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TCDD activates Mdm2 and attenuates the p53 response to DNA damaging agents

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In this study we investigated the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the p53 response to DNA damaging agents. Pre-treatment of rats with TCDD attenuated the p53 liver response to diethylaminosamine (DEN) and reduced levels of p53 and Ser15 phosphorylated p53. In addition, there were more slowly migrating p53 species, forming a ladder, which suggests an increased ubiquinization of p53 in TCDD-pre-treated rats. Terminal deoxynucleotidyl transferase-mediated X-dUTP nick-end labelling analysis indicated decreased apoptosis rates in the livers of these rats. Studies on aryl hydrocarbon receptor (AhR) knockout mice and their wild-type littermates confirmed this effect in AhR+/+ but not in AhR−/− mice, indicating that this effect may be AhR-mediated. Quantitative RT-PCR analysis revealed no increased mRNA levels in TCDD-treated rats, but immunohistological studies indicated that TCDD modulated Mdm2 protein levels, and in particular, increased nuclear levels in rat hepatocytes in situ. In vitro studies employing HepG2 cells confirmed the in vivo data. Thus, TCDD increased basal levels of Mdm2 protein, but not mRNA, and attenuated the p53 response to a variety of genotoxic and cytotoxic agents. The increase in Mdm2 protein levels was accompanied by rapid and highly sensitive phosphorylation of Mdm2 at Ser166, which has been associated to active Mdm2. In summary, TCDD is a potent inhibitor of p53 that may influence the liver’s ability to handle genotoxic agents in a safe way, and may play a role in TCDD-induced carcinogenesis.

Introduction

The tumor suppressor p53 is one of the best-studied genes. It is mutated, or inactivated by other means, in the majority of human tumors. Knockout mice for this gene (+/− or −/−) are susceptible to spontaneous as well as carcinogen-induced tumors (1). The gene product of p53 is a transcription factor that seems to be inactive in unstressed cells. In response to many types of stresses, including DNA damage, post-transcriptional modifications are introduced and p53 protein accumulates and trans-activates target genes, including Mdm2 and genes inducing growth arrest or apoptosis (1,2). Mdm2 is the main regulator of p53. It codes for an E3 ubiquitin ligase and can terminate the p53 response by inducing p53 ubiquitination and degradation (3). However, p53 function is also modulated by many types of protein modification induced by various factors including oxidative stress, energy metabolism and hormonal status, although the complexity of protein modifications in this signalling network is not fully understood (1). Furthermore, DNA damage signalling kinases, such as ATM, may target both p53 and Mdm2 (4–7). At least some of these changes in the Mdm2 phosphorylation status may precede changes in p53 (8), implying that Mdm2 may act as a primary target for at least some cellular stress signals.

Polychlorinated dibenzo-p-dioxins (PCDDs or ‘dioxins’), furans and biphenyls are widespread, persistent and highly toxic environmental contaminants (9). Due to their lipophilicity and stability they accumulate in the food chain and are present in foods, such as Baltic fish, and human tissues, including mother’s milk (10). Therefore, dioxins are a potentially significant environmental health problem, which is not merely a remote theoretical possibility (compare with ref. 11).

Although carcinogenicity and teratogenicity are characteristic features of dioxin toxicity in laboratory animals, the role of p53 in dioxin-induced carcinogenicity has not been studied thoroughly. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a model compound for all dioxin-like chemicals, and has been used in most experimental studies. It activates the cytosolic aryl hydrocarbon receptor (AhR), and most of the adaptive and toxic effects of TCDD (and other dioxins) and effects on, for example, xenobiotic metabolism (12) and carcinogenesis (13) seem to be mediated by AhR. Data on developmental toxicity are less clear (14).

In a recent study it was shown that AhR mediates the degradation of estrogen receptor α (ERα) through the activation of proteasomes. TCDD also induced a physical interaction between these two receptors (15). In a later study it was shown that ERα could be recruited to the Mdm2 promoter and increase cytosolic Mdm2 levels in MCF-7 mammary cancer cells. Degradation of nuclear receptors was coupled to an increased expression of Mdm2, and ubiquitin ligase activity mediated the degradation (16).

These data, as well as other previous data (17,18), indicate that Mdm2 may not only mediate p53 degradation but also the degradation of nuclear receptors. They also suggest that AhR-mediated signalling may cross-talk with p53 signalling. In this report we present data indicating that pre-treatment of rats with TCDD attenuates the p53 response to DNA damage in the liver. Our in vitro and in vivo studies reveal that TCDD...
modulates Mdm2 protein levels in hepatocytes and that this effect may lead to the rapid onset of p53 degradation. In addition, TCDD induces Ser166 phosphorylation of Mdm2. These alterations of the p53 response to DNA damage can be important for TCDD-induced liver carcinogenesis.

Materials and methods

In vivo studies

Treatment of donor animals. Adult female Han/Wistar (Kuoipo) rats (H/W rats) and Long-Evans (Turka/AB) rats (L-E rats) (compare with refs 19–21 and references therein) were divided into six groups for each rat strain and treated as follows. Group 1: corn oil (vehicle for TCDD) 3 days before death. Group 2: 1 mg TCDD/kg p.o. 3 days before death. Group 3: 10 mg TCDD/kg 8 days before death. Group 4: 0.6 mmol diethylthiobarbituric acid (DEN)/kg 24 h before death. Group 5: 1 mg TCDD/kg 3 days before death and 0.6 mmol DEN/kg 24 h before death. Group 6: 10 mg TCDD/kg 8 days before death and 0.6 mmol DEN/kg 24 h before death. Each group consisted of four rats.

Adult AHRKO mice, originally obtained from the Jackson Laboratories (USA) and their congenic wild-type littermates were treated as p.o. follows. Group 1: corn oil 3 days before death. Group 2: 300 mg TCDD/kg 3 days before death. Group 3: corn oil 3 days before death and 0.6 mmol DEN/kg 24 h before death. Group 4: 300 mg TCDD/kg 3 days before death and 0.6 mmol DEN/kg 24 h before death. Due to difficulty in obtaining these mice each group consisted of one to two animals per gender and genotype.

Immunohistological staining. Livers were fixed and slices were stained as described previously (22). In brief, livers were perfused with 3.7% buffered formaldehyde for 1.5 h and subsequently placed in formalin for 24 h. The slides were treated with microwaves for 5 min each time prior to staining. Antibodies used were mouse monoclonals SMP-14 (Santa Cruz, CA). Mdm2 was visualized using the EnVision+ peroxidase kit (DAKO, Denmark) with 3-diaminobenzidine tetrahydrochloride as substrate. The TdT FragEL apoptosis kit (Calbiochem) was used for TUNEL (terminal deoxynucleotidyl transferase-mediated X-DUTP nick-end labelling) staining.

Immunoprecipitation and western blotting. Livers were homogenized and subfractionated. Immunoprecipitation of p53 was then performed as described previously (23), using monoclonal pAb122 antibodies (Roche, Germany) and protein A-Sepharose (Pharmacia, Uppsala, Sweden). Mdm2 was immunoprecipitated with the polyclonal H-221 antibody (Santa Cruz) and protein A/G agarose (Santa Cruz, CA). Phosphatase treatment for western blotting: the PVDF membranes were incubated with 5 U of calf intestine alkaline phosphatase for 1 h at 37°C before blocking. p53 was detected with CM-1 polyclonal antibodies (Novocastra Laboratories, Newcastle, UK) and with rabbit antibodies directed against p53 phosphorylated at the residue Ser15 (New England Biolabs, CA). SMP-14 (Santa Cruz, CA) and 2A10 monoclonal antibodies (Oncogene™) directed towards Mdm2 were also employed. Cdk2 (M2) was from Santa Cruz. Proteins were visualized using the ECL procedure (Amersham Biosciences).

Quantitative real-time RT-PCR. Total RNA was isolated from flash-frozen rat liver samples with TRIzol solution (Invitrogen, Carlsbad, CA). cDNA was reverse transcribed from 1200 ng RNA with Omniscript (Qiagen, Hilden, Germany). About 200-bp fragments of β-actin and Mdm2 were amplifi ed by conventional PCR, isolated, purified and cloned in pCR Script Amp SK (+) cloning vectors (Stratagene, La Jolla, CA). Plasmid concentrations were determined with GeneQuant II (Pharmacia Biotech, Uppsala, Sweden) and 10-fold dilution series prepared. These series were then employed as external standards in quantitative real-time PCR on Rotor-Gene 2000 (Corbett Research, Mortlake, Australia) with Quantitect reaction mixture (Qiagen). For fluorescence data analysis, both dynamic tube normalization and noise slope correction were enabled. The linear correlation coefficients for the standards were over 0.988. β-Actin was intended to be employed as a housekeeping gene for normalization of Mdm2 data. However, β-actin levels turned out to be induced by DEN at both time points tested and in both rat strains in a statistically significant manner, and therefore this normalization was only used for comparison of basal Mdm2 mRNA concentrations. Mdm2 mRNA levels were compared among the different treatment groups by Kruskal–Wallis non-parametric ANOVA followed by the Mann–Whitney U test.

In vitro studies

Cell culture. HepG2 cells were grown in Minimum Essential Medium with Earle’s salts with 1-glutamine supplemented with 1 mM sodium pyruvate, non-essential amino acids, 10% inactivated fetal calf serum and penicillin-streptomycin. Serum-starved cells were cultured with medium supplemented with 0.5% serum for 24 h. Etoposide, benzo[a]pyrene (B[a]P), α-naphthoflavone (α-NF) and cycloheximide were obtained from Sigma and leptomycin B (LMB) and proteasome inhibitor (PSI) from Calbiochem. TCDD was obtained from Dow Chemicals, USA. The final concentration of DMSO added to the cells was <0.2%.

Western blotting. Cells were washed with PBS and lysed in IPB-7 (with NaF, NaVO₃, trypsin inhibitor, leupeptin, aprotenin, PMSF). The samples were subjected to SDS–PAGE and thereafter blotted onto a PVDF membrane (Bioread, Hercules, CA). The protein bands were subsequently probed using polyclonal antibodies towards p53 (CM-1, Novocastra, Newcastle, UK) and rabbit antibodies directed against p53 phosphorylated at the residue Ser15 (New England Biolabs, CA). Mdm2 was detected employing the antibodies 2A10 (Oncogene™), Mdm2 (phosphorylated at the residue Ser166: New England Biolabs, Beverly, MA) and 3G5. The monoclonal antibody 3G5 was kindly provided by Dr A. Levine. p21 was from Santa Cruz. Cdk2 was used as a loading control (M2, Santa Cruz).

Fig. 1. TCDD pre-treatment attenuated the p53 response to DEN in rat liver. Cells were pre-treated with TCDD (1 µg/kg 3 days or 10 µg/kg 8 days before death) and a challenging dose of DEN was given 24 h before death. (A) Nuclear liver fractions from seven L-E rats were immunoprecipitated for p53 and analysed employing western blotting. Antibodies for p53 or for Ser15 phosphorylated p53 were used. (B) A similar result obtained with nuclear fractions derived from 16 H/W rats.
corresponding to p53 as well as to Ser15 phosphorylated p53 were much smaller in rats treated with TCDD. In previous studies (8) we have shown that p53 levels, as well as Ser15 phosphorylated p53 levels, peaked at 24 h after DEN administration in Sprague-Dawley (SD) rats, suggesting that the effects obtained with DEN alone represent peak levels.

Similar experiments in H/W rats, which are resistant to some of the toxic effects of TCDD (19,24), also showed that TCDD pre-treatment attenuated the p53 response to DEN (Figure 1B), indicating no obvious inter-strain difference in response. Both the high dose and the low dose of TCDD induced a similar attenuation of the p53 response, suggesting that the lower dose of TCDD was sufficient to induce maximal effects on parameters studied here.

As expected, TCDD alone did not affect p53 levels at either 10 or 1 µg/kg dose (data not shown). However, TCDD treatment (1 µg/kg 3 days before death) increased the immunohistochemical staining for Mdm2 and resulted in a characteristic midzonal cytoplasmic staining (Figure 2B), similar to that described previously in SD rats (8). In addition there was increased nuclear staining, and increased staining in centrilobular hepatocytes. Similar changes but higher constitutive Mdm2 expression was observed in H/W rats (data not shown). It thus seems that TCDD treatment induced alterations in both strains of rats characterized by increased nuclear and cytoplasmic staining for Mdm2. In control experiments and in accordance with the data presented in Figure 2, western blot analysis indicated increased Mdm2 protein levels in L-E rats 3 days after TCDD administration (1 µg/kg; Figure 3). This effect was not clearly seen in H/W rats (data not shown), which can, for example, be explained by the high background level in these rats (Figure 2C) concealing smaller increases.

At the mRNA level, DEN increased Mdm2 expression >10-fold 24 h after administration (Figure 4). Inductions of a similar magnitude were seen previously in SD rats (8) and are consistent with the well-known transactivating effect of p53 on Mdm2 expression in L-E rats. TCDD (1 µg/kg) administered 3 days prior to death significantly potentiated DEN-induced Mdm2 expression, whereas TCDD had no effect alone. This effect was however not seen at the high dose of TCDD 8 days after administration.

Analysis of mRNA levels for Mdm2 in H/W rats indicated that control levels were slightly higher than in L-E rats (0.07 versus 0.04 relative to β-actin). Again, TCDD alone had no effect on mRNA levels (Figure 4). Likewise, DEN alone markedly increased mRNA levels and the lower dose potentiated this effect.

In order to further study p53-dependent transactivation we analysed the number of TUNEL-positive cells in these rats. It was found that pre-treatment with both the low and the high dose of TCDD decreased the number of TUNEL-positive hepatocytes, as analysed 24 h after DEN administration. Figure 5 shows that most TUNEL-positive cells were seen in centrilobular areas in DEN-treated rats. This was expected from previous studies (8). After TCDD pre-treatment there were very few positive cells in centrilobular areas. Similar results were obtained in both strains of rats.

As mentioned previously, Mdm2 exhibits E3 ubiquitin ligase activity and may terminate the p53 response by ubiquitination of p53 (3). Further analysis of p53 indicated that homogenates from TCDD pre-treated rats contained proteins that reacted with the p53 antibody but that had migrated with a slower speed than the main band (which is overexposed in Figure 6). The pattern of bands is similar to that interpreted previously to indicate ubiquitinated p53 (25,26), suggesting that there is an increased ubiquitination and degradation of p53 in TCDD + DEN-treated rats.

Taken together these in vivo data indicate that TCDD alone can up-regulate basal protein levels of Mdm2 in rat hepatocytes. This effect might explain the low levels of p53 seen at 24 h after a challenging dose of DEN. As Mdm2 acts as an ubiquitin ligase, it may also explain the relatively high levels of slowly migrating p53 species in TCDD pre-treated rats.

In vivo experiments with AhR knockout mice
In a pilot study we investigated the effect of TCDD on the DEN-dependent p53 response in AhR knockout mice.
G.P

TCDD alone for 24 h. TCDD increased Mdm2 levels in a

Cdk2 was used as a loading control in these western blot

experiments. In vitro experiments on these mice is needed.

was AhR dependent, even though a more comprehensive study

response (Figure 7). The data suggest that the effect of TCDD

functional changes. The data confirm the previous

for p53, p21, was decreased in proportion to the decrease in

(Figure 9B). Figure 9B also shows that the downstream target

to DNA damaging agents are shown. B[a]P, 5-Fu and etoposide, chosen to exhibit different toxicokinetics and/or toxicodynamics, were used to induce p53 accumulation. LMB, a blocker of the nuclear export of p53, was also used. Pre-treatment with 0.5 and 1.0 nM TCDD for 24 h attenuated the p53 response to 5-Fu, etoposide and LMB. The effect of TCDD on the B[a]P response was less consistent in this experiment. Similar results as shown in Figure 8B were obtained by using serum-starved (G0) cells (data not shown). This indicates that the effects of TCDD were independent of cell cycle changes. We also pre-treated cells with αNF, an AhR antagonist, and found that it partially prevented the effect of TCDD on LMB-induced p53 accumulation (Figure 8C).

Although TCDD is a strong inducer of xenobiotic metabolism (12), the fact that similar results were obtained with all stressors (Figure 8B) suggest that the TCDD-induced alterations in p53 response were not dependent on alterations in xenobiotic metabolizing enzymes. Furthermore, a previous study showed that TCDD, added 30 min after a UV dose, attenuated the p53 response in rat hepatocytes (28). We also performed experiments in which TCDD was added after the p53-accumulating agent. In this case Ser15-phosphorylated p53 was also analysed, and etoposide induced a response in p53 as well as in Ser15-p53, which is consistent with the fact that etoposide induces double strand breaks. PSI, a blocker of proteasomal degradation, also induced an accumulation of p53, however, without affecting levels of Ser15-p53 (Figure 9A). In both cases, TCDD attenuated the p53 response and the Mdm2 response in these cells (Figure 9A). Similarly, TCDD ameliorated the p53 response following exposure to B[a]P (Figure 9B). Figure 9B also shows that the downstream target for p53, p21, was decreased in proportion to the decrease in p53 levels, implying that the lowered p53 levels reflected functional changes. The data confirm the previous in vivo and in vitro data on p53 (see above). In addition, the finding that TCDD treatment resulted in similar effects when added after B[a]P, supports the notion that TCDD can induce alterations in the p53 response independently of, for example, changes in xenobiotic metabolism.

We also tested shorter exposure periods. When TCDD was added 20 h after B[a]P treatment and 3 h before termination it did not induce a clearly visible effect on Mdm2 levels in B[a]P-stressed cells (Figure 9C), which may relate to the short TCDD-exposure period. However, in the presence of cycloheximide, an inhibitor of protein synthesis, it could be seen that Mdm2 levels decreased more rapidly in TCDD-treated cells than in controls. A plausible explanation for this effect is that TCDD has the capacity to increase the degradation of Mdm2 in stressed cells with augmented levels of p53/Mdm2 complexes. However, it cannot be excluded that the degradation of the p53/Mdm2 complexes was partially dependent on protein synthesis, and that TCDD abolished this effect.

Since many post-translational modifications may affect Mdm2 function, we also investigated the effect of TCDD alone on Mdm2 phosphorylation. By employing an antibody specific for Ser166 phosphorylation of Mdm2 we detected increased levels of phosphorylated Mdm2 already after 15 min (Figure 10A), indicating a rapid induction of

Employing the antibody for Ser15 phosphorylated p53, it was found that −/− mice responded to DEN to the same extent as +/+ congenic littermates (Figure 7). However, there was no apparent difference after TCDD pre-treatment in −/− mice, whereas +/+ congenic littermates exhibited an attenuated p53 response (Figure 7). The data suggest that the effect of TCDD was AhR dependent, even though a more comprehensive study on these mice is needed.

In vitro experiments

In vitro experiments were performed employing HepG2 cells. Cdk2 was used as a loading control in these western blot studies. Figure 8A shows results obtained with exposure to TCDD alone for 24 h. TCDD increased Mdm2 levels in a
dose-dependent fashion, confirming the in vivo data shown in Figures 2 and 3. As reported previously (27) the Mdm2 antibody (2A10) gave two bands.

In Figure 8B, the effects of TCDD pre-treatment on responses to DNA damaging agents are shown. B[a]P, 5-Fu and etoposide, chosen to exhibit different toxicokinetics and/or toxicodynamics, were used to induce p53 accumulation. LMB, a blocker of the nuclear export of p53, was also used. Pre-treatment with 0.5 and 1.0 nM TCDD for 24 h attenuated the p53 response to 5-Fu, etoposide and LMB. The effect of TCDD on the B[a]P response was less consistent in this experiment. Similar results as shown in Figure 8B were obtained by using serum-starved (G0) cells (data not shown). This indicates that the effects of TCDD were independent of cell cycle changes. We also pre-treated cells with αNF, an AhR antagonist, and found that it partially prevented the effect of TCDD on LMB-induced p53 accumulation (Figure 8C).
Ser166 phosphorylation of Mdm2. In this case insulin was used as a positive control, as previous data showed that insulin rapidly induce Ser166 phosphorylation of Mdm2 (29). Even picomolar concentrations of TCDD were effective (Figure 10B) indicating that very low concentrations of TCDD have the capacity to rapidly induce Ser166 phosphorylation of Mdm2.

**Discussion**

The biological impact of dioxins is characterized by many apparently unrelated biological effects. Long-term effects include carcinogenesis, hyperkeratinization, malformations and also ischaemic heart disease. Other effects involve induction of xenobiotic metabolism, disturbances of lipid...
metabolism, immunotoxicity and endocrine effects (12,19). The AhR seems to mediate most toxic effects, but all targets for AhR may not have been identified and TCDD-induced carcinogenesis is not fully understood. In this study, we showed that TCDD alone rapidly induced phosphorylation of Ser166 on Mdm2 and modulated nuclear and cytoplasmic levels of Mdm2 protein in hepatocytes. In addition, it was demonstrated that TCDD could affect the p53 response to DNA damaging agents.

The p53 response to DNA damaging agents was attenuated by TCDD. It is well known that p53 and Mdm2 interact in an autoregulatory loop. Most studies of this loop focus on p53 accumulation, as triggered by, for example, DNA damage (30,31). Mdm2 is implicated as a downstream target for p53 accumulation, as triggered by, for example, DNA damage (8,29). We thus suggest that TCDD-induced alterations in Mdm2 might shorten the p53/Mdm2 autoregulatory loop (34,35) or activation of the CDK inhibitor p27 (36).

Increased protein level and post-translational modifications of Mdm2 might shorten the p53/Mdm2 autoregulatory loop (8,29). We thus suggest that TCDD-induced alterations in Mdm2 expression documented here curtailed the p53 response by a more rapid formation of p53/Mdm2 complexes and facilitated p53 degradation. Such a curtailed loop may explain the low levels of p53 seen in TCDD + DEN-treated cells (Figure 9C). It is also supported by the finding that TCDD attenuated the p53 response irrespective of the stress factor employed. Thus, PSI- and LMB-induced p53 accumulation were affected in the same way as B[a]P-, DEN-, 5-Fu- or etoposide-induced p53 responses. In a previous study it was shown that also a UV-induced p53 response was attenuated by TCDD (28). The data, as well as the fact that TCDD induced effects also when added after the stress factor, suggest that the TCDD-induced attenuation was not mediated by alterations in DNA-damage signalling, or by changes in xenobiotic metabolism, but by increased p53 degradation.

TCDD modulated Mdm2 protein levels, and that this effect was independent of p53. We also observed effects that can be described as secondary to the attenuated p53 response, such as decreased p21 levels and the decreased number of TUNEL-positive cells.

Mdm2 levels may vary during the cell cycle (33) and it is thus possible that the increase in basal levels of Mdm2 induced by TCDD in in vitro experiments reflected alterations in cell cycle stages. However, similar results were obtained in serum-starved cells as well as in the in vivo model in which major cell cycle alterations seem unlikely. Furthermore, the effect of TCDD on hepatocyte proliferation is usually described as inhibitory, involving, for example, the AhR binding of Rb (34,35) or activation of the CDK inhibitor p27 (36).
TCDD attenuates the p53 response to DNA damaging agents

Fig. 11. Schematic model for suppression of the p53 response to DNA damaging agents by TCDD.

Even though we cannot rule out, for example, early increases in mRNA levels, we only found evidence for TCDD-induced enhanced transcription of Mdm2 in DEN-treated rats. As discussed above, this effect may have facilitated p53 degradation in these rats, but further studies are needed to understand why for example it seemed restricted to the low TCDD dose/3-day exposure. It may be related to relatively late events such as post-translational alterations of p53, which has been shown to be phosphorylated by TCDD (37).

At least some, if not all, of the effects of TCDD on Mdm2 are best explained by post-translational modifications (Figure 11). Several observations suggest that the Ser166 phosphorylation of Mdm2 was of importance. It correlated in time with the rapid effects seen within 3 and 6 h (Figure 9). It has also been shown that Ser166 phosphorylation of Mdm2 enhances the ubiquitination-promoting function of Mdm2 and leads to a more effective degradation of p53 (26) and decreased apoptosis (38). Interestingly, Ser166 phosphorylation has been related to an increased stabilization/accumulation (39) and nuclear translocations of Mdm2 and a subsequent increased degradation of p53 (26,29,39), so the histological observations (Figure 2) are supported by the phosphorylation data presented in Figure 10. Ser166 phosphorylation is often described as a pAkt-mediated phosphorylation (26,29,38,39), but a recent study showed that ZIP kinase can phosphorylate Mdm2 at Ser166 (40), and the signal transduction pathway induced by TCDD remains to be characterized.

This study showed that pre-treatment not only attenuated the p53 accumulation in response to DNA damaging agents, but also decreased the number of apoptotic hepatocytes and the p21 response. Taken together the data indicate that TCDD has the capacity to attenuate the net response in downstream targets for p53. This attenuation might be important for cellular adaptations to DNA damage and may have an impact on TCDD-induced toxicity and carcinogenesis.

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