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Effect of *p,p'*-DDT and Estrogen on the Presence in the Circulation and Degranulation of Blood Eosinophil Leukocytes

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DDT (1,1,1,-trichloro-2,2-bis [chlorophenyl] ethane) is a pesticide still indiscriminately used in several Third World countries (Metcalf 1973). It is known that DDT contaminates the environment, including food. This contamination can affect human health as well as the survival of wild animals and thus makes important the investigation of its toxic effects in mammals. It is well known that o,p'-DDT exerts estrogen-like activity (Foster et al. 1975; Kupfer 1975; McBlain 1987; Galand et al. 1987), through interaction with cytosol-nuclear estrogen receptors (Kupfer and Bulger, 1977). Studies previously performed in our laboratory have demonstrated that the least toxic and most widely used of the DDT isomers, p,p'-DDT, also displays estrogenic action in the rat uterus (Bustos et al. 1988).

Estrogens are known to induce separate groups of responses through independent mechanisms of hormone action in which different kinds of estrogen receptors are involved. The increase in uterine RNA and protein synthesis are genomic responses to hormone stimulation induced through hormone interaction with cytosol-nuclear receptors in the various uterine cell-types (Jensen and DeSombre, 1972). Estrogen induced uterine edema, increase in vascular permeability and release of histamine are non-genomic responses (Tchernitchin and Galand 1982; Tchernitchin et al. 1985b) induced through hormone interaction with eosinophil leukocyte estrogen receptors (Tchernitchin et al. 1985b, 1989), which mediate the migration of these cells from the blood to the uterus (Tchernitchin et al., 1974, 1985b), their degranulation (Tchernitchin et al., 1985a, 1989), and the release of enzymes and agents involved in the development of the eosinophil-mediated responses (Tchernitchin et al. 1985b, 1989). The existence of multiple and independent mechanisms of estrogen action for the different responses to hormone stimulation causes the dissociation of such responses under a number of conditions (Tchernitchin and Galand, 1982; Galand et al. 1985; Tchernitchin et al., 1985b). Further, estrogenic compounds may interact selectively with some receptor systems, affecting some parameters of hormone stimulation only (Tchernitchin et al., 1985b). Therefore, the study of any agent displaying estrogen action should consider

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the different mechanisms and the wide spectrum of responses to hormone stimulation in the target organ under study.

Taking into consideration the role of eosinophils in estrogen action and the finding that changes in the number or degranulation of eosinophils in the blood affect eosinophil-mediated responses in target organs (Tchernitchin et al., 1985b, 1989), we investigated the effect of estrogen and DDT on eosinophil numbers and degranulation in the blood.

MATERIALS AND METHODS

Female rats from a Sprague-Dawley-derived colony bred at the vivarium of the Faculty of Medicine, University of Chile, were used in the present study. The animals were ovariectomized at the age of three months, and 15 days thereafter submitted to one of the following treatments: (a) p,p'-DDT, at a dosage of 250 µg/kg body wt, in dimethyl sulfoxide; (b) estradiol-17ß, at a dosage of 300 µg/kg body wt, in ethanol-saline 1:9 v/v, (c) p,p'-DDT, immediately followed by estradiol-17ß, (d) estradiol's vehicle, and (e) DDT's vehicle. All injections were performed intravenously, into the jugular vein, under ether anesthesia.

Blood samples were taken from each animal immediately before the treatments and at 6 and 24 h after the treatment. The blood from the tail of ether-anesthetized animals was collected into glass vials containing a 5% (w/v) solution of EDTA in distilled water, obtaining a 9:1 dilution of blood in EDTA solution. Eosinophil quantification and evaluation of eosinophil degranulation was performed according to the method of Tchernitchin et al. (1985a), as follows: the blood samples that have been collected into glass vials were incubated at 18°C. Aliquots were taken immediately after blood sample collection and at 15 min of subsequent *in vitro* incubation and diluted 1:10 with freshly prepared eosin stain solution (1 ml of 1% eosin Y stock solution in 100% ethanol diluted in 10 ml of distilled water and 1 ml of acetone). Subsequently, an alliquot of blood-stain solution was transferred to a Neubauer chamber for eosinophil quantification and evaluation of their degranulation. For this purpose, each eosinophil was classified as degranulated or non-degranulated according to its histological characteristics. Eosinophils that appeared with a marked decrease in the number of eosin-stained cytoplasmic granules, i.e., that contained less than half of the normal granule content, assessed visually, or that exhibited cytoplasmic areas free of granules, or that had lost almost all granules, were considered to be degranulated. Almost completely degranulated eosinophils were recognized because there was always a few eosin-stained granules in the vicinity of the nucleus that conferred a slight shade of pink color to the nuclei of the eosinophils but not to the nuclei of other white blood cells.

To compare the means between control and the experimental samples, the least significant difference (LSD) test was used. The common variance used in this test was obtained from the one way analysis of variance (ANOVA). The possible loss of eosinophils throughout the incubation period in the glass vials, which could alter the results on degranulation percentage, was monitored by comparing the eosinophil numbers before and after the 15 min *in vitro* incubation by ANOVA. In none of these comparisons a significant difference in the number of eosinophils was detected.

RESULTS AND DISCUSSION

The pesticide p,p'-DDT induced a significant increase in the number of blood eosinophils, 24 h after treatment (Fig. 1). Neither estradiol-17ß or the vehicles of DDT or the hormone induced any significant change in blood eosinophilia. The DDT-induced blood eosinophilia was not detected in animals treated with DDT plus estradiol. None of the above agents, alone or in combination, induced any significant effect at 6 h after treatment.

p,p'-DDT, estradiol-17ß or both agents together induced an increase in the *in vitro* degranulation of blood eosinophil leukocytes, at 6 or 24 h after treatment (Fig. 2). None of the vehicles induced any significant change in degranulation percentage. In the animals treated with DDT + estradiol, the degranulation values are significantly lower than those obtained with DDT or estradiol alone.

It is well known that any agent changing the number of eosinophils in the blood, or their state of degranulation, interferes with the different functions of these cells in physiological or pathological processes (Tchernitchin et al., 1985a, 1985b, 1989). In this context, the present finding of a three-fold increase in the number of eosinophils in the blood after exposure to DDT, and an increase in their degranulation, suggest that the pesticide may interfere with processes such as eosinophil-mediated non-genomic responses to estrogen in the uterus, eosinophil-mediated glucocorticoid-induced regulation of some immune processes, eosinophil-mediated defense against parasite infections, hypersensitivity reactions, sequellae from hypereosinophilic syndrome, etc. In the same context, the finding that estrogen completely abolishes DDT-induced blood eosinophilia suggest that estrogen may partially overcome some of the effects of the pesticide.

The mechanisms involved in DDT-induced blood eosinophilia or in the increase in eosinophil degranulation are not known. A relationship between effects of the pesticide and estrogen action may be suggested, based on the inhibition of DDT-induced blood eosinophilia by estrogen, and on the weaker eosinophil degranulation that follows treatment with

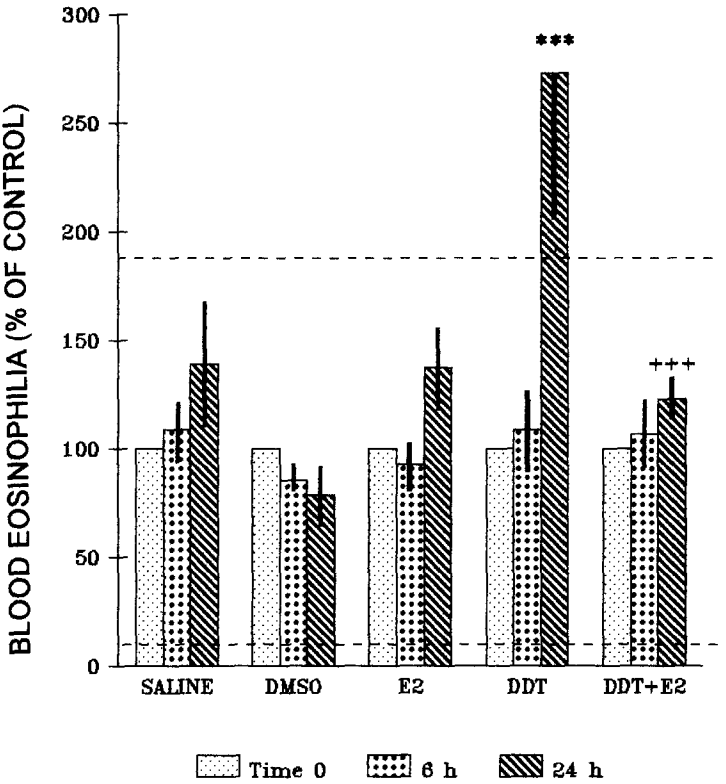

Figure 1. Effect of p,p'-DDT and/or estradiol-17ß on blood eosinophil levels. Values were obtained immediately before (time 0) and at 6 h and 24 h after treatment, and expressed as % of the values before treatment. For analysis of significance, the Least Significant Difference (LSD) test was used. *** $p<0.001$, compared to vehicle-injected animals within the same time of treatment; +++ $p<0.001$, compared to the homologous condition without estradiol. In comparisons between values before and after treatment, the limits of the significance at the level of $P=0.05$ are shown by the horizontal dashed lines.

DDT and estradiol together, when compared with the response obtained with the hormone or the pesticide alone. Further studies are necessary to demonstrate whether there exist a competitive interaction between both substances at receptor level, or an interaction of allosteric nature.

Estrogen-induced eosinophil degranulation is a response mediated by a second class of estrogen receptors in the eosinophils, that display low affinity for estradiol-17ß and high affinity for estradiol-17α (Tchernitchin et al. 1985b, 1989). Accordingly, the degranulation of the blood eosinophil leukocytes can be *in vitro* (Tchernitchin et al., 1985a) or *in vivo* (Tchernitchin

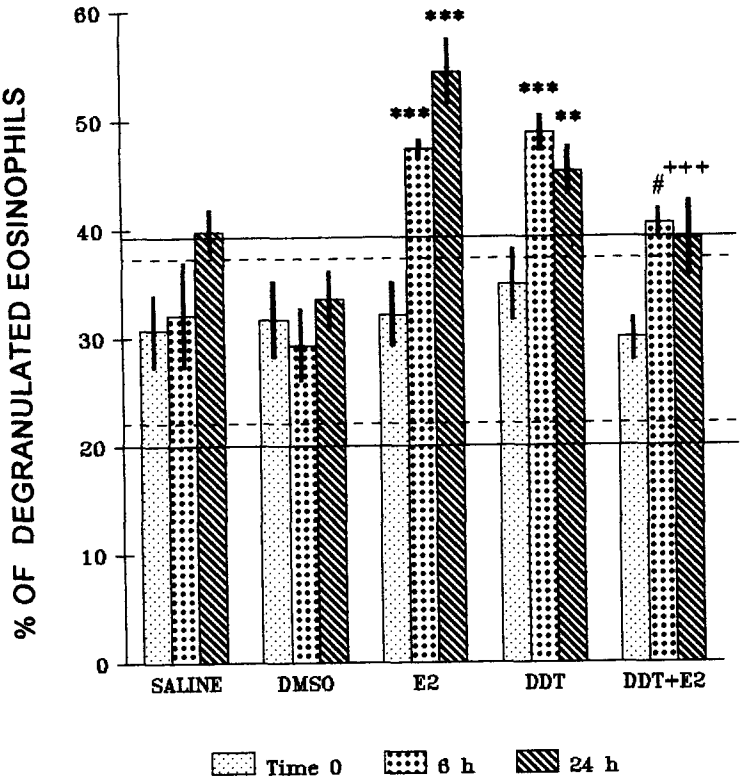

Figure 2. Effect of p,p'-DDT and/or estradiol-17ß on eosinophil degranulation *in vitro*. Blood samples were obtained immediately before (time 0) and at 6 h and 24 h after treatment, then incubated for 15 min in a solution of EDTA; values of degranulated eosinophils were obtained at the end of the incubation and expressed as % of total. For analysis of significance, the Least Significant Difference (LSD) test was used. ** $p<0.01$, *** $p<0.001$, compared with vehicle, within the same time of treatment; +++ $p<0.001$, compared to the homologous condition without DDT; # $p<0.05$, compared to the homologous condition without estradiol. In comparisons between values before and after treatment, the limits of significance at the level of $P=0.05$ are shown by the horizontal dashed lines and at the level of $P= 0.01$ by the horizontal solid lines.

et al., 1989) -induced by estradiol-17ß at high doses only, while a broad range of low to high doses of estradiol-17α causes strong eosinophil degranulation (Tchernitchin et al., 1989). Based on our previous finding that the synthetic estrogen diethylstilbestrol induces strong eosinophil degranulation at low to middle doses only, and that an unexplained reduction in eosinophil degranulation occurs at higher doses of this compound (Grunert et al., 1986; Tchernitchin et al., 1989), we propose

that other estrogens, or combination of estrogens, may display a similar behavior. Therefore, the effect of adding DDT to estrogen may be interpreted as an increase in overall estrogenic activity, causing an inhibition of eosinophil degranulation.

If DDT-induced blood eosinophilia is an estrogenic response, then estrogens themselves should induce blood eosinophilia, which was not detected in the present experiments or in prior reports. On the contrary, it was reported that estrogen treatment at some times induced a moderate eosino-penic response at 6 h after treatment (Steinsapir et al., 1978, 1982), while did not induce any significant response in other experiments (Grunert et al., 1986, present results). Migration of eosinophils from the blood to the uterus under the effect of estrogen explains estrogen-induced blood eosinopenia, since the drop in eosinophils numbers in the blood was apparent in intact animals only, but not in hysterectomized animals (Steinsapir et al., 1978). If migration of eosinophils to target organs under the effect of estrogens occurs, it should induce a much more pronounced drop in blood eosinophil levels, as happens with glucocorticoids (Sabag et al., 1978). The moderate eosinophil drop or the absence of eosinopenia may only be explained if there is a simultaneous eosinophil production increase or a substantial release of these cells from another compartment to the blood.

In this context, we propose that both DDT and estradiol induce an increase in eosinophil production or release from another compartment. Furthermore, estradiol but not DDT can induce a prominent migration of these cells to the uterus, decreasing blood eosinophils to normal levels. Therefore, the inhibition by estradiol of DDT-induced blood eosinophilia, may be due to the estrogen-induced migration of eosinophils to the uterus, resulting in decreased eosinophil levels in the blood.

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