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Progress Report: Evaluation of an In Vitro Assay for the Detection of "Dioxin-Like" Activity in the Binghamton State Office Building

by

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Abstract

In an earlier report the application of the cell keratinization assay (CKA) to BSOB soot extracts as a test for polychlorinated dioxins, dibenzofurans and biphenylenes was described. The ability of the test to rank order the extracts with regard to level of contamination suggested that the CKA may be used as a screen for dioxin-like activity. A problem arose however, concerning a decline in the ability of the cells to respond to TCDD. This problem has now been alleviated by technical modificatons of the original protocol.

Further development and verification of what appeared to be a TCDD-induced change in cell morphology ("flat cell" induction) as an alternative assay has also been carried out. Experimental results indicate that this assay shows the same sensitivity to 2,3,7,8 TCDD and 2,3,7,8 TCDF as the CKA. Furthermore, this morphological change appears to be specific for the particular epithelial cell line used in this procedure and is not induced by various other toxic compounds including inhibitors of DNA, RNA and protein synthesis, and mitosis. These results indicate the specificity of flat-cell induction for dioxin isomers and congeners.

Twenty nine benzene extracts of cotton gauze BSOB swipes, prepared by Versar, have been tested using the CKA and flat-cell assays. Results using an internal standard of 2,3,7,8 TCDD indicate loss of activity during the solvent exchange. This may be related to the appearance of a precipitate during the solvent exchange from benzene to DMSO.

Introduction

In the preliminary report of January, 1982 the development and verification of an assay for dioxin-like activity was described (1). The cell keratinization assay (CKA), is based on the ability of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) to induce keratinization in a specific line of epithelial cells grown in culture as originally described by Knutson and Poland (2). Extracts (3) of soot samples taken from ceiling panels of various floors of the BSOB were tested for dioxin-like activity in the CKA and the results were compared to the actual polychlorinated dibenzodioxin (PCDD) and polychlorinated dibenzofuran (PCDF) content of the samples as determined by high resolution GC/MS chemical analysis. The results of this comparison are not contained in the report of January, 1982 because the chemical analysis had not yet been completed. They were, however, presented at the meeting of the BSOB Expert Panel on March 29, 1982 in Binghamton (4). Briefly the level of dioxin-like activity, as determined in the CKA correlated well with that predicted by the chemical analysis. The results of the CKA permitted ranking of the samples with regard to dioxin-like activity, which corresponded accurately to the levels of PCDDs and PCDFs in the samples. These results suggested the potential use of the CKA to screen a large number of samples and rank them with regard to their degree of contamination with PCDDs and PCDFs.

A problem associated with the CKA also became evident, however, during the course of these experiments. The cells exhibited a progressive decline in the magnitude of their keratinization response to 2,3,7,8 TCDD exposure. This decline called into question the utility

of the CKA in an ongoing screening program. Described below is the progress which has been made in solving this problem.

The preliminary report also contained a description of a morphological change which the cells underwent, apparently as a result of exposure to PCDDs and PCDFs. It was suggested that this change might form the basis of an alternative assay ("flat cell assay") for dioxin-like activity. However, very few compounds had been tested using this endpoint; thus, although the results appeared to correlate well with results obtained from the CKA, the specificity of the alternative assay was very uncertain. Described below is progress which has been made with regard to this question.

Results

a) Modification of CKA

New starter cultures of the XB epithelial and 3T3 feeder layer cells were obtained from Dr. H. Green (5). These cultures were propogated and portions were frozen for later use. Previously, cells which had been frozen and thawed were not responsive in the CKA (1). The freezing procedure was modified from that used previously (1), resulting in the recoverability of responsive cells. It is thus now possible to maintain a large supply of frozen cells which can be thawed and propogated when needed. It was also found that an increase in the ratio of 3T3 to XB cells to 10 resulted in an enhanced keratinization response. It now appears that with these two technical modifications, the CKA can be used on an ongoing basis.

b) Validation of the Flat-cell Assay

Since the endpoint for this assay is determined by a subjective determination of altered cell morphology, the reproducibility of the assay as well as its ability to quantitate concentrations of 2,3,7,8 TCDD in various samples was established in a double blind experiment. The results indicated that the reproducability and sensitivity of this assay is comparable to that of the CKA. Various inhibitors of macromolecular synthesis and mitosis were examined for their ability to induce the flat cell morphological change. Hydroxyurea (a DNA synthesis inhibitor), actinomycin D (a RNA synthesis inhibitor), cycloheximide (a protein synthesis inhibitor) and colchicine (a mitosis inhibitor) were all found to be inactive over a broad range of concentrations. This indicates that the flat cell effect is not associated with a general toxicity but may be a specific effect, analagous to, and perhaps related to, keratinization. This possibility is supported by the finding that 2,3,7,8 tetrachlorodibenzofuran (TCDF) also induced the flat cell effect, but had approximately one tenth the potency as 2,3,7,8 TCDD, which is the relative potency which has been reported for these two compounds in the induction of keratinization (2). Furthermore, other cell lines, including fibroblasts and transformed epithelial cells, were tested for their ability to respond to TCDD with a change in morphology, and no flat cell effect was observed. Thus, the specificity of the flat-cell assay appears, from these preliminary experiments, similar to that of the CKA in terms of both inducing compounds and susceptible cells.

c) Application to BSOB samples

Twenty nine benzene extracts of cotton gauze swipes of various locations in the BSOB were received from Versar. These were processed in a manner analogous to the soot samples which had been analyzed previously (1): one ml of a benzene extract was solvent exchanged to 50 µl of DMSO and a dilution series applied to the cells. However, it was noted that after the solvent exchange, a precipitate appeared in the sample extracts and matrix blank extracts but not in the reagent blanks. The precipitate was removed (with some difficulty) by centrifugation. All samples, including those which had been spiked with authentic 2,3,7,8 TCDD gave either very low or no activity in the CKA and the flat-cell assay. Experiments are currently being done to determine the cause of this loss of activity. Among the possibilities which are being tested are that a) there is a loss of activity during the solvent exchange procedure, and b) material in the cotton gauze is extracted by the benzene, but is insoluble in DMSO and the active dioxins and furans adsorb to the precipitated material.

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