Influence of 1,2,3,4-TCDD and 2,3,7,8 -TCDD on the growth of JAR- Choriocarcinoma cells in vitro

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Objective of the study: In order to find out whether PCDDs could interfere with trophoblast function in vivo, choriocarcinoma cells in vitro were incubated with TCDD contaminated medium and their growth pattern recorded using cell count, viability estimates and radioactive thymidine incorporation. Choriocarcinoma cells are derived from non transformed trophoblast tissue. Apart from their immortalized growth kinetics they can serve as an in vitro model for trophoblastic tissue.

Introduction: The trophoblast is the main constituent of the fetal side of the placenta. The placenta is the interface between mother and child, a crucial site of immune reactions between the two systems and a powerful hormone production unit. The successful implantation of the fertilized egg and the survival of the implanted embryo depend on the undisturbed function of the placenta. Earlier we could demonstrate that PCDDs significantly impair the pre-implantation development of mouse embryos in vitro[1]. PCDDs and PCDFs are found in human placental tissue [2,3]. Generally human fertility appears to be decreasing in western societies over the last years. Many people blame polyhalogenated hydrocarbons for this development. Choriocarcinomas are the malignant transformed equivalents of normal trophoblastic tissue. JAR cells are an immortalized cell line derived from a human placental choriocarcinoma. As active hormone producers they may serve as an in vitro model for trophoblast cells. In this study we investigated the influence of TCDD-contaminated cell culture medium on the growth kinetics of JAR cells.

Material and Methods: JAR cells were obtained in a deep frozen vial from the American Type Culture Collection, Maryland, USA. The cell line was reestablished to form an immortalized cell culture, kept at 37°C in an humidified atmosphere of 5% CO_2.
RPMI 1640 cell culture medium (Biochrom) was supplemented with 10% heat inactivated FCS, 1% Glutamine, 1% Antibiotic/Antimycotic (Gibco). 100 ml prepared medium were each stirred overnight at rt with 1g sepharose G-10 (Pharmacia) bearing either 1,2,3,4-TCDD or 2,3,7,8-TCDD at 5 µg/g. Control medium was either used straight or stirred overnight with uncontaminated G-10 sepharose ("Sepharose medium"). The sepharose was finally removed from the media by sterile filtration through a 0.2 µm filter unit. An aliquot of the media was reserved for determination of TCDD concentration (below), the remainder stored frozen in appropriate aliquots until used. 1 ml of aliquoted medium was passed through Merck RP-18

columns which were dried, eluted with n-hexane after the addition of 2.5 ng $^{13}C_{12}$ labelled 2,3,7,8-TCDD standard, dried under a stream of N_2 , finally redissolved in toluene and HRGC/LRMS-analysis was carried out.

JAR cells were cultured in 96 well plates at a starting concentration of $8x10^3$ cells/ well in 50 µl medium plus 50 µl treatment medium (day 0). On day 2 of culture 20 µl tritium labelled thymidine were added at 0.5 µCi/well, 17 hours later (day 3) the cells were harvested on glass fibre paper. In preliminary experiments all culture conditions were found to give optimal results. The harvested cells on glass fibre paper were transferred into counting vials, 2.5 ml liquid scintillation fluid were added, unspecific chemoluminescence was avoided by cold incubation for 24hrs. Finally the vials were counted in a beta counter for 5 min. each. Each treatment group was represented by 10 repetitive wells (n=10). The results were recorded as counts per minute (CPM) +/- standard deviation. In parallel, control wells were used to count the cell number and determine cell viability at the time of harvesting after trypsinization and addition of 0.5% trypane blue solution.

Results: The concentrations of TCDD were as follows:
1,2,3,4-TCDD: 1.583 ng/ml;
2,3,7,8-TCDD: 0.3272 ng/ml.
Fig. 1 shows the results of the preliminary experiments: indicating cell count and corresponding radio thymidine incorporation as measured in counts per minute (CPM) over the duration of cell culture. The starting concentration of JAR cells was 8000 per well. The main experiment was carried out during the uninhibited proliferation phase of the cells until day 3 as indicated in Fig. 1 (below).

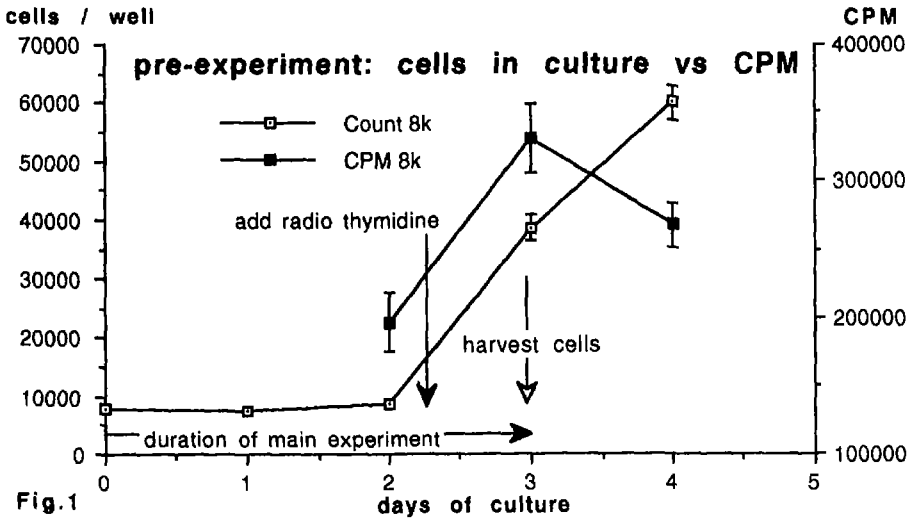

Fig.1

Main experiment: In the control wells treated with straight medium cell growth from day zero (start of culture $8x10^3$ cells/well) to day three (harvesting) corresponded with the findings of the pre-experiments. Mean final cell concentration on day 3 at harvesting was 32416 per well +/- 3621. Cell viability was in excess of 90 %. Fig. 2 demonstrates the incorporated radioactivity in the experimental set up, harvested on day 3.

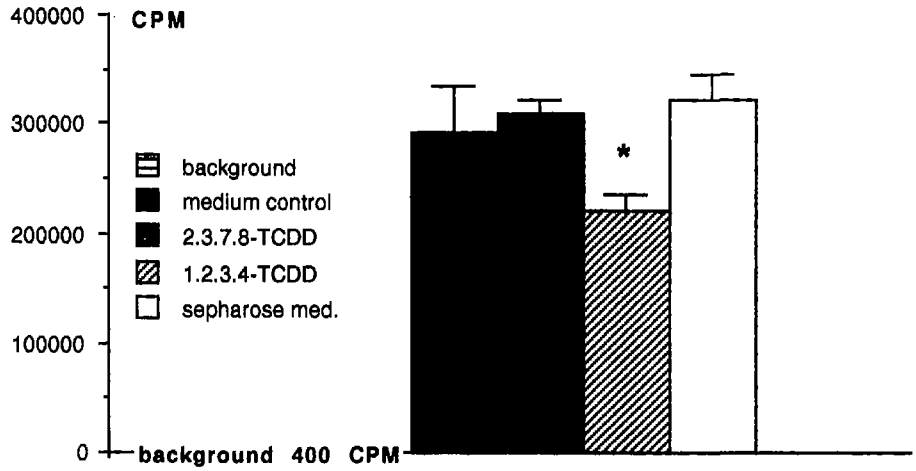

Fig. 2

Analysis of variance for repeated measures of the data was carried out with computer assistance. Significance at the 95% confidence interval was flagged for 1,2,3,4-TCDD vs. 2,3,7,8-TCDD, 1,2,3,4-TCDD versus straight medium and sepharose treated medium.

Discussion and conclusions: As expected the higher water solubility of 1,2,3,4-TCDD led to a higher concentration of this isomere in the medium as compared to the more toxic 2,3,7,8-TCDD. However, considering the TEF of 0.01 for 1,2,3,4-TCDD the 4.8-fold higher concentration of 1,2,3,4-TCDD does not explain the inhibitory effect on the ^3H -thymidine incorporation into JAR cells. Own previous results obtained in experiments[4] on the effects of various PCDD congeners on mouse preimplantation embryo development in vitro seemed to indicate a higher toxic potential of 1,2,3,4-TCDD than 1,2,3,7,8 Pe CDD. TCDD at 5.6 ng/ml yielded a significantly higher rate of degenerated embryos than Pe CDD at 1.6 ng/ml (TEF of 0,5). It appears that dioxin congeners could interfere with trophoblast activity, if the in vitro model character of JAR cells is accepted. However, it has to be pointed out that there are major differences between normal trophoblast in vivo and an immortalized cell line in vitro. Radio-thymidine incorporation as a parameter of cell proliferation and activation is only one aspect of trophoblast cell function. Therefore studies on the effects of dioxins on hormone production of JAR cells are presently under way.

Of particular interest is the repeated observation that 1,2,3,4-TCDD (TEF 0.01) exerts more pronounced biological effects than some 2,3,7,8-substituted congeners with higher TEFs. It will have to be investigated whether this observation describes a peculiarity of 1,2,3,4-TCDD, or whether other non-2,3,7,8-substituted congeners yield similar effects.

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