CARCINOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN IN EXPERIMENTAL MODELS

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1. Tumor formation/promotion in experimental animals

A major cause of concern related to exposure to 'dioxinlike' compounds is their possible carcinogenicity in humans. Carcinogenic affects were first reported for TCDD, and most experimental data are available on this compound. When administered by different routes and at low doses to rodents (rats, mice, hamsters) TCDD caused tumors at multiple sites. Tumors were observed in various tissues of both sexes, e.g. liver, lung, nasal turbinates, hard palate, thyroid and tongue. Tumor promotion experiments (prior exposure to a known carcinogen and subsequent exposure to TCDD) enhanced tumor incidence.
Reports describe i) squamous cell tumors in the lung, neoplastic nodules and cholangiocarcinoma in the liver in male rats[1]; ii) hepatocellular hyperplastic nodules and hepatocellular carcinoma as well as squamous cell carcinomas of the hard palate, lung, tongue and nasal turbinates in female rats, and thyroid tumors, liver tumors, squamous cell carcinoma of the hard palate, nasal turbinates and the tongue in male rats[2], iii) thyroid follicular cell adenomas and neoplastic nodules of the liver in male and female rats[3], iv) cholangiocarcinoma and hepatocellular adenoma of the liver, cystic keratinizing epithelioma of the lung, gingival squamous cell carcinoma of the oral mucosa, and squamous cell carcinoma of the uterus in female rats[4], v) fibrosarcoma of the integumentary system in female but not in male mice[5], vi) liver tumors (hepatocellular adenoma and carcinoma) in male mice[6], vii) hepatocellular carcinoma in male and female mice, follicular-cell adenoma of the thyroid, lymphoma and subcutaneous fibrosarcoma in femalemice, and lung tumors in male mice[3], viii) hepatocellular adenoma and carcinoma in female and male mice[7], ix) thymic lymphoma in both male and female mice, and hepatocellular carcinoma in male mice[7], and x) squamous-cell carcinoma of the facial skin in male Syrian golden hamsters (s.c. injection)[8].
Administration of TCDD after initiation with known carcinogens enhanced the incidences of various tumor types, e.g. skin papilloma, lung and liver adenoma, or hepatoblastoma in mice. In several rat strains the incidences of hepatic preneoplastic lesions was increased after initiation with *N*-nitrosamines[9].

2. Mechanistic aspects

Conflicting results on genotoxicity

In the Ames test either in the presence or absence of an exogenous metabolic system, TCDD failed to induce mutations[10]. Furthermore, unscheduled DNA synthesis was not induced in normal human mammary epithelial cells[11], while increased formation of sister chromatid exchange was observed in human lymphocytes in vitro[12]. An increase in DNA single-strand breaks was found in rat liver[13], and in rat peritoneal lavage cells[14]. A mixture of TCDD and other congeners caused increased DNA single-strand breaks in liver and brain of treated rats[15]. There is no evidence for the formation of TCDD-derived DNA adducts[16].

The aryl hydrocarbon receptor

A variety of studies have dealt with the effects of AhR polymorphisms or variants in either spontaneous or TCDD-mediated carcinogenicity. In a transgenic mouse line expressing a constitutively active AhR (CA-AhR) spontaneous development of stomach tumors was reported[17]. Treatment of CA-AhR mice with DEN resulted in a pronounced increase in liver tumors when compared to DEN-treated wildtype mice[18]. Han/Wistar rats being relatively resistant to the acute toxicity of TCDD, bearing an AhR with an altered transactivation domain, were exceptionally resistant to liver tumor promotion by TCDD when compared to the more sensitive Long-Evans strain[19].

The role of drug metabolism

Park et al.[20] reported an enhanced release of 8-oxo-guanine (8-oxo-dG) from TCDD-treated Hepa1c1c7 cells. The authors postulated that induced CYP1A1 is the source for reactive oxygen species (ROS) leading to oxidative DNA modification. Treatment of rats with TCDD resulted in a marked increase in 8-oxo-dG, a hallmark of oxidative DNA damage, in liver DNA. Since this effect was much more pronounced in the livers of female rats, which develop significantly more liver tumors at lower doses of TCDD, a correlation between 8-oxo-DG formation and TCDD-induced rat liver cancer was postulated[21]. In fact, the development of preneoplastic hepatic foci was significantly enhanced when male rats received TCDD together with 17b-estradiol (E2)[22]. Since ovariectomy led to a strong reduction in 8-oxo-dG formation in female rats[21], it could be speculated that 4-hydroxyestradiol, an estradiol metabolite formed by AhR-regulated CYPs, may undergo redox-cycling *via* the intermediate formation of the corresponding catechol/semiquinone and may thus act as a source for ROS. Alternatively, CYPs induced by TCDD treatment may release ROS directly and may thus lead to oxidative DNA damage[22,23] or TCDD-induced CYP enzymes may convert E2 into DNA-damaging species.

Effects on apoptosis

Effects of TCDD on apoptosis were found both *in vivo* and *in vitro*. In preneoplastic rat liver, TCDD treatment led to a decrease in the incidence of apoptosis, usually enhanced in preneoplastic vs. surrounding cells[24]. In rat hepatocytes in primary culture, TCDD led to a suppression of UV-induced apoptosis and to a concomitant inhibition of the increase in the tumor suppressor p53 usually seen after UV irradiation[25,26]. Similar results were obtained by Park and Matsumura27 in human MCF10A cells. The authors suggest that TCDD may act by mimicking the anti-apoptotic action of EGF through activation of the *c-Src*/ERK signalling pathway. In TCDD-treated rats, Paajarvi et al.[28] found an attenuation of the hepatic p53 response to DNA- damaging agents and a concomitant decrease in apoptosis in an AhR-dependent manner. Furthermore, TCDD induced the p53 antagonist Mdm2 accompanied by enhanced Mdm2 phosphorylation at Ser166.

3. Conclusions

A variety of studies demonstrated the carcinogenicity of TCDD in rodents. Major target organs are the liver and thyroid, oral cavity and lung in rats, and the liver, thymus, and skin in mice. Interestingly, in rats a reduced incidence of mammary tumors was found.

The striking sex-difference in liver carcinogenicity of TCDD in Sprague-Dawley rats, mainly observed in females, led to the suggestion that ovarian hormones play an important role in this effect. Enhanced formation of ROS probably originating from massive induction of CYPs may play a role as DNA-damaging, initiating event. Since CYP induction by TCDD in rats is not clearly sex-dependent, undefined, estrogen-dependent events must be involved. The better understanding of these mechanisms is crucial for the issue of species extrapolation of the liver carcinogenicity of TCDD. More work is also needed to elucidate the possible role of DNA damage in the other types and locations of tumors found in TCDD-treated rodents. Furthermore, TCDD acts as a liver tumor promoter in rodents pre-treated with genotoxic hepatocarcinogens. For this effect, a variety of mechanisms have been suggested as crucial including inhibition of apoptosis of preneoplastic hepatocytes, suppression of gap junctional intercellular communication, and release from intercellular/paracrine growth control. The molecular mechanisms responsible for these effects may have a common denominator, e.g., enhanced phosphorylation of signalling proteins crucial for growth regulation and apoptosis. It remains open, however, which kinase(s) are relevant for these effects and if the changes in phosphorylation are due to direct stimulation by the AhR and/or are how they are related to the TCDD-mediated oxidative or cell stress accused to result, e.g., in DNA damage.

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