table 1 it is seen that $\bar{d} = 0.12$, $s(\bar{d}) = 0.82$, $t = 0.144$, 9 DF; while from Table 2 the values are $\bar{d} = 0.48$, $s(\bar{d}) = 0.33$. $t = 1.454$, 14 DF. In both cases the t figures indicate that $\bar{d}$ is not significantly different from zero,

which indicates that the rate of metaphases was not affected by the different concentrations of DDS used.

No aneuploid cells were observed in 200 analyzed metaphases of either the controls or DDS-treated cultures of the individuals of Sample 1. Among the analyzed metaphases of Sample 2 controls, 8 (2.7%) of 300 revealed numerical chromosome abnormalities as represented by hypodiploid (6), hyperdiploid (1) and endoreduplicated (1) cells. This proportion was significantly lower than that found among the DDS-treated cultures (corrected $X^2 = 3.878$; 1 DF; $p < 0.05$) because among these, 19 (6.3%) of 300 metaphases showed numerical chromosome abnormalities (13 hypodiploid and 6 hyperdiploid cells).

In the controls of Sample 2, the affected chromosomes of the aneuploid cells were restricted to groups C (57.1%), D (14.3%) and G (28.6%), while in the DDS-treated cultures the aneuploidies were mostly caused by loss or excess of chromosomes in groups C (47.4%) and E (21.0%). The other chromosomes affected belonged to groups D (5.3%), F (5.3%) and G (10.5%), while 10.5% could not be recognized.

The structural aberrations detected in both samples were only represented by achromatic gaps. In Sample 1 they were observed in 2% of the metaphases of either the controls or the DDS-treated cultures, affecting chromosomes of groups A and E in the former, and A and B in the latter. In the controls of Sample 2, these gaps were observed in 7 (4.7%) of the 150 cells, the chromosomes affected being of groups A (42.9%) and C (57.1%). This proportion was significantly lower than that observed in the DDS-treated cultures of Sample 2 (corrected $X^2 = 11.032$; 1 DF; $p < 0.001$) because among them 26 (17.3%) of the 150 cells presented the same abnormality affecting chromosomes of groups A (50.0%), B (19.2%), C (26.9%) and D (3.9%).

DISCUSSION

Chromosome aberrations were not induced by 0.4 μg/ml DDS, but a higher concentration of this sulfone (4 μg/ml) was able to significantly increase the frequency of both the aneuploidies and achromatic gaps. Since the plasma levels of DDS in individuals receiving a daily dose of 50 mg of this drug seem to vary around 0.4 μg/ml (4), it is likely that most of the leprosy patients to whom low doses of sulfone are administered orally will not be under risk of showing chromosome aberrations induced by DDS. Nevertheless, taking into account that leprosy patients may receive larger doses of DDS and that considerable variation does exist in the mean plasma level of dapsone of different individuals receiving the same daily dose (4), it is advisable to conduct investigations on the types and frequency of chromosome aberrations in leprosy patients who are under treatment. The results of such research not being available, the data obtained in Sample 2 deserve some comments because of their possible heuristic value. Thus, it is widely accepted that drugs and viruses induce aneuploidies by depressing DNA and increasing the number of persisting nucleoli in dividing cells (5). However, this mechanism does not seem to have been followed by DDS for provoking numerical chromosome aberrations. As a matter of fact, neither a decreased rate of metaphases was detected (Table 2), nor were the acrocentric chromosomes (D and G groups), which are the nucleolus organizers (7), more frequently affected in the sulfone treated cultures.

Otherwise, little attention is usually paid to the achromatic gaps which are often found in normal cultures, with frequencies varying between 2.2% and 4.5% (6). Since these gaps reveal chromatin fibers linking their proximal and distal ends when examined under the electron microscope (3), they are considered to merely represent staining discontinuities of the chromatids (2). Though the findings in the DDS-treated cultures of Sample 2 have not thrown light on the nature of the achromatic gaps, they suggest that such chromosome abnormality is more likely to be a chromosome injury which deserves more attention in future studies.

SUMMARY

The proportion of accumulated metaphases, as well as the frequency of aneuploidies and structural chromosome aberrations were investigated in DDS-treated and untreated (controls) leukocyte cultures from two samples of healthy adult Caucasoid individuals. In one sample, the sulfone-treated cultures differed from the controls

in that they contained 0.4 μg of DDS per ml of tissue culture medium plus phytohemagglutinin. In the other sample, these cultures differed from the controls in that they contained a tenfold higher concentration of DDS.

The two concentrations of DDS used have not significantly affected the rate of metaphases, while 0.4 μg/ml DDS did not increase the frequency of chromosome aberrations. The same was not true for the cultures treated with 4 μg/ml DDS in which the proportion of aneuploidies and achromatic gaps increased significantly.

RESUMEN

Se estudió la proporción de metafases acumuladas, como también la frecuencia de aneuploides y aberraciones cromosómicas estructurales en cultivos de linfocitos tratados y no tratados (controles) con DDS, de dos muestras de individuos Caucasóides sanos. En una muestra, los cultivos tratados con sulfona diferían de los controles en que contenían 0,4 μg de DDS por ml de medio de cultivo de tejido, más fitohemaglutinina. En la otra muestra, estos cultivos diferían de los en que contenían una concentración de DDS diez veces mayor.

Las dos concentraciones de DDS utilizadas no afectaron en forma significativa la tasa de metafases, mientras que la concentración de 0,4 μg/ml de DDS no aumentó la frecuencia de aberraciones cromosómicas. Esto no fué así para los cultivos tratados con 4 μg/ml de DDS, en los cuales la proporción de aneuploides y hendiduras acromáticas aumentó significativamente.

RÉSUMÉ

On a procédé à une étude des cultures leucocytaires traitées par la DDS ou non traitées (témoins) et provenant de deux échantillons prélevés chez des adultes caucasiens en bonne santé. Le but de cette étude était de comparer la proportion de métaphases accumulées, ainsi que la fréquence d'aberrations chromosomiques structurelles et d'aneuploïdie dans les cultures traitées et dans les cultures témoins. Dans un échantillon, les cultures traitées par les sulfones se sont distinguées des cultures témoins par le fait qu'elles contenaient 0,4 μg de DDS par ml de milieux de culture tissulaire auquel on avait ajouté de la phyto-hémagglutinine. Dans l'autre échantillon, ces cultures différaient des cultures témoins par leur concentration en DDS, qui était dix fois plus élevée.

Les deux concentrations de DDS utilisées n'ont pas eu d'influence significative sur le taux de métaphases, alors qu'une concentration de DDS s'élevant a 0,4 μg/ml n'entraînait pas une augmentation de la fréquence des aberrations chromosomiques. Il en était autrement pour les cultures traitées par 4 μg/ml de DDS, dans lesquels la proportion d'aneuploïdie et les intervalles achromatiques se sont révélés significativement augmentés.

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