PX1805

Chapter 19

THE MECHANISM OF ACTION OF DIOXIN

Fumio Matsumura
Pesticide Research Center
Michigan State University

TOXIC EFFECT OF TCDD

There is no question that TCDD is a very unique poison. The first time I gave TCDD to animals I expected to see some immediate symptoms. We waited and waited and we could not see usual signs of toxicity. The animals did look a little quieter but there was no obvious toxicity until about thirty days later; at that point the animals just died. In addition, we could find no clear-cut dose response. Even when we gave high doses, not all animals died and we had no idea what the difference was among the population. Not only are there differences within species, there are very large differences between species as Dr. McConnell pointed out (McConnell, 1984).

TCDD is also different in not showing organ specific toxicity. Although it does produce organ damage, the target organs differ from species to species (Moore, 1973). In addition, you cannot say what effect leads to the death of the animal. If you give something like DDT, the animal starts showing convulsions so you suspect that the nervous system must be affected. But in the case of TCDD you cannot say why they die. It is really a mysterious compound.

ENZYME INDUCTION

Now, some good news. There has been significant progress in one area; the area of induction. TCDD is a very potent inducer of certain detoxification enzymes, particularly in the liver, which is the detoxifying organ for foreign compounds. When you administer a small quantitiy of TCDD you notice that the liver (hepatocyte) microsomes start increasing. This is the first step in the increase of the detoxification enzymes which reside in the microsome. Several groups, especially Dr. Poland's and Dr. Nebert's groups, have contributed to the understanding of TCDD's mechanism of action. It appears that TCDD binds with a receptor in the cytosolic portion of the cell and this complex migrates into the nucleus. This somehow activates the synthesis of the detoxifying enzymes: cytochrome P448 and related systems. There is a pleiotropic response; that is, one change (TCDD-receptor complex) leads to many, many different changes (induction of many proteins) (Poland et al., 1974; Nebert, 1979).

Although phenomenal progress has been made in this area, there is one problem: induction, per se, probably has nothing to do with toxicity. There are many other compounds, such as DDT, phenobarbital, 3-methylcholanthrene, which are good inducers but do not cause the same symptoms as TCDD (Madhukar and Matsumura, 1981). They are toxic but they do not cause weight loss, chloracne and other signs of toxicity which are specific to TCDD-type compounds. The best evidence for the lack of relationship of induction and toxicity is the case of the guinea pigs. Guinea pigs are the most sensitive

animals to TCDD but liver induction is not seen. In fact, when you look at the liver of the guinea pigs, you hardly see any effects of TCDD even at the time of death (Gupta et al., 1973; McConnell et al., 1978). This is not to say that induction studies are unimportant; they reflect some profound biochemical changes that are taking place. However, what I would like to emphasize here is that we still do not have the whole picture.

PLASMA MEMBRANE ALTERATIONS

In the absence of a clear understanding of TCDD's toxicity, our group started looking at the problem from a different angle. Around 1975-77, I had the pleasure to work with Dr. Dick Peterson and his group which had found that the excretion of neutral substrate in the liver was hampered by TCDD (Yang et al., 1977). This phenomenon was confirmed by Dr. Curt Klaassen's group. Not knowing about the mechanisms of neutral substrate transport in the liver, we suspected that it could be due to sodium-potassium ATPase. We found that after about 10 days of TCDD administration the level of ouabain transport starts to decrease. At the same time, sodium-potassium and magnesium ATPase also start decreasing. It was a beautiful correlation. Unfortunately, we also found that sodium-potassium ATPase had nothing to do with the transport of neutral substrate, since by using steroid hormone analogs the ouabain transport activity could be uncoupled from that of ATPases (Peterson et al., 1979a; Peterson et al., 1979b).

However, we later realized that sodium-potassium ATPase is a good marker for plasma membrane; i.e., cell surface membrane as opposed to any other membrane. This came to our attention when we were studying the isolated hepatocytes. When you start culturing the hepatocytes from an animal treated with TCDD, you realize that those cells do not stick to each other in contrast to normal hepatocyte cultures where the cells are attached to each other. In addition, normal cells start forming a monolayer so that when you pour off the supernatant, you can see beautiful layers formed. This is not observed in cells from TCDD-treated animals. Thus we started to suspect that there was some change in the plasma membrane around 1979.

At that point, we started to look at the plasma membrane and fortunately there is a good biochemical method for isolating relatively pure plasma membrane from the rat liver. When we examined SDS gel-electrophoretograms of the plasma membrane proteins we noticed that some of the protein bands started disappearing after TCDD treatment. There wasn't much effect in the first few days but by the tenth day, intensities of some bands started decreasing. We used one band, at 48,000, as a marker, because it is a structural protein which always gives two or three percent of the total protein. We sometimes used markers at the microsomal enzymes and, so that if there is any contamination there, we will find it. Using these markers, you can see a great decrease in some of the bands, particularly after day 20. It was clear that some profound changes were taking place in the plasma membranes (Brewster et al., 1982).

LIPOPROTEIN ACTIVITY

At the same time, other investigators were looking at various biochemical changes in the guinea pig in an attempt to better understand the mechanism of toxicity. One noticeable sign of TCDD's toxicity is an unusual accumulation of cholesteryl ester and triglyceride carrying lipoproteins in the serum; particularly low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) (Swift et al., 1981). In light of the known alterations in plasma membranes due to TCDD administration, and the presence of low-density lipoprotein receptors in liver cell plasma membranes, we decided to look at this phenomenon more closely.

We administered TCDD by injection to the guinea pigs and isolated their hepatocytes ten

days later. We first looked at the uptake of low-density lipoprotein by these cells as compared to cells from untreated animals. We found that there was a significant decrease in both the rate of uptake and total uptake in the cells from the TCDD-treated animals. The next step was to try to see why this reduction in uptake was occurring. To approach this, we looked at how the different liver cell fractions responded to low-density lipoprotein. It was clear that the greatest reduction in lipoprotein uptake occurred in the plasma membrane fraction (Bombick et al., 1984).

A further refinement was to isolate the plasma membranes from both TCDD-treated and untreated guinea pigs and also treated and untreated rats (which also show increased serum cholesterol in response to TCDD administration). There was a clear decrease in low-density lipoprotein binding in both guinea pig and rat hepatic plasma membranes. To ensure that these decreases were not due to changes in nutritional status (i.e. TCDD poisoned animals eat less),we also looked at untreated animals which were fed exactly the same amount of food as the treated ones. Again, the results were the same (Bombick et al., 1984).

RECEPTOR SYSTEMS

As a check on these findings, we decided to look at the internalization of low-density lipoprotein receptors in hepatocytes. It is known that when low-density lipoprotein interacts with its receptor, it is then carried inside the cell where it is metabolized. Thus, if there is less uptake and thus fewer lipoprotein-receptor complexes, there should be less low-density lipoprotein internalized. Studies of cells from TCDD-treated animals and untreated animals showed that this was, indeed, the case. There was a significant decrease in internalization in the treated as compared to the untreated animals (Bombick et al., 1984).

Although it appears clear that there is an association between TCDD and low-density lipoprotein receptor activity, it might be argued that this is a secondary effect resulting from the increased cholesterol levels (i.e., "down regulation"). We do not feel this is the case for a number of reasons. One is the low level of TCDD at which the binding decreases occur in the guinea pig and the lack of overt toxic signs at these levels. It would thus appear we are seeing the early toxic effects of TCDD rather than some indirect action. Second, if the reduction of LDL receptors is due to down regulation there should be a decrease in VLDL production by the liver, whereas VLDL levels in TCDD treated guinea pigs are elevated, rather than depressed. There are a number of observations which support our theory that serum low-density lipoprotein can increase as a result of a reduction in the activity of low-density lipoprotein receptors. This has been seen not only in animals such as rabbits (e.g., Watanabe rabbit) (Goldstein et al., 1983) but also in humans who have a genetic disorder known as familial hypercholesterolemia.

These results are obviously of interest in providing clues as to the mechanism of action of TCDD. Moreover, they also tie in with the possible hypercholesterolemia in humans exposed to TCDD that was mentioned earlier in Dr. Suskind's presentation (Suskind, 1984). Although this link is based on epidemiological data from limited populations, the similarity of effect found in experimental animals suggests the need for further studies in this area.

We are continuing to look at the plasma membrane to see if we can detect other changes which might reflect TCDD's mode of action. We have looked at several enzyme systems and have found that many are affected, mostly depressed. One which seems to show an increase in activity is the group of protein kinases.

In addition, we are looking at receptor systems other than the low-density lipoprotein receptors. Again, we find effects which seem to be related to TCDD. For example, the insulin receptor seems to be stimulated at lower doses but depressed at higher ones. The

epidermal growth factor (EGF) receptor also seemed to show a marked depression after TCDD administration. Indeed, EGF receptor was the one which was most sensitive, i.e., that showed an effect at the lowest TCDD dose so far as we have studied. We are presently following up on that finding.

Even if we do find these changes, we are still faced with the task of determining if these are related to toxicity and, if so, in what way. What we are seeing so far are little hints or clues to keep us going. We are far from an understanding of the unique action of TCDD but we are hoping that the combined efforts of many biochemical toxicologists will eventually provide the answers we seek.

REFERENCES

Bombick, D.W., Matsumura, F. and Madhukar, B.V. 1984. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes reduction in the low density lipoprotein (LDL) receptor activities in the hepatic plasma membrane of the guinea pig and rat. Biochem. Biophys. Res. Comm. 118:548-554.

Brewster, D.W., Madhukar, B.V. and Matsumura, F. 1982. Influence of 2,3,7,8-TCDD on the protein composition of the plasma membrane of hepatic cells from the rat. Biochem. Biophys. Res. Comm. 107:68-74.

Goldstein, J., Kita, T. and Brown, M. 1983. N. Engl. J. Med. 309:288-296.

Gupta, B.N., Vos, J.G., Moore, J.A., Zinkl, J.G. and Bullock, B.C. 1973. Pathologic effects of 2,3,7,8-tetrachloro-p-dioxin in laboratory animals. Environ. Health Perspectives 5:125-140.

Madhukar, B.V. and Matsumura, F. 1981. Differences in the nature of induction of mixed function oxidase systems of the rat liver among phenobarbital, DDT, 3-methylcholanthrene and TCDD. Toxicol. Appl. Pharmacol. 61:109-118.

McConnell, E.E. 1984. This volume.

McConnell, E.E., Moore, J.A., Haseman, J.K. and Harris, M.W. 1978. The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44:335-356.

Moore, J.A. 1973. Chlorinated dibenzodioxins and dibenzofurans. Environ. Health Perspectives, Issue No. 5, pp. 313, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Nebert, D.W. 1979. Genetic differences in the induction of monooxygenase activities by polycyclic aromatic compounds. Pharmacol. Ther. 6:395-417.

Peterson, R.E., Madhukar, B.V., Yang, K.H. and Matsumura, F. 1979a. Depression of ATPase activity in isolated liver surface membranes of 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. J. Pharmacol. Exp. Ther. 210:175-282.

Peterson, R.E., Hamada, N., Yang, K.H., Madhukar, B.V. and Matsumura, F. 1979b. Reversal of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced depression in ouabain biliary excretion by pregnenolone-16α-carbonitrile and spiranolactone in isolated perfused rat livers. Toxicol. Appl. Pharmacol. 50:407-416.

Poland, A., Glover, E., Robinson, J.R. and Nebert, D.W. 1974. Genetic expression of aryl hydrocarbon and hydroxylase activity. Induction of monooxygenase activities and cytochrome P-450 formation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice genetically "nonresponsive" to other aromatic hydrocarbons. J. Biol. Chem. 249:5599-5606.

Suskind, R.R. 1984. This volume.

Swift, L.L., Gasiewicz, T.A., Dunn, G.D., Soule, P.D. and Neal, R.A. 1981. Characterization of the hyperlipidemia in guinea pigs induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 59:489-499.

Yang, K.H., Croft, W.A. and Peterson, R.E. 1977. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on plasma disappearance and biliary excretion of foreign compounds in rats. Toxicol. Appl. Pharmacol. 40: 485-496.