CHROMOSOMAL ABERRATIONS IN LEUKOCYTE METAPHASES OF LEPROSY PATIENTS UNDER DAPSONE THERAPY (*)

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SUMMARY — Chromosome analyses were made on leukocyte metaphases of 18 leprosy patients who were ingesting daily doses of 50 mg or 100 mg of DDS and of 40 healthy individuals used for control.

These analyses have shown that the proportion of numerical chromosomal aberrations in the leukocyte metaphases of the leprosy patients did not differ significantly from that observed in the cells of the controls. In contrast, the frequency of cells with chromatid or chromosome breaks and gaps was significantly increased in the leukocytes of leprosy patients.

Multiple regression analysis applied to the data recorded has shown that the increase of breaks and gaps in the chromosomes of leprosy patients can not be attributed to age, years under sulfonetherapy or to concentration of DDS in blood.

Termos índice: Hanseniase. Terapêutica. Dapsona. Genética. Análise cromossômica. Aberrações cromossômicas.

Key words: Hanseniasis. Therapy. Dapsone. Genetics. Chromosome analysis. Chromosomal aberrations.

It was previously observed that dapsone (4,4' diaminodiphenylsulfone or DDS) is able to increase the frequency of aneuploidies and achromatic gaps when added in a concentration of 4 sg/m1 to leukocyte cultures of healthy individuals.

Taking into account that levels of DDS higher than 4 µg/ml are frequently found in the blood of leprosy patients six hours after the ingestion of 100 mg DDS (2) it seemed important to investigate the frequency of chromosomal aberrations in leukocyte cultures of leprosy cases who are submitted to dapsone therapy.

(*) This investigation received financial support from the World Health Organization.
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MATERIAL AND METHODS

Chromosome analyses were made on metaphases obtained by culturing leukocytes of 18 Brazilian leprosy patients (7 lepromatous, 5 indeterminate and 6 tuberculoid cases) and 40 healthy Brazilian individuals used for control. All the leprosy cases (11 males and 7 females), aging between 23 and 45 years (mean = 35.3; s.d. = 5.94) were outpatients under dapsone therapy for periods ranging from 3 months to more than 12 years (mean = 4.8 years; s.d. = 3.42), who were ingesting daily doses of 50 mg or 100 mg DDS and not receiving corticoids, thalidomide, aspirin on any immunosuppressive therapy at the time of this investigation. The control sample was composed of 23 males and 17 females with ages varying from 16 to 61 years (mean = 24.6; s.d. = 8.01) who affirmed that they were not ingesting any drug at the time of this investigation.

Venous blood samples of 10 ml were collected in heparinized syringes from the leprosy patients 6 hours after the ingestion of their daily dose of sulfone, and allotted to two sterile tubes. One of these was destined for separation of the leukocyte-rich plasma for culturing these cells. The other served to determine the level of DDS in blood according to Simpson's method (8). A smaller sample of blood (5 ml) was collected from each healthy individual, since the investigation of the concentration of DDS in their blood was not needed.

The cultures were performed by incubating the plasma with the leukocytes for 72 hours at 37°C in sterile 100 ml prescription bottles containing 9 ml of tissue culture medium (60% Hanks BSS enriched with 0,5% lactalbumin hydrolisate, 20% fetal bovine serum, 20% ascitic fluid, 100 IU/ ml penicillin and 100 μg/ml streptomycin) plus 0.1 ml of phytohemagglutinin prepared in the authors' laboratory. The cell divisions were arrested in metaphase by adding 0.1 ml of colchicine (Houdé) 4 x 10^{-5}M to each bottle 2 hours before harvesting the cultures.

After transferring the contents of the bottles to tubes which were centrifuged for 5 minutes at 800 *rpm*, the hypotonic treatment was initiated by adding gently 4 ml of distilled water at 37°C to the sediment suspended in one ml of the supernatant. This 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 5 minutes at 800 *rpm*.

Fixation of the cells was achieved by resuspending the sediment in 4 ml of fixative and by maintaining the tubes in the refrigerator overnight. The tubes were centrifuged again for 5 minutes at 800 rpm and the sediment resuspended in 0.5 ml freshly prepared fixative for being distributed on frozen and moisted slides, which were rapidly flamed shortly afterwards. The chromosomes were stained with Giemsa's reagent for 10 minutes, after which the slides were rinsed in running tap water, air-dried and mounted.

The metaphases were selected under low power magnification on the basis of the quality of chromosome spreading and examined under high magnification, the

chromosomes being sketched with the help of a *camera lucida*. From each culture of the leprosy patients 30 metaphases were selected for chromosomal counts and 20 for the investigation of structural aberrations, while 10 leukocyte metaphases of each healthy individual were used for analysing both the numerical and the structural aberrations.

The structural abnormalities of the chromosomes were considered as chromatid lesions if only one of the two chromatids of a metaphase chromosome was affected and as chromosomal lesions if both chromatids of such a chromosome were injured in corresponding sites. Both the chromatid and the chromosomal lesions were subclassified as gaps or breaks according to the size of the discontinuity seen in the chromatids, as well as the position of the distal fragment. Thus, the discontinuities smaller than the width of one chromatid, that maintained the distal and the proximal ends in the same direction were considered as gaps. Otherwise, the discontinuities larger than the width of one chromatid and/or showing a change of the direction of the distal fragment were recorded as breaks. When two similar free chromatid fragments lacking a centromere were found in a cell, they were considered as chromosome lesions, but described as acentric fragments.

RESULTS

In table 1 there are assembled the data concerning sex, leprosy form, age, number of years under sulfone treatment and concentration of DDS in blood of the 18 leprosy patients studied, as well as a summary of the cytological findings in their leukocyte metaphases.

TABLE 1

Data recorded on the 18 leprosy patients studied

Patient	Sex	Leprosy form	Age	Years of treatment	DDS (μg/ml)	% of cells with Chromosomal aberrations	
						Numerical	Structural
A.G.	M	T	38	6.08	3.6	10.0	5.0
P.G.D.	M	T	39	5.17	1.6	—	5.0
G.S.	M	T	32	8.17	2.2	—	25.0
M.E.	M	I	27	0.92	0.0	—	10.0
J.T.R.	M	L	38	12.25	1.6	—	10.0
A.G.O.	M	I	30	1.67	1.0	3.3	25.0
O.M.	M	L	32	8.75	3.3	6.7	15.0
B.I.S.	F	L	30	10.92	2.9	—	25.0
A.T.	M	T	37	4.33	2.8	—	10.0
N.A.R.	F	I	45	3.00	3.2	—	10.0
M.L.O.	F	I	23	1.58	6.3	—	15.0
A.P.L.	F	L	44	5.75	4.3	13.3	20.0
E.A.C.	F	T	36	3.33	5.0	3.0	25.0
L.A.C.	M	L	38	3.58	3.3	3.3	0.0
G.P.S.	M	I	39	1.92	1.9	—	15.0
M.I.S.R.	F	I	38	3.08	4.6	—	10.0
A.M.S.	F	L	41	5.42	4.6	—	20.0
S.B.A.	M	T	29	0.08	5.1	3.3	5.0

The chromosomal counts on 540 leukocyte metaphases of these patients showed that 13 (2.4%) exhibited aneuploidies represented by 5 hypodiploid, 6 hyperdiploid and 2 endoreduplicated cells (three 45,XX, -C, two 45,XX,-E, two 47,XY,+C, one 47,XX,+C, one 47,XY,+D, two 47,XY,+G and two *end* 46,XY cells). The same counts performed on 400 metaphases of the controls showed that 4 (1%) disclosed chromosomal numerical aberrations (one 46,XY,-C, one 44,XY, -C, -C, one 47,XY,+G and one *end* 46,XY).

The types of structural aberrations seen in the leukocyte metaphases of both the leprosy and the healthy individuals are specified in table 2, while in table 3 the distributions of gaps and breaks according to the chromosome groups are compared.

TABLE 2

Structural chromosomal aberrations observed in leukocyte metaphases of 18 leprosy patients and 40 healthy individuals

Type of cells	Patients cells (N.° = 360)	Controls cells (N.° = 400)	
With chromatid lesions	37 (10.3%)	12 (3.0%)	(a)
One break	10	3	
Two breaks	1	—	
One gap	22	9	
Two gaps	2	—	
One break and one gap	2	—	
With chromosome lesions	13 (3.3%)	3 (0.8%)	(b)
One break	2	—	
Two acentric fragments	2	—	
One gap	6	3	
One gap and one chromatid gap	1	—	
One break and one gap	1	—	
One break and two acentric fragments	1	—	
Total n.° of cells with structural chromosome aberrations	50 (13.9%)	15 (3.8%)	(c)
Total n.° of cells with chromatid or chromosome breaks	19 (5.3%)	3 (0.8%)	(d)
Total n.° of cells with chromatid or chromosome gaps	35 (9.7%)	12 (3.0%)	(e)

Differences:

(a) $\chi^2_{(1)} = 15.454$; $P < 0.001$

(b) $\chi^2_{(1)} = 6.202$; $P < 0.05$

(c) $\chi^2_{(1)} = 23.624$; $P < 0.001$

(d) $\chi^2_{(1)} = 12.255$; $P < 0.001$

(e) $\chi^2_{(1)} = 13.622$; $P < 0.001$

TABLE 3

Distribution of gaps and breaks according to the affected chromosome groups of the leprosy patients

CHROMOSOME GROUP	GAPS	BREAKS	TOTAL N.º	TOTAL %
A	15	8	23	41.8
B	9	2	11	20.0
C	11	4	15	27.3
D	3	3	6	10.9
TOTAL	38	17	55	100.0

$$\chi^2_{(3)} = 2.044; \quad 0.50 < P < 0.70$$

The results of the multiple regression analysis applied to the data in table 1, in which the frequency of structural aberrations was considered as the dependant variable on sex, age, years under sulfone therapy and amount of DDS in blood are shown in table 4. For this analysis the data on the frequency of structural aberrations were transformed by using the square root of each value, to which 0.5 was previously added. Males were coded as 1 and females as 0.

TABLE 4

Results of the multiple regression analysis applied to the data of table 1, in which the proportion of metaphases with structural chromosomal aberrations was considered as the dependant variable.

Independant variable	Regression coefficient	Standard error	t-Ratio D.F. = 16	P
DDS (μg/ml)	— 0.293	0.205	— 1.429	> 0.10
Years of treatment	0.079	0.077	1.026	> 0.30
Age	— 0.070	0.044	— 1.591	> 0.10
Sex	— 1.759	0.666	— 2.641	< 0.05

DISCUSSION AND CONCLUSIONS

The chromosomal counts performed on the leukocyte metaphases of the individuals of both samples examined do not support the hypothesis that the frequency of chromosomal numerical aberrations is increased among leprosy patients. As a matter of fact, the proportions of aneuploid cells seen in these samples do not differ significantly ($X^2_{(1)} = 1.S32$; $0,10 < P < 0.20$) and are much lower than the average frequencies of aneuploidies usually found in leukocyte cultures of healthy individuals sampled from several populations (4, 5, 6, 10). Otherwise, taking into account the frequencies of aneuploid cells that are considered in the pertinent literature (6) as being normal variations of the chromosomal counts in leukocyte metaphases, it may also be

concluded that the proportion of aneuploidies in the cultures of the seven patients who presented cells with abnormal chromosomal counts (table 1) are compatible with the hypothesis that they occurred mostly as consequence of artifacts of cell culture.

Therefore, the present results do not confirm the hypothesis that the concentrations of DDS which are able to increase the frequency of aneuploidies in vitro have this effect in vivo. Here it seems important to stress that the levels of DDS listed in table 1 refer to the total blood of the patients. In their leukocyte cultures these amounts were diluted at least ten times, because each culture was prepared by adding to 9 ml of tissue culture medium about one ml of plasma free of erythrocytes, which may have a certain amount of DDS linked to them (2).

Concerning the structural chromosomal aberrations, it is seen in table 2 that, whatever type taken into consideration, the frequency of such abnormalities in the chromosomes of the leprosy patients was significantly higher than that found in the chromosomes of the healthy individuals. Among the latter, either the breaks or the gaps were below the limits accepted as being due to normal variations of cell culture, that is to say, up to 2% of breaks (4) and 4.5% of gaps (7).

Before going further it seems important to emphasize that several investigators consider the chromatid and chromosome gaps merely as staining discontinuities of the chromosomes (achromatic gaps) (4), while others, including the present authors (3), sustain that gaps should be considered as true chromosome aberrations. The data on tables 2 and 3 favor the latter because they confirm that gaps and breaks increase or decrease in parallel (9) and suggest that this parallelism involves the chromosomes that are affected by these abnormalities.

The increase of breaks and gaps in the leukocyte metaphases of the leprosy patients cannot be attributed to the treatment with DDS because, according to the figures in table 4, the proportion of these abnormalities neither is correlated to the concentration of DDS in blood, nor to the number of years under sulfonetherapy. In the same table it is also shown that structural chromosomal aberrations are not correlated to age, and that they are slightly increased among females, these data being in agreement with those of other authors (6).

Since DDS cannot be considered as responsible for the increase of structural chromosomal aberrations *in vivo*, it could be supposed that the high frequency of such abnormalities seen in the leukocyte metaphases of leprosy patients is a consequence of leprosy itself. Nevertheless, the possibility that conditions closely related to this disease or to its treatment are responsible for the increase of structural aberrations in the chromosomes of leprosy patients cannot be excluded before further investigation.

At the moment it is also too early to advance speculations on the significance of the excess of structural chromosomal aberrations in the leukocytes of the leprosy patients from the individual viewpoint, because it is not known whether most of the chromatid and chromosomal lesions observed

are naturally repaired or not. The same is not true concerning the possible genetical implications of these aberrations, in spite of not knowing whether or not the chromosomes of the germinative cells are affected in the same intensity as those of the leukocytes.

As a matter of fact, if structural chromosomal aberrations were increased in the germinative cells of leprosy patients, a high frequency of spontaneous abortions would be expected among couples composed of, at least, one leprosy individual, because most of the zygotes with these abnormalities are not able to develop viable embryos. However, in a previous paper (1) it was pointed out that the frequency of spontaneous abortions among couples composed by lepromatous women married to healthy or to lepromatous men was similar to that found in couples of the general population.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. H. Sansarricq, Chief Medical Officer, Leprosy, from the Division of Communicable Diseases of the World Health Organization, for the critical review of the manuscript.

RESUMO

ABERRAÇÕES CROMOSSôMICAS EM METÁFASES DE LEUCóCITOS DE PACIENTES DE LEPRA SOB TERAPIA COM DAPSONA

As metáfases obtidas por cultura de leucócitos de 18 doentes de lepra, que ingeriam doses diárias de 50 mg a 100 mg de DDS, e de 40 indivíduos sadios tomados para *controle foram submetidas a estudos cromossômicos.*

Nessas análises verificou-se que a proporção de aberrações numéricas dos cromossomos nas metáfases dos doentes de lepra não *diferiu significativamente da observada nas células dos controles.*

Em oposição, a freqüência de metáfases com quebras e falhas cromatídicas e cromossômicas foi significativamente mais alta nos leucócitos dos doentes de lepra.

A análise dos dados estudados por regressão múltipla permitiu constatar que o aumento das quebras e falhas cromossômicas nas metáfases dos doentes de lepra não *pode ser atribuído à idade, aos anos sob sulfonoterapia, nem aos níveis sangüíneos de DDS.*

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Received for publication 20 August 1975.