THE EFFECTS OF DIOXIN ON ESTRADIOL MODULATION OF OXIDATIVE BURST METABOLISM BY HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Introduction. The endocrine disruptor Dioxin (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin or TCDD) can be both agonistic and antagonistic to the effects of estradiol in a number of biological systems. Dioxin has been shown to exhibit potent effects on the immune system both *in vivo* and *in vitro*[1] following interaction with the aryl-hydrocarbon receptor (AhR). Alterations in immune functions include effects on polymorphonuclear leukocyte (PMN) functions such as chemotaxis, phagocytosis, and oxidative burst metabolism. Estradiol has been shown to influence PMN oxidative burst metabolism[2-5]. In the current study we have evaluated the effects of estradiol on oxidative burst activity by PMN cultured in the presence and absence of dioxin. These studies show that low doses of dioxin dramatically alter the modulatory effects of estradiol on PMN oxidative burst. In addition we demonstrate that both peripheral blood PMN and PMN present in human tissues, such as endometrium and Fallopian tubes, express the AhR.

Methods. PMN were isolated from normal male donors and cultured in defined low estrogen media for 24 hours in the presence and absence of varying doses of estradiol (10^{-11}M to 10^{-7}M), dioxin (1ppt to 5ppt), or both estradiol and dioxin. Following culture, oxidative burst capacity in response to stimulation with phorbol myristate acetate (PMA) was measured by chemiluminescence (CL). Lucigenin (NADPH oxidase associated) enhanced CL and luminol (myeloperoxidase dependent) enhanced CL were measured at 5 minute intervals for 1 hour[6]. Results were expressed as the mean total CL in relative light units ± 1 s.d. (n=3).

AhR expression of peripheral blood PMN and tissue PMN was evaluated by confocal microscopy following multi-color immunofluorescent staining using a polyclonal rabbit anti-human AhR specific antibody and a monoclonal antibody against the PMN specific cell surface marker CD66b[7].

Results and Discussion. The effects of estradiol, dioxin and both estradiol and dioxin on CL responses showed complex but reproducible dose responses. Although temporally similar, dose response curves varied in the magnitude of changes in CL response between donors. All culture conditions resulted in constantly high levels of viability in the recovered cells (>95%). 24 hour culture with estradiol showed a low dose (10^{-11}M) inhibition of PMA induced CL using lucigenin enhancement and minimal increseases in luminol dependent CL (Figure 1). In contrast, higher estradiol doses (10^{-7}M) inhibited both lucigenin and luminol enhanced CL, although this effect

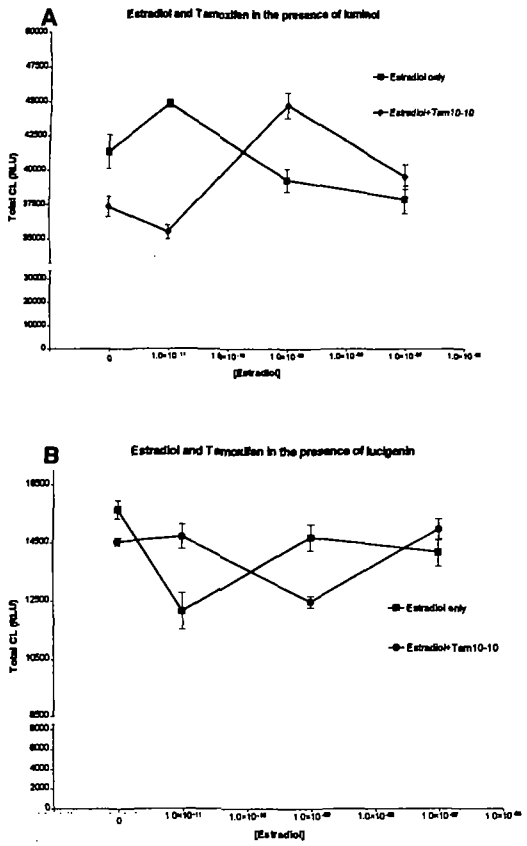

Figure 1. *The effects of estradiol and tamoxifen on luminol and lucigenin enhanced CL.*

A. Low dose estradiol (10^{-11}M) caused a significant increase in luminol enhanced CL, whereas higher doeses had no effect or an inhibitory effect. Tamoxifen (10^{-10}M) reversed the estradiol effects. **B.** In contrast, low doses of estradiol (10^{-11}M) caused a significant decrease in lucigenin enhanced CL, whereas higher doses had no effect or an inhibitory effect. Tamoxifen (10^{-10}M) again reversed the estradiol effects. Tamoxifen in the absence of exogenous estradiol had a slight but statistically significant inhibitory effect on both luminol and lucigenin enhanced CL.

was minimal with lucigenin. Both inhibition and enhancement of CL responses by estradiol were reversed by the preincubation of cultures with the estrogen antagonist tamoxifen (10^{-10}M-10^{-6}M), indicating that the estradiol effects were estrogen receptor mediated. Furthermore, tamoxifen caused a donor variable inhibition of CL from untreated PMN, suggesting that endogenous estrogenic activity influences baseline PMN CL responses (Figure 1).

As shown in Figure 2 (for this particular donor) luminol enhanced CL by PMN, cultured in the presence of estradiol (10^{-9}M), was inhibited to unstimulated levels. This was also observed for lucigenin enhanced CL (data not shown). Dioxin, in the absence of exogenous estradiol, inhibited both lucigenin (not shown) and luminol (Figure 2) enhanced CL in an inverse dose dependent manner, such that low doses (1ppt) inhibited CL to unstimulated levels whereas higher doses (5ppt) were less inhibitory. However, in the presence of estradiol (10^{-9}M), this effect of dioxin was reversed. Higher doses of dioxin (5ppt) in the presence of estradiol inhibited the CL response to unstimulated levels. Whereas in the presence of estradiol concentrations that alone inhibited oxidative burst activity, low doses of dioxin abolished this inhibition.

When peripheral blood PMN were assessed for AhR receptor expression by immunofluorescent staining, an average of 30% of PMN were positive on confocal analysis, but this percentage varied considerably between donors. When AhR receptor expression was evaluated in tissue sections from endometrium and Fallopian tube, PMN showed a similar frequency of receptor expression.

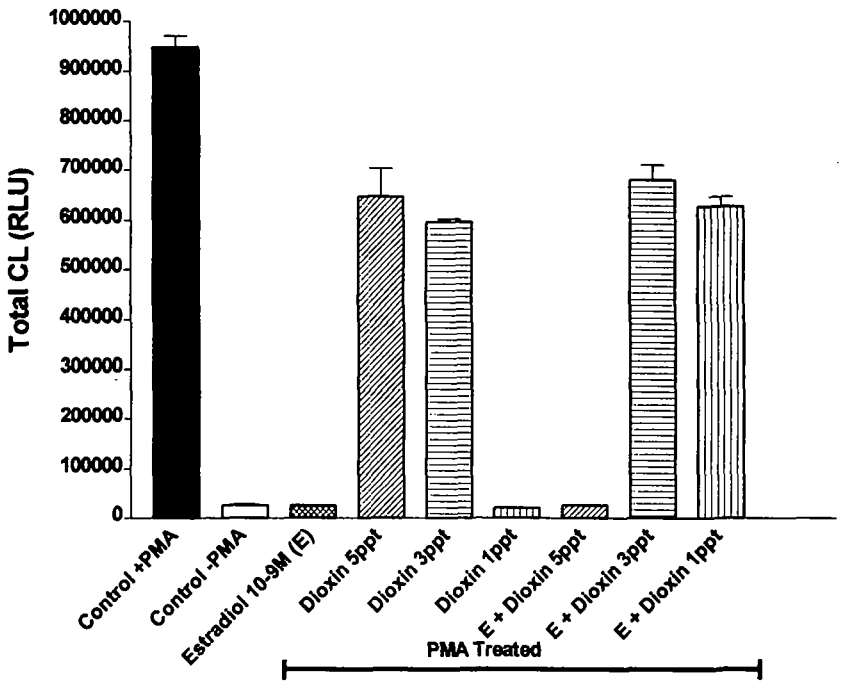

Figure 2. *The effects of estradiol, dioxin and both estradiol and dioxin on luminol enhancd CL.* Estradiol at (10^{-9}M) inhibited luminol enhanced CL to unstimulated (no PMA) levels. Dioxin inhibited CL in an inverse dose dependent manner, i.e. low doses (1ppt) inhibited luminol enhanced CL to unstimulated levels. However, dioxin in the presence of estradiol (10^{-9}M) inhibited CL in a dose dependent manner i.e. 5ppt inhibited CL to unstimulated levels, however, the presence of dioxin at 1ppt or 3ppt (but not 5ppt) partially reversed the effects of estradiol alone. CL is expressed as the total light emission over a 1 hour period. Error bars represent the mean (n=3) ± 1 s.d.

The heterogeneity of AhR expression in PMN from different donors may account for the variability in dioxin effects on PMN function. Whether estradiol receptor expression in PMN varies in a similar manner remains to be determined.

These findings suggest that PMN effector function is modulated by estradiol, and that estradiol modulation is highly sensitive to dioxin exposure. The estradiol effects reported here are at the low end of the physiological range. Similarly, the dioxin levels used here are within the range of environmental exposure. One explanation for the profound effects on PMN oxidative burst at these low levels of estradiol and dioxin, maybe that acute exposure leads to shifts in equlilibria resulting in altered cellular responses. Alternatively, under these culture conditions the bioavailability of both estradiol and dioxin may be greater than under *in vivo* conditions. We are currently investigating the mechanism of control of oxidative burst modulation. The fact that these differences are seen in response to PMA stimulation indicates, that the effects are

downstream of protein kinase C activation (PKC). A number of potential mechanisms may be involved in this modulation of oxidative burst activity. Firstly, modulation could arise from a switch in PKC isoform from a PMA responsive to a PMA non-resposive PKC isoform. Secondly, estradiol and dioxin treatment may alter expression levels of cytoplasmic or membrane cytochrome oxidase components. Or thirdly, degranulation and/or membrane mobilization which are necessary for the assembly of a functional NADPH oxidase and for myeloperoxidase degranulation may be altered. These possibilities are currently being investigated. We believe that this *in vitro* system may form the basis for a bioassay useful in the evaluation of dioxin and dioxin conjoiner mediated effects at the cellular level.

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References.

1. Holsapple, M.P., Snyder, N.K., Wood, S.C. & Morris, D.L. A review of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced changes in immunocompetence: 1991 update. *Toxicology* 69, 219-55 (1991).
2. Angel, K.L. *et al.* Effects of estradiol 17 beta implants on hematologic values and the chemiluminescence response of neutrophils of steers. *Am J Vet Res* 53, 78-82 (1992).
3. Shibuya, T. *et al.* Study on nonspecific immunity in pregnant women: II. Effect of hormones on chemiluminescence response of peripheral blood phagocytes. *Am J Reprod Immunol* 26, 76-81 (1991).
4. Pertynska, M.P., Tchorzewski, H., Lewkowicz, P., Pasnik, J. & Pertynski, T. [Evaluation of the hormonal replace therapy (HRT) effect on generation of reactive oxygen intermediates (ROI) by neutrophil in peripheral blood of menopausal women]. *Ginekol Pol* 71, 55-62 (2000).
5. Olinescu, R., Hertoghe, J., Savoiu, D., Matachescu, C. & Nita, S. Steroid hormones may modulate the chemiluminescence emission produced by polymorphonuclear leukocytes. *Rom J Intern Med* 32, 37-46 (1994).
6. Yeaman, G.R., Froebel, K., Galea, G., Ormerod, A. & Urbaniak, S.J. Discoid lupus erythematosus in an X-linked cytochrome-positive carrier of chronic granulomatous disease. *Br J Dermatol* 126, 60-5 (1992).
7. Yeaman, G.R. *et al.* IFN-gamma is produced by polymorphonuclear neutrophils in human uterine endometrium and by cultured peripheral blood polymorphonuclear neutrophils. *J Immunol* 160, 5145-53 (1998).