

IN VITRO EFFECT OF DDS ON PHYTOHAEMAGGLUTININ (PHA)— INDUCED LYMPHOCYTE TRANSFORMATION

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ABSTRACT — A study to find out the **in vitro** effect of diamino diphenyl sulphone (DDS) on phytohaemagglutinin (PHA)-induced lymphocyte transformation was carried out in three phases using a wide range of DDS concentrations. Lymphocytes from healthy volunteers were investigated. Volunteers were divided into three groups to conduct the study in three phases. In each phase in addition to 0.02 ml of PHA, 4 different concentrations of DDS were added per 10^6 lymphocytes in tissue culture system.

A statistically significant depression ($P < 0.05$) in the per cent of blast cell formation induced by PHA was observed in the cultures with all the concentrations of DDS except with the lowest concentration (0.01 μg) of DDS. While the depression observed in the first and the third phase was found to be dose dependent, no significant correlation was noted between the DDS concentration and depression in PHA induced blastogenesis in the second phase. The significance of these observations are discussed.

Key words: Diamino diphenyl sulphone. Phytohaemagglutinin. Lymphocyte transformation test.

1 INTRODUCTION

In recent times there has been accumulation of a great deal of references suggesting that sulphone may have some immunosuppressive effect in addition to the anti-bacterial effect. From our laboratory SENGUPTA *et al.*, (1979) demonstrated that diamino-diphenyl sulphone (DDS) when administered at a dosage of 100 mg daily for 7 days in volunteers, the lympho-

cyte blastogenesis to PHA was significantly lowered. Further these authors showed that after administration of DDS in volunteers, a significant amount of the drug could be detected in the lymphocytes. Therefore, one could possibly speculate that this depression in PHA-induced blastogenesis might be due to the blocking of PHA-lymphocyte-receptor sites by the drug. If the above hypothesis is correct then DDS should not necessarily depress

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any other cell-mediated immune reaction (like in *in vivo* skin delayed hypersensitivity response to lepromin) except PHA-blastogenesis of lymphocytes. However, RAMU *et al.*, (1980) showed that DDS at 100 mg/day dosage continued for a month when administered in tuberculoid and borderline-tuberculoid leprosy patients, could suppress the lepromin skin reaction up to a great extent. Conversely after a month of administration of acedapson, lepromin reactions in the patients were significantly stimulated. The present study was, therefore, undertaken to find out the effect of DDS *in vitro* at various dose levels on PHA-induced blast cell transformation. Although such a study has already been conducted by BEIGUELMAN & PISANI (1974), these authors worked only within a limited high dose ranges of DDS (0.4 μg to 16 μg) in which they noted depression in PHA induced blastogenesis at all concentrations. However, the present study was carried out in several phases within a wide range of DDS concentration (0.01 μg to 15 μg) to find out the end point of DDS dilutions having depressive effect on the PHA-induced blastogenesis of lymphocytes obtained from healthy volunteers.

2 MATERIALS AND METHODS

Volunteers: Normal adult volunteers who were not taking any drug at the time of investigation were selected for the present study. The study was carried out in three phases. Fifteen, 14 and 15 volunteers were selected for first, second and third phases of investigations respectively.

Lymphocyte Culture: From each volunteer 15 ml of venous blood was drawn in preservative-free heparin (10 units/ml of blood). Lymphocytes were purified by differential centrifu-

gation on ficoll-isopaque (sp. gr. 1.077) using the method of BOYUM (1968). The technique employed for lymphocyte culture has been described in an earlier report SENGUPTA *et al.*, (1979). In brief, the cultures were performed at cell concentration of $2 \times 10^6/\text{ml}$ in 2 ml volumes in TC 199 (Centron, Bombay, India) containing 25% autologous plasma in tissue culture tubes. The cultures were always performed in duplicate and 0.02 ml of PHA (Difco Laboratories, USA) was added per million cells per culture.

In each phase of investigation, in addition to PHA, four different concentrations of DDS (Wellcome Laboratories) were added as follows: in the first phase 15 μg , 10 μg , 5 μg and 2.5 μg DDS/culture; in the second phase 1.0 μg , 0.5 μg , 0.25 μg and 0.1 μg DDS/culture; and in the third phase 0.075 μg , 0.05 μg , 0.025 μg and 0.01 μg DDS/culture. Control cultures were set without PHA and DDS and with PHA alone. The cultures were incubated at 37°C in a humidified atmosphere with 5% CO₂ and 95% air mixture. After 72 hours of incubation the cultures were harvested. The blast cells were finally counted under a fluorescent microscope (Model No. FM 200, Tiyoda, Tokyo) after staining with acridine orange using the criteria of LAMVIK (1968). The results were expressed as per cent blast cells after deducting the figures of the number of blast cells in control cultures.

The data on blast cell transformations obtained from lymphocyte cultures with PHA alone and with PHA and DDS together of all the three phases of investigations are detailed in tables 1, 2 and 3 respectively.

From the observations obtained in the first phase of experiment it is noticed that normal blastogenesis of lympho-

cytes by PHA was significantly suppressed by the addition of all the four concentrations of DDS (15 μ g, 10 μ g, 5 μ g, and 2.5 μ g) (Table 1). When a correlation coefficient (r) between the DDS concentrations and the decrease in number of blast cells was calculated, it was noted that the reduction in the percentage of blast cells is dose dependent ($r = -F-0.9463$, $P < 0.05$) (Fig. 1). The results obtained in the second phase of experiment showed a significant depression in PHA-induced lymphocyte blastogenesis at all concentrations of DDS (1 μ g, 0.5 μ g, 0.25 μ g and 0.1 μ g). However, it did not reveal any dose dependent depression in blast cell transformation ($r = +0.854$, $P > 0.05$; Table 2, Fig. 2). In the third phase of study where DDS has been added at very low concentrations (0.75 μ g, 0.5 μ g, 0.025 μ g and 0.01 μ g) a significant depression in blastogenesis was still noted in all concentrations except in the lowest concentration, i.e., with 0.01 μ g of DDS. With this concentration no significant difference in PHA-induced blast cell transformation was

noted when compared to that of control (Table 3). Here again there was a significant dose dependent correlation in depression in blastogenesis (Fig. 3).

3 DISCUSSION

In the present study all the concentration of DDS (from 0.025 μ g to 15 μ g /10⁶ lymphocytes) induced a significant reduction in the blastogenesis of lymphocytes in peripheral blood of volunteers. The lowest concentration of 0.01 μ g did not show any depressive effect on blastogenesis. BEIGUELMAN & PISANI (1974) however, performed experiments on the above lines but the doses of DDS used by them were much higher than those used in the present study. As the present study was aimed at in finding out the end point of DDS concentration which does not show any depressive effect on PHA-induced lymphocyte blastogenesis therefore, the investigations were carried out at lower dilutions of DDS than those used in earlier studies. The

TABLE 1 — Percentage (mean) of blast cells in lymphocyte cultures treated with PHA only and PHA + DDS (Phase I)

Sl. No.	Type of culture	Per cent blast cells (Mean)	S.D.
1	With PHA only ^(a)	39.13	± 4.942
2	With PHA + 15 μ g DDS ^(b)	1.85	± 1.585
3	With PHA + 10 μ g DDS ^(c)	7.00	± 1.673
4	With PHA + 5 μ g DDS ^(d)	10.46	± 2.777
5	With PHA + 2.5 μ g DDS ^(e)	19.20	± 2.286

P value between (a) and (b) < 0.05

P value between (a) and (c) < 0.05

P value between (a) and (d) < 0.05

P value between (a) and (e) < 0.05

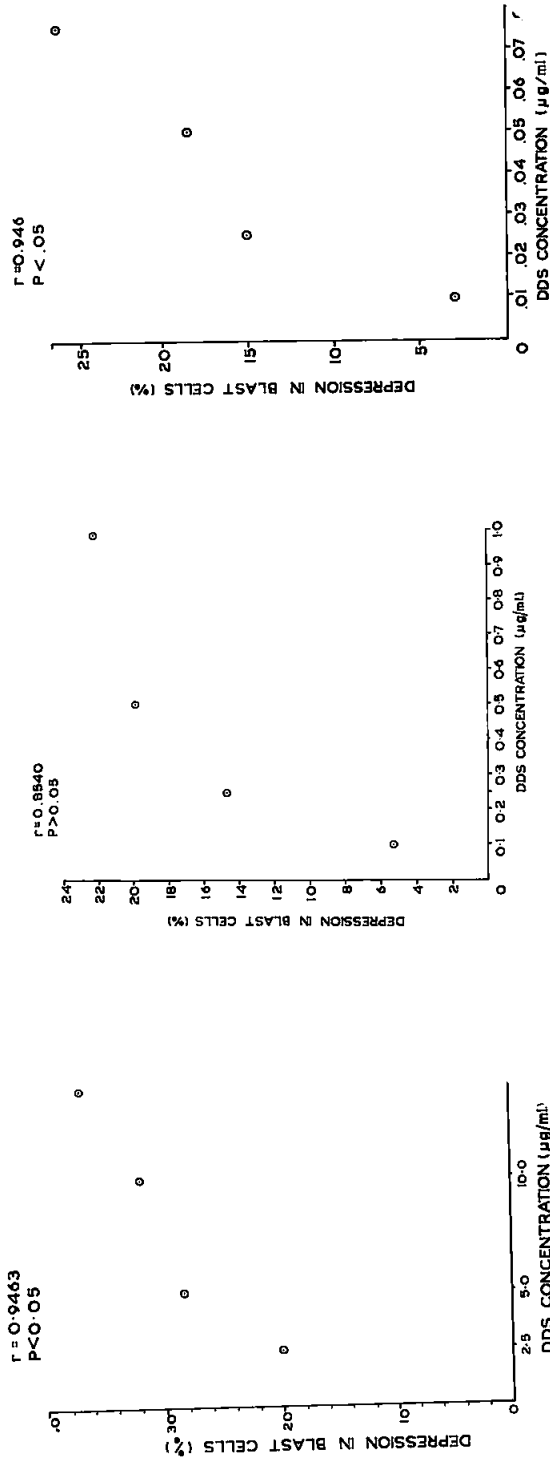


FIGURE 1 — Showing dose dependent correlation between the DDS concentration and the per cent depression in blast cell. Each point represents the average figure of fifteen volunteers except in Fig. 2 wherein the points represent the average of 14 volunteers.

TABLE 2 — Percentage (mean) of blast cells in lymphocyte cultures treated with PHA only and PHA + DDS (Phase II)

Sl. No.	Type of culture	Per cent blast cells (Mean)	S.D.
1	With PHA only ^(f)	40.710	± 5.656
2	With PHA + 1.00 µg DDS ^(g)	18.357	± 3.577
3	With PHA + 0.50 µg DDS ^(h)	20.785	± 2.335
4	With PHA + 0.25 µg DDS ⁽ⁱ⁾	25.928	± 3.514
5	With PHA + 0.10 µg DDS ^(j)	35.357	± 6.466

P value between (f) and (g) < 0.05

P value between (g) and (h) < 0.05

P value between (h) and (i) < 0.05

P value between (j) and (k) < 0.05

TABLE 3 — Percentage (mean) of blast cells in lymphocyte cultures treated with PHA only and PHA + DDS (Phase III)

Sl. No.	Type of culture	Per cent blast cells (Mean)	S.D.
1	With PHA only ^(m)	50.47	± 5.36
2	With PHA + 0.075 µg DDS ⁽ⁿ⁾	25.00	± 9.12
3	With PHA + 0.05 µg DDS ^(o)	31.80	± 7.66
4	With PHA + 0.025 µg DDS ^(p)	35.53	± 9.83
5	With PHA + 0.01 µg DDS ^(q)	47.60	± 9.62

P value between (m) and (n) < 0.05

P value between (m) and (o) < 0.05

P value between (m) and (p) < 0.05

P value between (m) and (q) > 0.05

present study has although showed a depressive effect in PHA-blastogenesis of DDS at high doses but does not necessarily mean that the patients' capacity to induce DH to lepromin (an indirect probable measure of cell mediated immunity (CMI) to *Mycobacterium leprae*) will get depressed

while being treated with DDS. However, work of RAMU *et al.*, (1980) in tuberculoid (TT) and borderline-tuberculoid (BT) patients indicated that administration of 100mg of DDS daily for a month reduces the intensity of

lepromin reaction to a great extent Hence, the possibility in inducing a depressive effect by DDS towards CMI expression by other antigens should not be disregarded. RAMU *et al.*, (1980) further showed that treatment of TT/BT patients with acedapsona resulted in enhancement in the intensity of lepromin reaction to a significant level. Although the present study showed abolition of any depressive effect of DDS at low level of 0.01/, $\mu g / 10^6$ cells *in vitro* but did not indicate any stimulatory effect of DDS on PHA-blastogenesis. However, to understand these mechanisms fully in *in vitro* situation, further work is needed in culture system with lower dilutions of DDS than the dilutions used in the present study.

Although BEIGUELMAN & PISANI (1974) did not observe any DDS-dose dependent depression in PHA-induced blastogenesis, the present study however, showed a dose dependent depression in the first and third phases of study. In the second phase as at only one dose level ($1 \mu g / 10^5$ cells) the value of depression is quite away from

the straight line of correlation coefficient the level of significance (P) did not fall below 0.05 (Fig. 2). This may be explained due to some experimental error because the first and third experimental phases of study showed a significant positive correlation between the DDS levels and blastogenesis.

The mechanism of depression by DDS in PHA-induced blastogenesis may be due to either the blocking of PHA-receptors on lymphocytes by DDS or the drug may combine with any nuclear component of lymphocyte and thereby inhibiting the DNA synthesis. A preliminary study already conducted in our laboratory has indicated that DDS do not compete with PHA at membrane level of lymphocytes (data not shown in the text). Further work is in progress to find out the mechanism of depressive effect of DDS on lymphocyte blastogenesis by PHA.

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RESUMO: Estudou-se o efeito do DDS. sobre a transformação linfocitária estimulada pela PHA, **in vitro**, utilizando-se concentrações variáveis de DDS. Os linfócitos foram doados por voluntários, divididos em três grupos, e o estudo foi desenvolvido em três fases. Foram empregadas 4 concentrações diferentes de DDS para 10^6 de linfócitos em cultura de tecidos além de 0,02 ml de PHA. Foi observada uma diminuição estatisticamente significativa ($P < 0,05$) no percentual de formação de células blásticas induzida pela PHA com todas as concentrações de DDS, exceto com a concentração mais baixa ($0,01 \mu g$ de DDS). Enquanto que a diminuição observada na primeira e na terceira fases mostrou-se dependente da dose de DDS, na segunda fase não se encontrou correlação significativa entre a concentração de DDS e a depressão da blastogênese induzida pela PHA. Discute-se a significância destas observações.

Palavras chave: Diamino-difenil-sulfona. Fitohemaglutinina. Transformação de linfócitos.

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