Cytogenetic Effect of Dapsone, an Antileprotic Drug, in the Mouse *in Vivo* System[1]

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Dapsone (4',4'-diaminodiphenylsulfone), commonly known as DDS, was the first drug to be introduced in the treatment of leprosy in the early 1940s, and it is still the first drug of choice in the triple-drug regimen recommended by the World Health Organization (WHO) [27]. In the case of monotherapy DDS is the only choice. It is the most potent sulfone, having a strong bacteriostatic and a mild bactericidal effect. Dapsone is prescribed for regular use for a prolonged period—a minimum of 2 years; sometimes continued for 10–15 years. The production units for the drug in India, having a specific production quota, can hardly meet the country's total requirement which exceeds 50 metric tons per year [15]; this gives some idea of the large-scale use of the drug.

Most epidemiological studies [6, 17, 24] have shown that the incidence of cancer in leprosy patients is no higher than that in leprosy-free individuals. But epidemiological studies related particularly to dapsone therapy are extremely limited. One report [11] notes that malignant tumors have occurred more frequently in leprosy patients since chemotherapy was introduced. In another report [21], based on histopathological examinations of skin biopsies, a higher incidence of skin cancer was noted in leprosy patients, particularly of the lepromatous type. Bergel [5] was the first to demonstrate the induction of cancer in 8 of 13 male Wistar rats which received progressively higher levels of DDS (0.025%–0.3%) in the diet for 17 to 25 months. Subsequently, his finding has been confirmed in rodents by other investigators [12, 26]. The literature also provides a number of negative reports in rodents [12]. The association of cancer with chromosome aberrations is well established. But to date not much work has been done on the possible genotoxic effect of DDS. Beiguelman and his associates [3, 4, 13] have reported higher frequencies of chromosome aberrations in peripheral lymphocytes and skin fibroblasts of patients under DDS therapy. However, the Ames *Salmonella*/microsome assay system has revealed no mutagenic effect of the drug [23].

In view of the large scale use of DDS regularly for long periods, its carcinogenic potency identified recently in rodents, and the conflicting reports on its potential genotoxicity, the present study was undertaken to evaluate further its clastogenic capacity in mice using three *in vivo* assay systems: a) bone-marrow metaphase analysis, b) bone marrow micronucleus test (MNT), and c) spermatocyte chromosome analysis. The analysis of chromosomes in mitotic as well as meiotic cells as a test system for recognizing genotoxicity is well established. The MNT involves scoring of the micronucleus (MN) which results from lagging acentric(s) and/or whole chromosome(s) in mammalian anucleated poly- and normochromatic bone-marrow erythrocytes [25]. Polychromatic erythrocytes (PCEs) are the young anucleate erythrocytes which result from erythroblasts through erythroblastosis and differentiate into normochromatic erythrocytes (NCEs) after about 24 hours.

MATERIALS AND METHODS

Inbred Swiss mice 10–12 weeks of age (at the time of starting treatment) having a body weight of 25–30 g were used for this study.

A base-free sample of dapsone (Batch no. 36065) was a gift from Burroughs Wellcome (India) Ltd. In the entire experiment the drug was administered to the experimental animals as an aqueous suspension via the oral route by gastric intubation. For the dose-response study, the dilution of the suspen-

sion was adjusted and the volume of the suspension received by each animal was kept constant (0.25 ml). The suspension was prepared every day for all the animals to be treated that day by mixing the required amount of the micropowdered drug with the required amount of water and shaking vigorously. Before every use the suspension was shaken thoroughly. Since the drug is recommended for long-term use, only the repeated treatment regimen was followed in our experiment.

A DDS dose of 40 /mg/kg/day was fed to a group of adult males for 2, 4, or 8 weeks, and the animals were killed 24 hr after the last dose was administered. This regimen was referred to as a time-response analysis for convenience. In another set, some males were fed a DDS dose of 20 or 80 mg/kg/day for 4 weeks, and killed 24 hr after the last treatment. Thus, for week 4, three dose levels were used and this comparison was referred to as a dose-response study.

As the treated mice were receiving the drug with water as a suspension week by week, age-matched males which were untreated served as controls. The mice were treated intraperitoneally with 4 mg/kg of colchicine 1½ hr before sacrifice. The bone marrow and testes were collected from each of the control and treated animals for analyses of bone marrow and spermatocyte chromosomes, respectively. Thus, materials for both mitotic and meiotic chromosome analyses were provided by the same animals. Cytological preparations of the bone marrow and the spermatocyte chromosomes were made following the conventional hypotonic-flame-drying-Giemsa method.

For the bone marrow micronucleus test (MNT) mice of both sexes (two males and two females for each dose level) were employed and the dose-response study with three different doses (20, 40, and 80 mg/kg/day) was conducted. The animals were treated with the drug in the same way, i.e., via the oral route, for 15 consecutive days. They were killed 24 hr after the last treatment, and their bone-marrow cells were processed for a micronucleus preparation following the technique developed by us ([7]) with certain modifications. Age- and sex-matched untreated mice were used as controls.

TABLE 1. *Incidence of structural changes of chromosomes induced in bone-marrow cells of mice treated with dapsone.*

Dose (mg/kg/d)	Period of treatment (weeks)	Cells scored/ no. mice	Break-type aberrations						Gaps	Total aberr. including gaps (% ± S.E.)
			Chromatid	Isochromatid[a]	Fragment of uncertain origin	Ring	Exchange[a]	Total (% ± S.E.)		
Controls	—	1200/12	4	—	3	—	—	0.58 ± 0.18	15	1.83 ± 0.25
20	4	400/4	7	—	1	—	—	2.25 ± 0.25[b]	6	4.00 ± 0.61[c]
40	2	400/4	2	—	3	—	—	1.75 ± 0.22[c]	5	3.00 ± 0.35[d]
	4	400/4	8	1	8	—	—	4.00 ± 0.61[b]	21	9.25 ± 2.27[c]
	8	400/4	4	2	2	—	—	2.00 ± 0.35[c]	8	4.00 ± 0.00[b]
80	4	400/4	6	2	4	—	—	4.00 ± 0.00[b]	20	9.00 ± 0.79[b]
						For dose		r = + 0.876		r = + 0.865
						For treatment period		r = + 0.088		r = + 0.04

[a] Each of the isochromatid breaks and exchanges was counted as two breaks.
[b] p < 0.001, significantly higher than control value (*t* test).
[c] p < 0.01, significantly higher than control value (*t* test).
[d] p < 0.05, significantly higher than control value (*t* test).

TABLE 2. *Results of micronucleus (MN) test in bone-marrow cells of mice treated with dapsone.*[a]

Dose (mg/kg/d)	No. mice	PCE with MN	NCE with MN	PCE + NCE with MN	PCE/NCE ratio	% dividing cells (mitotic index)
Controls	6	0.21 ± 0.02	0.01 ± 0.01	0.11 ± 0.01	0.92 ± 0.06	0.49 ± 0.03
20	4	0.50 ± 0.06[c]	0.30 ± 0.03[b]	0.40 ± 0.03[b]	0.97 ± 0.02	0.45 ± 0.06
40	4	0.47 ± 0.04[b]	0.20 ± 0.03[b]	0.33 ± 0.03[b]	1.20 ± 0.07[d]	0.37 ± 0.03
80	4	0.50 ± 0.06[c]	0.42 ± 0.04[b]	0.46 ± 0.01[b]	1.09 ± 0.04[d]	0.36 ± 0.07
		r = + 0.704	r = + 0.847	r = + 0.812	r = + 0.638	

[a] Values are mean per 100 cells ± S.E.; 1000 polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored from each individual.

[b] $p < 0.001$, significantly higher than control value (t test).

[c] $p < 0.01$, significantly higher than control value (t test).

[d] $p < 0.05$, significantly higher than control value (t test).

RESULTS

Numerical changes of chromosomes were not marked. The structural chromosome aberrations encountered in bone-marrow metaphases were categorized under two main heads: gaps and break-type aberrations. Because of the uncertain significance of the gaps, a statistical evaluation was done separately for break-type aberrations, and for gap and break-type aberrations taken together (referred to here as "total aberrations"). Table 1 summarizes the frequencies and kinds of chromosome aberrations in bone-marrow metaphases. Since the analyses of variance did not reveal any statistical differences among the control values for different weeks (for break-type aberrations F = 6.50, and for total aberrations F = 3.03), they were pooled and data from all the treated mice were compared with the pooled control values. The incidences of break-type aberrations showed significant increases over the control values for all of the treatment periods and for all of the doses tested. The same tendency was also noted for total aberrations. The analyses of correlation coefficients showed a significant positive correlation of these increases with the increasing dose levels but not with the period of treatment.

The dose-response study revealed significantly elevated frequencies of micronuclei (MN) compared to controls in the polychromatic erythrocytes (PCEs) as well as normochromatic erythrocytes (NCEs), taken separately or together, for all dose levels tested (Table 2). However, the frequencies failed to show any statistical correlation with the doses (analysis of correlation coefficient).

Again, for spermatocyte chromosome analysis, since the control values for break-type aberrations obtained in different weeks fluctuated within a very narrow range (0.50%–0.66%), the treated values were compared with the pooled control mean (0.54%) (Table 3). Significantly higher incidences of breaks were noted only for the highest dose (80 mg/kg/day for 4 weeks) and for the longest period of treatment (40 mg/kg/day for 8 weeks). The incidence of univalent formation exceeded the control limit only with the highest dose (80 mg/kg/day) for autosomes and sex chromosomes, taken separately or together.

DISCUSSION

Significantly higher incidences of chromosome aberrations, including as well as excluding gaps, noted in bone-marrow metaphases for all of the treatment periods and for all of the doses clearly revealed the clastogenic capacity of DDS, although the changes were not severe. Our results are in very good agreement with those of Beiguelman and his co-workers. They have earlier demonstrated not only the positive clastogenic effect of the drug but also the absence of correlation between the period of DDS treatment and the aberration yield from metaphase chromosome analyses in peripheral blood lymphocytes ([4]) and skin fibroblasts ([13]) of leprosy patients under DDS therapy. Our data on the dose-response analysis of bone-marrow metaphase chromosomes also corroborate the results ob-

tained in human peripheral blood lymphocytes exposed to the drug *in vitro* ([3]).

The MNT has been proven to be comparable to, or even more sensitive and reliable than, direct metaphase analysis in the screening of environmental agents for potential mutagenicity ([14]). Both clastogens and spindle poisons are known to induce MN ([8, 25]). But the absence of any influence of the drug on the percent of dividing cells (mitotic index) and the occurrence of MN of small size indicate a lack of a spindle-poisoning effect of the drug. The high incidence of MN in the treated series may, therefore, be attributed to the clastogenic effect of the drug. A slight increase of the PCE/NCE ratio, particularly at higher doses, is very likely due to the hemolytic activity of DDS ([19]), which is more marked on NCEs than on PCEs.

In only two instances—with the highest dose and with the longest period of treatment—did spermatocyte chromosomes show significantly higher frequencies of aberrations. In both of these cases, the cells scored were expected to remain at the spermatogonia stem cell stage at the time of starting the treatment ([22]). So, in view of this significant effect, the possible susceptibility of stem cells cannot be ruled out. However, we have certain reservations in considering this possibility, because very few chemicals are known to induce cytogenetic effects in stem cells that can be measured in meitoic cells ([2]). The less-frequent occurrence of chromosome aberrations in spermatocytes compared to chromosome aberrations in bone-marrow metaphases may be explained along this line. The drug seems to have little effect on the pairing behavior of the chromosomes as judged by the univalent formation, the incidence of which increased slightly with the highest dose following chronic treatment for 4 weeks.

Although it is clearly evident from earlier studies ([3, 4, 13]) and our present study that DDS induces chromosome damage in the eukaryotic system, the Ames *Salmonella/* microsome assay failed to show any mutagenic effect of the drug or a number of its metabolites and derivatives; only two of its derivatives—the sulfoxide (DDSO) and sulfide (DDSD) analogs—showed a positive response in the presence of liver microsomes

TABLE 3. *Incidence of structural changes of chromosomes and univalent formation induced in spermatocytes of mice treated with dapsone.*

Dose (mg/kg/d)	Period of treatment (weeks)	Cells scored/ no. mice	Break-type aberrations				Total (% ± S.E.)	Univalent formation		
			Chromosome	Chromatid	Fragment	Exchange		Auto.	X–Y	Total
Controls	—	550/11	—	3	—	—	0.54 ± 0.26	4.00 ± 0.99	3.27 ± 0.90	7.27 ± 1.53
20	4	200/4	1	3	—	—	2.00 ± 0.70	5.00 ± 1.66	5.00 ± 1.12	10.00 ± 2.55
40	2	200/4	—	3	1	—	2.00 ± 0.71	3.00 ± 1.50	4.00 ± 1.00	7.00 ± 2.29
	4	200/4	1	2	—	—	1.50 ± 0.43	6.50 ± 2.16	8.50 ± 2.26	15.00 ± 3.32
	8	200/4	1	4	—	—	2.50 ± 0.43[a]	6.50 ± 1.78	3.50 ± 0.43	10.00 ± 1.41
80	4	250/5	—	6	—	—	2.80 ± 0.97[b]	11.00 ± 2.29[b]	8.00 ± 1.22[a]	19.00 ± 3.20[a]

[a] $p < 0.01$, significantly higher than control value (t test).
[b] $p < 0.05$, significantly higher than control value (t test).

([23]). That DDS is nonmutagenic was also reported earlier ([18]) from an identical experiment. The sensitivity and reliability of the Ames *Salmonella* test for the detection of mutagenicity and/or carcinogenicity is well established ([20]). Most of the chemicals that have a positive clastogenic effect in eukaryotes, *in vivo* or *in vitro,* have been shown to be mutagenic in the *Salmonella* test system. However, such types of differential results, i.e., a positive response in a eukaryotic chromosome analysis and a negative response in the Ames bacterial assay, are not uncommon in the field of chemical mutagenesis. In this context, we can refer to the cases of procarbazine, hexamethylphosphoramide, isoniazid, etc., which induce positive effects in several eukaryotic systems but not in the Ames test ([1, 9, 16]).

With regard to dose, if one calculates on a per kg basis, the lowest dose tested by us (20 mg/kg/day) would be 10 times the human therapeutic dose recommended by WHO ([27]). But if it is calculated on the basis of the surface area of the individual, as usually done for most drugs and chemicals, the equivalent mouse dose would be 12 times the human dose ([10]) on a per kg basis; the lowest dose tested would then be close to the human dose.

Further studies using other *in vivo* mammalian test systems are needed to confirm the genotoxic capacity of dapsone.

SUMMARY

Potential genotoxicity of dapsone was evaluated in mice following *in vivo* cytogenetic assays. Adult male mice treated with different doses (20 mg, 40 mg, or 80 mg/kg/day for 4 weeks) and for different periods (40 mg/kg/day for 2, 4, or 8 weeks) provided bone marrow and testes for mitotic and meiotic chromosome analyses, respectively. A dose-response (20 mg, 40 mg, or 80 mg/kg/day for 2 weeks) analysis was done with a separate set of mice using a micronucleus test (MNT). Untreated mice served as controls. Both the metaphase analysis and MNT in bone-marrow cells revealed significantly higher incidences of clastogenicity for all of the dose levels and treatment periods. Chromosome aberrations, with and without gaps, in bone-marrow metaphases showed a positive correlation with the doses, but not with the treatment periods. Correlation was also lacking in the MNT. In the meiotic cells, the incidences of chromosome aberrations increased significantly with the highest dose and with the longest period of treatment.

RESUMEN

Se evaluó la genotoxicidad potencial de la dapsona en ratones efectuando estudios citogenéticos in vivo. Los ratones adultos, machos, tratados con diferentes dosis (20 mg, 40 mg, u 80 mg/kg/día, por 4 semanas) y por diferentes periodos (40 mg/kg/día, por 2, 4, u 8 semanas) fueron la fuente de médula ósea y de testículos para los análisis de cromosomas mitóticos y meióticos respectivamente. En un grupo separado de ratones se hizo un análisis de dosis-respuesta (20 mg, 40 mg, u 80 mg/kg/día) usando una prueba de micronúcleo. Como controles se usaron ratones no tratados. Tanto el análisis de metafase como la prueba de micronúcleo en las células de médula ósea revelaron incidencias de clastogenicidad significativamente más altas para todas las dosis y todos los periodos de tratamiento. Las aberraciones cromosómicas, con y sin huecos, en metafases en médula ósea mostraron una correlación positiva con la dosis pero no con los periodos de tratamiento. Tampoco hubo correlación en la prueba de micronúcleo. En las células meióticas, la incidencia de aberraciones cromosómicas aumentó significativamente con la dosis más alta de dapsona y con el tiempo más prolongado de tratamiento.

RÉSUMÉ

On a évalué la génotoxicité potentielle de la dapsone chez la souris au moyen d'épreuves cytogénétiques in vivo. Des souris adultes mâles ont été traitées par différentes doses de sulfone (20 mg, 40 mg, ou 80 mg/kg/jour pendant 4 semaines) et ceci pendant des périodes différentes (40 mg/kg/jour pendant 2, 4, ou 8 semaines). Des échantillons ont été prélevés au niveau de la moëlle osseuse et des testicules et soumis respectivement à des analyses chromosomiques mitotiques et méiotiques. Une analyse de la réponse en fonction de la dose (20 mg, 40 mg ou 80 mg/kg/jour pendant 2 semaines) a été pratiquée sur un groupe séparé de souris en utilisant une épreuve micronucléaire (MNT). Des souris non traitées ont servi de témoins. Pour toutes les doses utilisées, et quelles qu'aient été les durées de traitement, tant l'analyse en métaphase que l'épreuve MNT sur les cellules de moëlle osseuse ont révélé une incidence significativement plus élevée de clastogénicité. Les abérrations chromosomiques, avec ou sans délétion, dans les cellules de moëlle osseuse en métaphase, ont montré une corrélation positive avec les doses mais non avec la durée de traitement. Cette corrélation manquait également pour l'épreuve MNT. Dans les cellules méiotiques, l'incidence d'abérrations chromosomiques a montré une augmentation significative à la dose la plus élevée et après la durée la plus longue de traitement.

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