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THE EFFECTS OF 2,3,7,8-TETRACHLORO-DIBENZO-p-DIOXIN ON HUMAN POLYMORPHONUCLEAR LEUCOCYTES

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Suspensions of human polymorphonuclear leucocytes were treated with 2,3,7,8--tetrachlorodibenzo-p-dioxin (TCDD) at concentrations 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M. The TCDD provoked a progressive extracellular release of the lysosomal enzyme beta-N-acetylglucosaminidase and the cytoplasmic enzyme lactate dehydrogenase in a dose-dependent fashion. At all concentrations TCDD was much more effective in releasing beta-N-acetylglucosaminidase than lactate dehydrogenase. Different responses of the two enzymes to TCDD might be explained by differential structural affinities of beta-N-acetylglucosaminidase for the lysosomal membrane and of lactate dehydrogenase for the cytoplasm. It is likely that TCDD affected the solubility of beta-N-acetylglucosaminidase to a higher extent than that of lactate dehydrogenase, which is probably firmly attached to the cytoplasm.

Key terms:
cytoplasmic enzyme, extracellular enzyme release, lysosomal enzyme

The polyhalogenated aromatic hydrocarbon, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is among the most ubiquitious and persistent environmental pollutants (1-4). Studies of its toxicological properties point to carcinogenicity, immunotoxicity, teratogenicity, liver lesions and thymic atrophy (5-7).

Although the mechanism of TCDD action is not fully understood, a major toxic effect seems to be mediated by a cytosolic protein, aryl hydrocarbon (Ah) receptor. The latter has been identified in organs and tissues of several animal

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species as well as in mammalian cultured and isolated cells such as human fibroblasts and lymphocytes. TCDD initially binds to the Ah-receptor and the liganded complex undergoes a transformation to a lower molecular weight heterodimer that exhibits an increased DNA binding affinity. This complex translocates into the nuclear fraction of the target cell and acts as a nuclear ligand-responsive transcriptional factor (8–10).

There are also other types of TCDD effects which are related to its lipophilic properties. One such effect is damage to biological membranes, as was observed for some other halogenated aromatic hydrocarbons e.g. gamma-hexachlorocyclohexane, dieldrin, and dichlorodiphenyltrichloroethane (11–14).

The aim of the present study was to obtain more information on the effects of TCDD upon the integrity of cellular and lysosomal membranes by monitoring the release of cytoplasmic and lysosomal enzymes from human polymorphonuclear leucocytes (PMNL).

MATERIAL AND METHODS

Chemicals. TCDD was graciously provided by Dow Chemical (Midland, MI, USA). All other chemicals (analytical grade) were obtained from Koch-Light Lab. Ltd (Haverhill, Suffolk, Great Britain).

Preparation of cell suspension. PMNL were isolated from heparinized venous blood of adult healthy donors by means of Hypaque/Ficoll gradients, followed by dextran sedimentation and hypotonic lysis of erythrocytes (15). The cells were washed twice and resuspended in a buffered salt solution consisting of 138 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $MgCl_2$ and 0.6 mM $CaCl_2$, pH 7.4, hereafter referred to as phosphate-buffered saline.

Cytoplasmic and lysosomal marker enzymes. Before deciding on whether a given agent acts by inhibiting or increasing the release of cellular enzymes, one should examine its effect upon activities of particular enzymes (16). Our preliminary observations (unpublished data) indicate that neither TCDD nor dimethylsulphoxyde (DMSO), used as solvent for TCDD in the final concentrations of 0.2% in media, interfere with the activities of beta-N-acetylglucosaminidase (beta-Glm, EC 3.2.1.30) and lactate dehydrogenase (LDH, EC 1.1.1.27). These enzymes were therefore chosen as lysosomal and cytoplasmic marker enzymes.

Test for the extracellular enzyme release in vitro. Washed human PMNL (5 x 10^6 in 0.9 ml phosphate-buffered saline) were incubated with 0.1 ml TCDD of various final concentrations, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M, or with equal volume doses of DMSO at 37 °C for 10 min.

At a defined time, the cell suspensions were removed and centrifuged at 1500 x g at 4 °C for 20 min. The resulting supernatants were assayed for the released beta-Glm and LDH activities. Total activities of these enzymes were

measured in selected reaction mixtures after the cells had been lysed by addition of 0.1% (v/v) Triton X-100. Appropriate control experiments were performed by measuring the release of enzymes tested in the specimens incubated with equal volume doses of DMSO, or without DMSO or TCDD, for 10 min at 37 °C. The enzyme activities determined in the supernatants were expressed as the percentages of their total activities in cell suspensions as described previously (17–19). This parameter served as a measure for the *in vitro* release of beta-Glm and LDH under the experimental conditions described. Also, the LDH release from PMNL served as an indicator of cell viability in the investigations *in vitro*.

Enzyme assays. The activity of beta-Glm was determined according to the procedure of *Sellinger and co-workers* (20), as modified by *Baccino and Zuretti* (21). The activity of LDH was measured by the method of *Wacker and co-workers* (22). In all instances the experiments were carried out in duplicate. Specific enzyme activity was expressed as units/mg protein.

Protein determination. Protein concentrations were measured by the method of *Lowry and co-workers* (23).

Statistical analysis was performed by means of Student's t-test with a significance level of $P<0.05$.

RESULTS

The Figure shows the extracellular release of lysosomal beta-Glm and cytoplasmic LDH enzymes from human PMNL suspensions incubated with increasing concentrations of TCDD (10^{-8}–10^{-5} M) or with equal volume doses of DMSO for 10 min at 37 °C. The data obtained with the specimens which were incubated in the

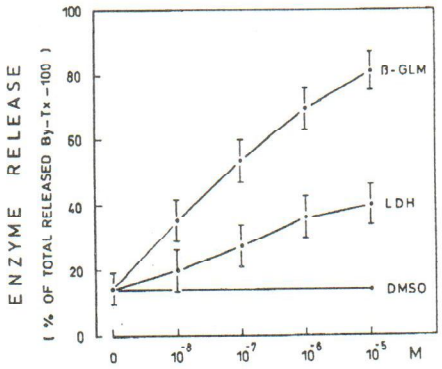

Figure. *The TCDD induced release of lactate dehydrogenase and beta-N- acetylglucosaminidase from human polymorphonuclear leucocytes (point represents the mean ± standard error of the mean of three experiments)*

same manner but without TCDD or DMSO were used as background levels. It should be noted that there were no significant ($P>0.05$) differences between the background levels and those following treatment with DMSO. Therefore the background levels were subtracted from the results for TCDD- and DMSO-treated specimens and are not included in the Figure. On the other hand, the TCDD induced, concentration-related release of the lysosomal enzyme beta-Glm (10^{-8}–10^{-5} M) and the cytoplasmic enzyme LDH (10^{-7}–10^{-5} M) from human PMNL suspensions was significant ($P<0.01$) in comparison with that from the DMSO-treated specimens. The TCDD concentration of 10^{-8} M did not induce a significant ($P>0.05$) leakage of LDH. However, the TCDD effect upon the release of beta-Glm was stronger than upon that of LDH, so that the differences in release between the two enzymes were more significant ($P<0.01$) at the concentrations from 10^{-7} to 10^{-5} and less significant ($P<0.05$) at 10^{-8} M.

DISCUSSION

A striking observation in this study was the extracellular release of lysosomal enzyme beta-Glm and cytoplasmic enzyme LDH from human PMNL treated with TCDD. The TCDD effect was dose-dependent. However, the enzymes exhibited different responses. Namely, at the concentrations of 10^{-8}–10^{-5} M TCDD was much more effective in releasing beta-Glm from human PMNL than LDH. Because of the very complex nature of the factors influencing extracellular enzyme release the present results are not sufficient to explain the mechanisms by which TCDD enters the cell and induces discharge of beta-Glm and LDH. However, it is well documented that TCDD binds the Ah-receptor and enters the nuclear fraction of the target cell. On the other hand, it has been shown that in some cell types the complex formed between drug and receptor follows rapid internalization into endosomes via coated pits. Once internalized, most such complexes are transported to lysosomes where the linkage between drug and receptor is split by lysosomal hydrolases (24–26). A certain amount of the receptor can be recycled back to the cell surface, while the drug liberated from the complex is set free to exert its effects upon lysosomal membrane.

Considering these findings and our present results one gains the impression that TCDD is bound by the Ah-receptor in the form of a complex which is then taken up within lysosomes by endocytosis. After intralysosomal separation from the complex TCDD could alter the integrity of the lysosomal membrane and induce the leakage of acid hydrolases, including beta-Glm, into the cytoplasm. In such conditions lysosomal enzymes are capable of producing autolysis and cell death which leads to the extracellular release of both lysosomal and cytoplasmic enzymes. Simultaneously TCDD diffuses out of the lysosomes into the cytoplasm and penetrates into the nucleus, its subcellular target.

The different enzyme responses to the action of TCDD might be explained by the differential structural affinities of beta-Glm for the lysosomal membrane

and of LDH for the cytoplasm. Thus it seems possible that TCDD may affect the solubilization of beta-Glm to a higher extent than that of LDH, which is probably firmly attached to the cytoplasm.

Since the proposed interpretations of the present results are highly speculative, further studies on the mechanisms by which TCDD enters the cell and modifies the structural properties of the lysosomal membrane need to be performed.

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Sažetak

UČINAK 2,3,7,8-TETRAKLORDIBENZO-p-DIOKSINA NA POLIMORFONUKLEARNE LEUKOCITE U LJUDI

2,3,7,8-tetraklorodibenzo-p-dioksin (TCDD) u koncentracijama od 10^{-8}, 10^{-7}, 10^{-6} i 10^{-5} M uzrokuje progresivno izvanstanično oslobađanje lizosomskog enzima beta-N-acetil-glukozaminidaze i citoplazmatskog enzima laktat-dehidrogenaze iz humanih polimorfonuklearnih leukocita. Učinak TCDD-a ovisio je o primijenjenim dozama. TCDD je u svim koncentracijama u mnogo većoj mjeri oslobodio lizosomski enzim beta-N-acetil-glukozaminidazu u usporedbi s laktat-dehidrogenazom. Razlika u odgovoru između ispitanih enzima može biti povezana s različitim strukturnim srodnostima, primjerice beta-N-acetil-glukozaminidaze s lizosomskom membranom i laktat-dehidrogenaze s citoplazmom. Nije isključeno da je laktat-dehidrogenaza jače vezana za citoplazmatsku strukturu od vezivanja beta-N-acetil-glukozaminidaze za lizosomsku membranu.

Ključne riječi:
citoplazmatski enzim, izvanstanično oslobađanje enzima, lizosomski enzim

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