LETTERS TO THE EDITOR

Di-octyl Phthalate Induced Altered Host Resistance : Viral and Protozoal Models in Mice

Key words: Di-octyl phthalate—EMCV—Plasmodium—Mouse

Many environmental contaminants are detrimental to health by methods other than direct toxicity leading to compromised immune system and which occur at dosages unaccompanied by clinical signs of disease. This may eventually render the host increasingly susceptible to infectious agents. Recently the incorporation of host resistance assay has been emphasized for better understanding and improvement of the predictive value of current immunotoxicologic testing of chemicals.¹⁾ Among industrially important chemicals, the effect of phthalate ester plasticizers on host resistance and immune surveillance to disease has not been well studied. Our recent studies with Di-octyl phthalate (DOP) have demonstrated lymphoid organotoxicity, alteration in the functioning of immune system and altered host resistance to a hookworm parasite (*Nippostrongylus brasiliensis*) in rodents.^{2,3)} These observations suggested that DOP, probably through its effect on immune system, could result in altered host resistance to infection. The present studies were, therefore, undertaken to further assess the altered host resistance in DOP treated mice when challenged with either a virus (encephalomyocarditis) or a protozoal (plasmodium) infection, to delineate the possible contribution of phthalate-induced state of immunomodulation to infections.

Three-month-old swiss albino mice obtained from Central Drug Research Institute, Lucknow were housed in plastic cages and provided with pellet diet (Hind Lever, India) and water *ad libitum*. Di-octyl phthalate (DOP: $C_8H_4-1,2(COO(CH_2)_7CH_3)_2$; MW 390.57) an isomer of Di-2-ethylhexyl phthalate (DEHP) was procured from Ranbaxy Laboratories Ltd. (India). One hundred twenty mice were divided into 3 equal groups and were administered orally DOP at dosages of 0, 0.05 and $0.2 \times LD_{50}$ in 0.2 ml of groundnut oil daily for 5 consecutive days (LD_{50} : 13.0 g/kg). Forty-eight hours after the last dose the animals were employed for infection models of virus and plasmodium.

The encephalomyocarditis virus (EMCV) originally obtained from Dr. R. K. Maheshwari (Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA) was maintained in swiss mice. Brains from mice showing paralytic symptoms were collected aseptically and homogenized in PBS as a 1 g/ml (W/V). The mixture was spun at 4000 rpm for 30 min in a refrigerated centrifuge, supernatant collected, dispensed into 0.5 ml aliquots and stored at $-20^\circ C$. For use, virus was thawed rapidly in a $37^\circ C$ water bath. Twenty mice

Fig. 1. Accumulative mortality rate in various treatment groups of mice inoculated with encephalomyocarditis virus (1×10^{-6} dilution).

Fig. 2. Malaria lethality in mice treated with DOP for 5 days.

Table 1. Malaria parasitaemia in mice inoculated with *P. berghei* after 5 days treatment with DOP^{a,b}

Day posinoculation	Parasitaemia (%)		
	Oil control	0.05×LD ₅₀ DOP	0.2×LD ₅₀ DOP
5	0.0537±0.047 (16)	0.11±0.17 (15)	0.0214±0.0348 (7)
7	0.6813±0.067 (16)	1.27±0.80 (15)**	1.3833±0.8953 (6)**
8	2.187 ±0.963 (16)	2.09±0.85 (15)	3.1667±1.1255 (6)*
9	3.187 ±1.459 (16)	2.60±0.93 (15)	4.00 ±1.3784 (6)*
11	3.312 ±1.436 (15)	3.35±1.12 (15)	4.8333±1.8619 (6)*
14	4.400 ±1.681 (13)	5.23±2.62 (11)	7.00 ±2.5495 (5)**

^a All mice were inoculated intraperitoneally with 1×10^3 *P. berghei* infected erythrocytes. Parasitaemia (% infected erythrocytes) were determined in a thin blood Giemsa-stained smear.

^b All data are presented as a mean±S.E. Group size is indicated by the number in parenthesis.

** $p < 0.01$; * $p < 0.05$.

in each group were then inoculated intraperitoneally with 0.5 ml of 1×10^{-6} dilution (LD₂₀) of EMCV per mouse and animals observed daily for 16 days for mortality.

The strain of *Plasmodium berghei* obtained from the National Institute of Communicable Diseases, New Delhi was maintained by passaging weekly by i.p. injection of 1×10^3 infected erythrocytes in swiss mice. Sixty animals pretreated with DOP (20 mice/group) were inoculated i.p. with 1×10^3 *P. berghei* infected syngenic erythrocytes per mouse. The endpoints examined included cumulative mortality over a period of 19 days in all groups while the degree of parasitaemia in blood was assessed upto 14 days. The data from both the models were analysed statistically using chi square and ANOVA. The level of statistical significance was determined at $P < 0.05$ and $P < 0.01$.⁴⁾

The mortality rate and time of death in mice from various groups challenged with EMCV (10^{-6} dilution) are shown in Fig. 1. Although the percent mortality was significantly higher in 0.2×LD₅₀ DOP group than the 0.05×LD₅₀ DOP and control groups, yet both the doses of DOP (0.2 and 0.05×LD₅₀) caused significantly higher mortality than the oil control group. The lethality pattern and percentage parasitaemia in mice from various groups challenged with 1×10^3 *P. berghei* infected erythrocytes are shown in Fig. 2 and Table 1 respectively. Not much difference was seen in the mean survival times of control group and 0.05×LD₅₀ DOP group (12.15 to 13.50 days). However, treatment with higher dose of DOP (0.2×LD₅₀) caused a drop in mean survival time (6.25 days). This was further supported by time dependent significant increase in the percentage parasitaemia in comparison to control from 7 to 14 days postinfection specially in higher dose group ($P < 0.01$). Occasional differences also occurred between the two dose levels of DOP.

The present study has utilized two infection models, EMCV and *P. berghei* in

mice to evaluate the influence of subtle toxic effect of DOP on host defence capacity. Infection with EMCV has been used extensively as models in immunotoxicologic studies of several environmental chemicals.^{5,6)} The host defense mechanisms involved in the pathogenesis of EMCV have been characterized, which include an early rise in interferon followed by induction of systemic neutralizing antibody.⁷⁾ In the present study DOP treatment at sublethal doses impaired the resistance of mice to challenge of EMCV. The percent mortality caused by the highest dose of DOP ($0.2 \times LD_{50}$) was significantly higher than other groups. The increased mortality observed could have relevance to altered interferon and/or antibody by DOP which would cause enhanced viral replication and dissemination as reported with other chemicals.^{6,8)}

In addition to microbial pathogens, *P. berghei*—a lethal intracellular sporozoan malarial parasite of rodents has also been reported useful in immunotoxicity assessment.⁹⁾ The pathogenesis and immune mechanisms involved in *P. berghei* have been extensively studied and related to the impairment of antibody production and to some extent the altered functions of T-cell and macrophages.⁹⁻¹¹⁾ The results of present study of a low inoculum of *P. berghei* challenge to DOP-treated animals indicated impaired host resistance as reflected by increased malaria lethality with decrease in mean survival time as well as time dependent increased parasitaemia. Efforts were not done to study the effect of DOP on the biology of malarial parasite. In conclusion, the studies described here demonstrated that exposure of mice to DOP can markedly impair host resistance to EMCV and *P. berghei* challenge and supported the notion that a host resistance assay is crucial for evaluating the *in vivo* immunotoxic hazard associated with chemical exposure.¹²⁾

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