In vivo Effect of DDS on Phytohemagglutinin (PHA)-Induced Lymphocyte Transformation Cultures in Normal Healthy Volunteers[1]

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Unlike chemotherapeutic drugs which are used against tuberculosis, 4-4'diaminodiphenylsulfone (DDS) takes a long time to cure leprosy patients. Even in cases of tuberculoid leprosy, where the immune system of the host is not much disturbed ([2, 3, 5, 9]), the drug has to be administered for years. The immune system of the host is unable to overcome the disease quickly in spite of this antileprotic drug therapy. One possibility that could be considered regarding the slow therapeutic effect of DDS is that the drug might be causing a side-effect of depressing the host's immune system while it is exerting a bacteriostatic effect on *Mycobacterium leprae*.

Beiguelman and Pisani ([1]) have noted that DDS at certain concentrations, when added directly to *in vitro* cultures, depresses PHA-induced lymphocyte transformation. Direct addition of DDS to *in vitro* lymphocyte cultures obviously may not adequately reflect the *in vivo* situation when DDS is administered clinically by mouth, is absorbed by the biological system, is metabolized, etc.

Therefore an attempt has been made in the present study to explore the possibility of DDS producing an immuno-depressive effect after oral administration to normal humans. PHA-induced lymphocyte transformation is due to a selective stimulation of T cells *in vitro* ([6]). The present study has been conducted on the peripheral blood lymphocytes of volunteers who were administered DDS.

MATERIALS AND METHODS

Volunteers. Fifteen healthy volunteers from the staff members of the Central JALMA Institute for Leprosy at Agra participated in the study. PHA-induced lymphocyte blast transformation studies were performed on the blood of all volunteers before they received DDS. DDS was then administered orally to each volunteer at the dosage of 100 mg/day for seven days, after which PHA-induced lymphocyte blast transformation studies were repeated. Simultaneously, blood DDS concentrations were also determined in each individual by the method of Shepard *et al* ([8]). After clearance of DDS from blood, the PHA-induced lymphocyte blast transformation was again determined in these volunteers.

Lymphocyte transformation. Essentially, the method of Godal *et al* ([4]) was followed. In brief, leukocyte rich plasma was obtained by unit gravity sedimentation from venous blood. The leukocyte rich plasma was layered on Lymphoprep® (Nyegaard, Oslo, Norway) and centrifuged for purification of mononuclear cells (lymphocytes and monocytes). The purified mononuclear cells ($2x10^6$/ml) were cultured in test tubes in 2 ml volumes in TC 199 medium containing 25% autologous plasma. The cultures were always set up in duplicate, and 0.02 ml of PHA (Difco Laboratories, USA) was added per 10^6 cells. The cultures were performed in flat-bottomed culture tubes (5 ml capacity) and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After 72 hours of incubation, the cultures were harvested by centrifugation, and the cells were stained with acridine orange. The transformed cells were counted according to the criteria described by Lamvik ([7]). The results were expressed as percent blast cells in the PHA-stimulated cultures after deducting the figures of percent transformation in control cultures (without PHA).

Purification of lymphocytes and estimation of DDS in them. Carbonyl iron powder (Fluka AG, Switzerland) was sprinkled on 10 ml samples of heparinized whole blood, and every five minutes for one hour the sample was shaken to insure that all phagocytic cells phagocytized the carbonyl iron particles. The sample was

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then placed on a magnet, and after an hour the plasma layer was removed and layered on Lymphoprep®. After centrifugation, the lymphocyte layer thus obtained was washed and suspended in 2 ml of saline and a total cell count made. This saline suspension of lymphocytes was then sonicated in an ultrasonicator (Model UR-200P, Tomy Seiko Co., Ltd., Japan). DDS estimation in this sample was performed with a spectrophotofluorometer (Aminco-Bowmann, USA).

RESULTS

After seven days of DDS administration (100 mg/day) the results of blast transformations with PHA were compared with their base levels of lymphocyte transformation (Table 1). The DDS regimen significantly reduced the blast transformation in these normal individuals ($p<0.02$). After clearance of DDS from the individuals, PHA-induced lymphocyte transformation was again estimated, and the results

TABLE 1. *PHA-induced lymphocyte transformation in peripheral blood.*

Serial number	Percent blast cell before DDS administration (a)	Percent blast cell after DDS administration (100 mg/day for 7 days) (b)	Percent blast cell after DDS clearance from blood (c)
1	46	11	37
2	35	9	43
3	54	19	38
4	55	15	20
5	42	14	27
6	40	17	27
7	52	42	53
8	52	18	50
9	20	10	16
10	53	40	55
11	20	14	21
12	42	32	ND
13	47	23	57
14	35	17	ND
15	49	21	ND
Mean ± S.D.	42.8 ± 11.3	20.1 ± 10.2	37.0 ± 14.7

p value between (a) and (b) < 0.02.
p value between (a) and (c) > 0.05.
ND = not done.

TABLE 2. *PHA-induced lymphocyte transformation in peripheral blood in volunteers after administration of 100 mg DDS/day for seven days.*

Serial number	Percent blast cell with autologous plasma (a)	Percent blast cell with AB serum (b)
1	15	16
2	16	14
3	26	37
4	20	19
5	13	16
6	30	23
Mean ± S.D.	20.0 ± 6.7	20.8 ± 8.5

p value between (a) and (b) is > 0.05.

were compared with the base levels (Table 1). It is observed that the percent blast cells came back up to normal levels ($p > 0.05$).

To find out whether this change in blast cell transformation has any correlation with the DDS levels in blood, a correlation coefficient (r) was calculated. No correlation was observed between the alteration in percent blast cells and the blood levels of DDS (Fig. 1).

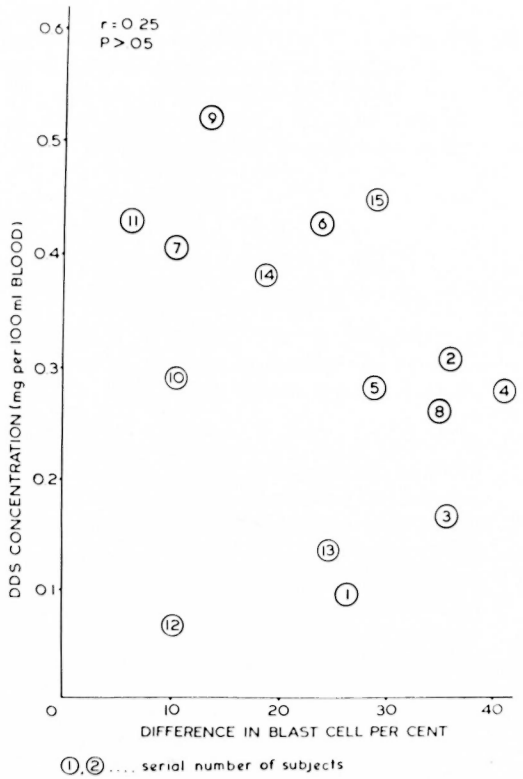

FIG. 1. Scattergram of the relationship between blood DDS concentration and difference in blast cell percent.

In another experiment the lymphocytes of the volunteers who had received 100 mg/day of DDS for seven days were cultured in autologous serum and also in AB serum obtained from a healthy donor who was not taking sulfonamides or sulfones. It is noted in Table 2 that normal AB serum treatment did not increase the levels of blast cells when stimulated by PHA. This indicated that DDS when administered *in vivo* has in some way affected the lymphocytes so that the cells are unable to undergo blastogenesis when stimulated by PHA.

At this stage, it may be speculated that DDS *in vivo* may combine with some proteins and consequently inhibit the pathway for PHA to act on lymphocytes, or DDS in some way may be blocking DNA synthesis. To understand whether DDS is associated with the lymphocyte *in vivo*, DDS was estimated in purified lymphocytes in seven individuals. It is noted that the lymphocytes contained a significant amount of DDS (Table 3).

DISCUSSION

The present study indicated that oral administration of DDS at the dosage of 100 mg per day for seven days significantly reduced normal PHA-induced blast cell transformation. Beiguelman and Pisani [1] conducted an *in vitro* study and noted that with an increase in DDS concentration in cultures, there was a decrease in percent blast cell transformation. However, these authors added DDS directly to the *in vitro* cultures of normal lymphocytes. This may not represent the situation *in vivo* after oral administration of DDS. In our study, lymphocyte blastogenesis with PHA was assessed after administration of DDS to healthy volunteers. In the *in vivo* situation

TABLE 3. *DDS concentrations in lymphocytes and plasma after seven days of DDS administration (100 mg/day).*

Serial number	$\mu g/10^6$ lymphocytes	μg/ml of plasma
1	1.4	4.4
2	0.4	4.2
3	0.4	5.1
4	1.8	4.4
5	3.2	3.2
6	0.5	1.5
7	1.1	6.9
Mean ± S.D.	1.26 ± 1.01	4.24 ± 1.66

DDS may combine with different tissue proteins, and the PHA-induced lymphocyte transformation may be quite different from what is observed in the *in vitro* situation. Further, this study, unlike that of Beiguelman and Pisani, failed to show any correlation between the DDS levels in blood and the PHA-induced lymphocyte transformation. However, since at present only one dose level of DDS (100 mg/day) has been tried, no conclusion can be drawn regarding the alterations in lymphocyte transformation to PHA at various dose levels of DDS *in vivo*.

With PHA there is a selective stimulation of T cells ([6]). It might be possible that DDS *in vivo*, after conjugating with some tissue proteins, is either blocking the receptor for PHA or inhibiting the synthesis of DNA. Although in the present study DDS has been found in the blood lymphocyte at different concentrations, it is not possible to indicate the exact site of conjugation of DDS in the lymphocytes. Further work along these lines is in progress.

SUMMARY

Depression in PHA-induced lymphocyte transformation in peripheral blood has been observed in 15 healthy volunteers after administration of DDS (100 mg/day) for seven days. *In vitro* culture of lymphocytes obtained from these volunteers in DDS-free normal AB serum has not altered the blast cell numbers. Lymphocytes of these volunteers have been found to contain a significant amount of DDS, ranging from 0.42 to 3.2 μg per 10.6 lymphocytes.

RESUMEN

Se estudió el grado de transformación de los linfocitos de sangre periférica inducido con fitohemaglutinina (FHA) en 15 voluntarios sanos que habian recibido 100 mg diarios de DDS durante 7 dias. Se observó una depresión en la respuesta. El cultivo *in vitro* de los linfocitos obtenidos de estos voluntarios en presencia de suero AB libre de DDS, no modificó el número de células blastoides. Se encontró, además, que los linfocitos de estos voluntarios contenian una cantidad significativa de DDS que osciló entre 0.42 y 3.2 μg por 10^6 células.

RÉSUMÉ

Chez 15 volontaires en bonne santé, on a observé, après administration de DDS à la dose de 100 mg par jour pendant 7 jours, un abaissement dans la transformation lymphocytaire induite par la phytohémagglutinine (PHA) dans le sang périphérique. La culture, dans du sérum AB sans DDS, des lymphocytes recueillis chez ces volontaires, n'a pas modifié le nombre de cellules blastiques. On a constaté que les lymphocytes des ces volontaires contenaient une quantité significative de DDS, variant de 0,42 à 3,2 μg par millions de lymphocytes.

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