Significance of TCDD-induced, Ah Receptor-mediated Activation of Protein Kinases in the process of Toxic Expression of TCDD

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TCDD (2,3,7,8-tetradilorodibenzo-p-dioxin) has been used as a molecular probe and a model compound for a large class of chemicals here collectively designated as dioxin-type chemicals.[1] While the most studied subject in the field of TC DD Action mechanism is TCDD-induced transcriptional activation process of cytochrome P450 1A1 type genes[1-3], there are other types of TCDD's effects which are unexplainable by the above mechanism. In 1984 it was reported by Matsumura et al[4] that *in vivo* administered TCDD (25μg/kg single i.p. male rats, sacrified after 10 days) causes a spectacular rise in cAMP-dependent and independent protein kinases in the rat liver plasma membrane. Such biochemical changes were found to be accompanied by down-regulation of a number of receptors, particularly the epidermal growth factor receptor (EGFR). A subsequent study[5] has shown that such a set of biochemical changes are caused by only toxic congeners of dioxin-type chemicals and are clearly Ah-receptor dependent. This phenonmenon of TCDD-induced EGFR down-reguation has been confirmed by other groups[6,7]. It is now well established that the initial activations of EGFR by its ligands and its subsequent down-regulation involve activation of protein tyrosine kinases (PTK's) and protein kinase C (PKC).[10] This was shown to be also the case with the TCDD-induced EGFR changes by our research group[8,9], the time sequence of events being the initial rise of PTKs folowed by a marked rise in PKC activity[10]. One of the protein tyrosine kinases we studied in 1987[11] was src products, $pp60^{c\text{-}src}$ and $pp60^{v\text{-}src}$. TCDD was found to cause a rapid rise in src protein levels both in the guinea pig

hepatic plasma membrane (*in vivo* study) and in NIH 3T3 fibroblast cells (*in vitro* study). It is also clear now that such a series of EGF-induced signal transduction pathway must involve ras proteins,[12] a family of PTKs specific G-protein. We could also show that TCDD c auses a rise in ras by using the *in vivo* guinea pig and mouse model[13] as well as the *in vitro* fibroblast cell model[14].It is important to stress here that in all cases we have scrupulously proven that these changes are clarly Ah receptor-dependent.

In 1988[10] we have reported that quercetin administered simultaneously with TCDD *in vivo* could prevent the latter's action to cause thymic atrophy. Quercetin is a bioflavinoid known to inhibit both PTKs and PKC. Indeed under the identical test condition, thymic protein kinases were found to be inhibited. More recently Puga et al.[14] have found that staurosporin, another PKC inhibitor could totally abolish the action of TCDD in Hepa 1 cells. while the specificies of these inhibitors are not totally proven, the fact that they abolish the action of TCDD must not be ignored. Our recent study results also indicate that two additional inhibitors are capable of abolishing the TCDD's effects on EGFR in NIH 3T3 fibroblast cells. Genistein is a specific PTKs inhibitor and neomycin is an agent which prevents inositol triphosphate from forming and thereby blocks the PKC/phosphoinositide pathway. Along the same line of logic we have tested the effect of various protein kinase inhibitors on the expression of lipoprotein lipase (LPL) in cultured adipose tissues from male guinea pig. The results clearly indicate that TCDD's effect on LPL activities could be reduced or abolished by various protein phosphorylation inhibitors such as neomycin (200μM)and geneistein μM), but not by stimulaotrs such as vanadate (30 μM) and TPA (1μM) (all added one hour prior to a 3 hour TCDD incubation study).

The main question we must raise now is how the Ah receptor mediated events could be abolished by protein kinase inhibitors. One possibility is that these chemicals directly interfere with Ah receptor. A second possibility, which we favor, is that the binding of TCDD to the cytosolic Ah receptor itself triggers PTK activation in the cytosol. The mechanism by which such ligand-induced activation of PTK is not known. However, there are precendents of steroid hormones activating pp60^{src} by

interacting with their receptors which are associated with heat shock proteins. Such a possibility has also been pointed out by L. Birnbaum in a recent meeting. Recently we have tested this possibility by directly incubating TCDD with a nuclear free cytosolic preparation from adipoytes of guinea pigs *in vitro*. To our surprise 10nM TCDD could significantly stimulate the overall protein kinase activities within 1 to 5 minutes. Immunopurification of heatshock protein (Hsp) 90 complex showed that the TCDD induced rise in phosphorylation was very prominant on this protein along with accompanying 50 and 60 KDa proteins which were recognized upon SDS-polyacrylamide gel-electrophoresis. A significant portion of this increased protein phosphorylation activity could be eliminated by genistein, a specific inhibotor of protein tyrosine kinases The probability that such a protein kinase mediated pathway could play significant roles in the expression of toxic action of TCDD must be seriously considered in the future.

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