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Effects of TCDD [2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD)] on the Early Stage of Pancreatic Carcinogenesis Induced by Azaserine in the Rat Pancreas

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Abstract: TCDD (2,3,7,8-tetrachlorodibenzo -p-dioxin), an addictive substance in cigarettes, has been implicated in the pathogenesis of pancreatic carcinoma. This compound has not been tested previously for carcinogenicity towards the pancreas, although TCDD is a known liver carcinogen. The present study was designed to ascertain whether TCDD may have a promoting effect on the development of precursor pancreatic lesions on the rat exocrine pancreas. For this purpose, 30 out of a total of 60 male Leeds rats aged two weeks received a single weekly i.p. injection of azaserine (30 mg/kg body weight) for 5 weeks. The rest of rats (n=30) were used as control groups during the injection period. At 6 weeks of age, azaserine-initiated (n=30) and untreated control (n=30) rats were divided into 4 groups separately each, as follows; Group: 1, UnCt (Untreated control rats) (n=15); Group 2, AzCt (Azaserine-initiated control rats) (n=15); Group 3, TCDD (untreated normal rats fed TCDD, 0.038 µg/kg diet TCDD) (n=15); Group 4, AzTCDD (Azaserine-initiated rats fed TCDD, 0.038 µg/kg diet TCDD) (n=15) for 6 months. Rats were killed and pancreata weighed and prepared for quantitative histologic analysis of atypical acinar cell foci (AACFs), which are putative preneoplastic lesions. Both the number and size of AACFs were analysed. In rats fed TCDD, the ACFF burden was higher than in control rats ($P<0.05$). TCDD feeding to rats injected with azaserine led to a significant increase in AACF burden over control values. The increased size and number of acidophilic AACF of rats fed TCDD (Group 3) and the pancreata of AzTCDD treated rats fed TCDD (Group 4) may indicate an enhancing effect of TCDD. This limited experiment suggests promoting effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), but further studies of longer term and with large doses of TCDD are needed to allow the assessment of whether TCDD affects growth of azaserine induced preneoplastic pancreatic lesions.

Key Words: TCDD, azaserine, pancreas

Introduction

Cigarette smoking is the most consistently reported risk factor for pancreas cancer, yet the dose-response relationship in many pancreas cancer studies is weak. In the present experiment our purpose was to evaluate whether a component of tobacco smoke 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may have promoting effects on the development of precursor lesions of rat pancreatic carcinogenesis, although TCDD is a known liver carcinogen.

In recent years 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (also called dioxin) has been intensively studied (1, 2) as the protype compound for a family of highly toxic and structurally related chemicals known as polyhalogenated aromatic hydrocarbons. Chemically 2,3,7,8-tetrachlorodibenzo-p-dioxin is lipophilic, almost insoluble in water (3). Furthermore, TCDD may arise during the heating or combustion of many polychloronited biphenyls and is also a combinant of some pesticides (4). It is extremely toxic, with an LD^{50} of 0.045 mg/kg for rats and 0.006 mg/kg for guinea pigs. It has also been found that cigarette smoking (20/day) induces the intake of approximately 50 μ/m^3 of various dioxins (5). It has been estimated that a typical person in most industrialised countries ingests as much as 500 fg/kg/day (1fg=1x10-6) of TCDD (4), and epidemiological studies indicate that a life time daily

uptake of 100 and 1000 fg/kg/day poses a minimal cancer hazard (6). It has been demonstrated that there is an increased incidence of tumours at multiple sites, but especially in the liver, in animals exposed to TCDD (7). Although it was suggested that TCDD was a tumour initiator (8), a study performed by Pitot (9) showed that TCDD alone could not initiate liver foci or liver tumours in rats. However, other studies on the mechanism of 2,3,7,8-TCDD carcinogenecity have found it also to be a cocarcinogen (10) or a weak carcinogen (11), depending on the animal model studied. It has been shown that two years of skin painting in mice with TCDD resulted in fibrosarcomas at the side of application, and it is now considered by some to be a complete carcinogen rather than just a promoter (12). A recent study performed on zebrafish (*Danio rerio*) has shown that after TCDD application to fish eggs, a variety of epithelial tissue and pancreatic lesions are seen (1). There is strong evidence that TCDD is a promoter, is not mutagenic and probably acts through a receptor mediated mechanism (13). Any interaction of TCDD with nucleic acids would most likely be through the formation of a physical complex (intercalation) rather than by direct chemical reaction (14).

Hitherto, there has been no direct evidence of an effect on pancreatic carcinogenesis, although its presence in cigarette smoke makes it a candidate factor in smoking-related pancreatic cancer. Use of the azaserine-rat model to evaluate the effect of TCDD on exocrine pancreas carcinogenesis may lead to a better understanding of the action of this substance.

Material and Methods

Animals

Inbred male Leeds strain rats were obtained from our breeding colony and were housed and kept five animals to a cage under standard conditions (room temperature 23°C; lighting 7am-7pm), on sawdust bedding. A standard diet (Paterson and Christopher Hill Group, Porton-Rat diet PRD) and tap water were supplied *ad libitum*.

Chemicals

Azaserine (98% purity), 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was purchased from Diverse Analyticals Ltd, Manchester, UK, and was determined by gas chromatography-mass spectrometry to be 99% pure. Formalin was obtained from BDH Chemicals Limited, UK.

Treatment

Starting at two weeks of age, 30 out of a total of 60 male Leeds rats received a single weekly i.p. injection of azaserine (30 mg/kg body weight) for 5 weeks, dissolved in 0.9% NaCl solution to a final concentration of 30 mg/ml on the day of injection, and untreated rats (n=30) were used as control groups. The pups were then returned to their respective dams and allowed to continue suckling until 21 days of age. At 6 weeks of age, azaserine-initiated (n=30) and untreated control (n=30) rats were divided separately into 4 groups, as follows: Group 1,UnCt (untreated control rats) (n=15); Group 2, AzCt (Azaserine-initiated control rats) (n=15); Group 3, TCDD (untreated normal rats fed TCDD, 0.038 µg/kg diet TCDD) (n=15); Group 4, AzTCDD (Azaserine-initiated rats fed TCDD, 0.038 µg/kg diet TCDD) (n=15). During the experiment, control groups (Groups 1 & 2) were fed the standard diet for 6 months after the last dose of azaserine, while Groups 3 and 4 were fed the same diet containing 0.038 µg/kg of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) for the same time. TCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was dissolved in a minimal quantity of acetone before thorough mixing with standard diet. All the animals were killed at 6 months after the start of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) feeding.

Stereological analysis

At the end of 6 months the rats were sacrificed by decapitation. The entire rat pancreas was excised at autopsy and all adherent fat, mesentery and lymph nodes were carefully trimmed off. The wet weight of each pancreas was recorded before fixation in 10% buffered neutral formalin for approximately 8-18 h. Before immersion in the fixative solution, each pancreas was spread out on a piece of porous paper to ensure maximal transectional area for subsequent sectioning. Thus, a single section of maximal area was obtained for stereology from each pancreas. Sections were then cut at 5mm on a microtome and stained with haematoxylin and eosin and were examined by light microscopy. Acidophilic foci in the sections were identified and classified according to the established criteria (15). The total area of exocrine pancreatic tissue was measured directly in a single histological section from each pancreas by means of a VIDS III video image analyser (Analytical Measuring systems, Cambridge). The same instrument was used to count acidophilic and basophilic AACF and to measure their transactional area. The observed data were processed numerically by a computer software package

(Volugen), which uses an algorithm based upon the mathematical formula of Campbell (16), as modified by Pugh and his co-workers (17).

Statistical analysis

Numbers of pancreatic foci, mean values and either standard errors of means were determined for all data. Non-parametric statistical analyses were performed by one-way analysis of variance and the Mann-Whitney U-test.

Results

Body and Pancreas weights

All rats were healthy and thriving at the end of the experiment. The rats fed TCDD alone (Group 3) had a significant mean body weight increase compared with UnCt (1) rats. The mean body weights of AzTCDD, UnCt and AzCt rats were very similar to each other, as seen in Table 1. Mean pancreatic weights were markedly increased by TCDD, both in TCDD (Group 3) and AzTCDD rats (Group 4). The variation was statistically significant between the TCDD (Group 3) and UnCt (Group 1) rats, but not between AzCt rats (Group 2) and AzTCDD rats (Group 4).

Quantitative Analysis of AACF

Two phenotypically different types of atypical acinar cell foci, basophilic and acidophilic AACF were observed in all groups.

Basophilic AACF

The quantitative stereological data are presented in Table 2. Quantitative analysis of tissue sections showed that there was an enhancing effect of TCDD on basophilic

*=P<0.05

Table 1. Effect of 2,3,7,8-TCDD Feeding, Following Initiation with Azaserine, on Rat Body and Pancreatic Weights. (Mean Value±Standard Error of Mean)

Group No.of rats	(1) UnCt 15	(2) AzCt 15	(3) TCDD 15	(4) AzTCDD 15
Body weight (g)	326.0±19.8	362.9±27.8	391.4±40.3 *vs UnCt	368.4±25.6
Pancreatic weight (g)	0.700±0.131	1.298±0.163	1.230±0.179 *vs UnCt	1.362±0.192

Abbreviations
UnCt : Untreated control rats
AzCt : Azaserine-initiated control rats
TCDD : Untreated rats fed only TCDD
AzTCDD : Azaserine-initiated TCDD fed rats

*=P<0.05

Table 2. Effect of 2,3,7,8-TCDD Feeding, Following Initiation with Azaserine, on Induction of Pancreatic Basophilic Atypical Acinar Cell Foci (AACF). (Mean values±Standard Error of Mean)

Group No.of rats	(1) UnCt 15	(2) AzCt 15	(3) TCDD 15	(4) AzTCDD 15
No. of AACF per pancreas	0.57±1.82	282.17±120.07	7.17±14.15 *vs UnCt	318.72±163.91 *vs AzCt
Volume of AACF as %of pancreas	0.002±0.006	0.144±0.78	0.002±0.005	0.185±0.123
Mean focal diameter (mm)	0.23±0.423	0.30±0.04	0.25±0.03	0.24±0.085
Mean focal volume (mm3)	0.003±0.021	0.068±0.0058	0.0044±0.0014	0.0106±0.0117 *vs AzCt

AACF incidence. At six months, AzTCDD rats exhibited a significantly increased abundance of basophilic AACF compared to AzCt rats, reflected in the number of AACF per pancreas (318.72 vs 282.17) and mean focal volume (0.0068 vs 0.0106). In addition, the number of AACF per pancreas in rats fed TCDD was significantly higher than in UnCt animals.

Acidophilic AACF

As shown Table 3, azaserine initiated rats fed TCDD had a higher number of AACF per pancreas (98.02 vs 117.94), and volume of AACF as % of pancreas (0.741 vs 1.306) than AzCt rats, and the differences were significant ($P<0.05$) between the two groups. There was a statistically significant increase in most of the quantitative stereological parameters of acidophilic AACF in the exocrine pancreas of TCDD rats compared with UnCt rats. The differences were statistically significant ($P<0.05$) except for the mean focal diameter of AACF.

Discussion

The body and pancreatic weights of rats fed TCDD without azaserine initiation were significantly increased in this study, in contrast to previous reports (17). It has been suggested that TCDD may induce multiple endocrine-mediated metabolic alterations (18). In a previous study it was suggested that body weight loss in TCDD-induced rats could be attributed to a decrease in pancreatic insulin secretion, since this was not assayed. However, it seems that the body weight changes in rats fed TCDD may be related to hormone release. It has been shown that TCDD-induced somatostatin levels coincided with an increase in the body weight of rats (19), which suggests that TCDD administration may alter pancreatic function and weight. TCDD is known to produce hepatic tumours in rats (20, 21). However, TCDD has little if any mutagenic activity and does not bind appreciably to DNA in vivo (22). It has been suggested, therefore, that this compound is not a complete carcinogen, but rather that it acts as a tumour promoter, enhancing neoplastic expression in already initiated cells (9). A recently work suggests that the pathological changes induced by TCDD in the liver and thymus are mediated entirely by aryl hydrocarbon receptor (AhR) (23). Kociba (24) found that TCDD at the dose range of 0.07-0.1 µ/kg/day elicited a positive carcinogenic response in rats. An intermediate dose range of 0.007-0.01 µ/kg/day elicited a less than definitive carcinogenic response in his studies. In this study, the quantitative stereological analysis of basophilic AACF showed that the number of AACF per pancreas was significantly higher in rats fed only TCDD. This suggests that TCDD enhances the development of basophilic foci. However, this type of acidophilic foci is not generally considered to be of great importance in pancreatic carcinogenesis (15). The present findings suggest that in azaserine initiated rats TCDD seems to promote the growth of basophilic foci more strongly than does TCDD fed alone. In this investigation, the number of AACF per pancreas and mean focal volume of basophilic foci were significantly higher in AzTCDD rats than in AzCt animals.

Basophilic AACF seems to have only a low potential for growth and progression to neoplasm, whereas a higher fraction of lesions that are classified as acidophilic

*=P<0.05

Group No.of rats	(1) UnCt 15	(2) AzCt 15	(3) TCDD 15	(4) AzTCDD 15
No. of AACF per pancreas	0.55±1.52	98.02±48.01	12.94±13.83 *vs UnCt	117.94±66.049 *vs AzCt
Volume of AACF as %of pancreas	0.005±0.019	0.741±0.511	0.049±0.424 *vs UnCt	1.306±1.082 *vs AzCt
Mean focal diameter (mm)	0.71±0.81	0.60±0.08	0.426±0.256	0.64±0.082
Mean focal volume (mm3)	0.011±0.003	0.143±0.136	0.044±0.038 *vs UnCt	0.147±0.073

Table 3. Effect of 2,3,7,8-TCDD Feeding, Following Initiation with Azaserine, on Induction of Pancreatic Acidophilic Atypical Acinar Cell Foci (AACF) (Mean values±Standard Error of Mean)

seem to have the potential for such progression. The results of present study indicate an enhancing effect of TCDD in azaserine initiated rats. The number of AACF per pancreas, volume of AACF as % of pancreas and mean focal volume were significantly higher in AzTCDD rats than in the AzCt group. Furthermore, the number of AACF per pancreas, volume of AACF as % of pancreas and mean focal volume quantitative parameters were higher in TCDD rats than in the UnCt group. This suggests that TCDD is a significant factor in the promotion of pancreatic AACF during the post-initiation stage in azaserine initiated rats. The observed increase in the quantitative parameters of the acidophilic AACF of AzTCDD rats in the present study may indicate that there is an effective increase in the incidence of foci in the early stage. Long-term studies conducted on TCDD in mice and rats have shown higher dosages to be associated with an increase in hepatocellular tumours (12, 24). The data reported here are consistent with a promoting effect of TCDD on azaserine-induced pancreatic carcinogenesis. So far the carcinogenic effects of TCDD on the exocrine pancreas have not been sufficiently evaluated to permit us to interpret the present limited findings as indicative of a potential carcinogenic effect. However, the results of present study show that the low level of TCDD (close to environmental levels) is associated with an increased acidophilic AACF incidence. Since the dosage of TCDD used was very low, this result indicates the need for further studies in this area. The data obtained from the present experiment only support the view that TCDD may promote azaserine induced carcinogenesis in the pancreas; however, more exposure and time is necessary for a definitive conclusion. Thus, TCDD remains a plausible candidate as a causative factor in smoking-related pancreatic cancer.

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