EARLY APPEARANCE OF GLUTATHIONE-*S*-TRANSFERASE PLACENTAL FORM-POSITIVE LIVER FOCI AFTER A SINGLE ADMINISTRATION OF 2,3,7,8-TETRABROMODIBENZO-*P*-DIOXIN IN RATS

Ohbayashi H[1], Takanobu K[1], Saito M[1], Aiso S[1], Nagano K[1], Arito H[1], Yamamoto S[1], Fukushima S[1]

[1]Japan Bioassay Research Center, Japan Industrial Safety and Health Association, 2445 Hirasawa, Hadano, Kanagawa 257-0015, Japan

Abstract

An early hepatocellular event as enzyme-altered foci relating to a preneoplastic lesion was examined by immunohistochemical staining of the liver tissue in 15 Crj:Wistar rats of both sexes each administered 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) by gavage at a single dose of 0, 10, 30, 100 or 300 μg/kg body weight. The liver tissue was stained with anti-glutathione-*S*-transferase placental form (GST-P) antibody and examined for microscopic lesions. Two different types of GST-P-positive hepatocytes were found in the TBDD-dosed rats. One type formed a focus composed of several to 60 hepatocytes stained clearly and homogeneously with anti-GST-P antibody, and clearly distinguishable from the adjacent area. Another type formed an area composed of the aggregated hepatocytes stained heterogeneously with anti-GST-P antibody and found predominantly in the centrilobular region. Incidences and severities of the foci and the GST-P-positive areas were increased dose-dependently in the rats of both sexes sacrificed on Day 36 after a single administration. Both the GST-P-positive foci and areas were more profoundly affected in females than in males, indicating a gender difference in the induction of these hepatic lesions. Thus, the present findings suggest that TBDD may exert hepatocarcinogenicity and induce morphological alteration in the liver.

Introduction

Polybromodibenzo-*p*-dioxins (PBDDs) and polybromodibenzofurans (PBDFs) are present in occupational settings and in the general environment, since these compounds are unintentionally produced during thermal decomposition reactions of brominated flame retardants[1]. There are few medical case reports and epidemiological studies on PBDD/PBDF-exposed humans or animal toxicology studies of PBDD/PBDF available for human health risk assessments, as compared with the vast knowledge on chlorinated analogues. Experimental toxicology studies have revealed that 2,3,7,8-tetrabromodibenzo-*p*-dioxins (TBDD) caused systemic, hematological, hepatic, teratogenic and myelogenic toxicities [2-5].

We previously reported that a single oral administration of TBDD by gavage in rats induced long-lasting hepatotoxicity characterized by multinucleated hepatocytes, disarranged hepatocytes and hepatocellular hypertrophy, in addition to increased hepatic levels of aryl hydrocarbon hydroxylase (AHH), ethoxycoumarin *O*-deethylase (ECOD) and ethoxyresorufin *O*-deethylase (EROD)[6]. However, a preneoplastic lesion of altered hepatocellular foci was not detected in the liver tissue by hematoxylin and eosin staining[6].

Glutathione-*S*-transferase-placental form (GST-P) is an accurate marker enzyme for the different histogenetic stages participating in the development of hepatocellular tumors of rats[7-9], but is latent and hardly detectable in normal rat liver[10].

The present study explored the possibility of TBDD-induced hepatocarcinogenicity by detecting any early hepatocellular event as enzyme-altered foci relating to a preneoplastic lesion, using an immunohistochemical method of GST-P. We examined whether GST-P-positive hepatocytes occur as aggregated forms of enzyme-altered foci in the liver shortly after a single administration of TBDD in rats.

Materials and Methods

TBDD (purity >98%) was purchased from Cambridge Isotope Laboratories, Inc. (MA, USA). TBDD was predissolved in degassed toluene and added to corn oil. The toluene concentration in corn oil was 5% for all treated groups. TBDD was orally administered by gavage to groups of 6-wk-old Crj:Wistar rats of both sexes

each at a dose of 10, 30, 100 or 300 µg/kg body weight. Control groups of both sexes received corn oil containing 5% toluene. The total volume administrated to rats was 5 ml/kg body weight. Animals surviving to the scheduled necropsy on Days 2, 7 and 36 or found dead during the 36-day observation period underwent complete necropsy. In addition to the conventional hematoxyline and eosin staining, the liver tissues of the TBDD-dosed and control rats of both sexes were stained with anti-GST-P antibody (Medical & Biological Laboratories, Nagoya, Japan)[9-12].

Results

Immunohistochemical examination of the liver tissues revealed the presence of foci composed of several to 60 hepatocytes stained homogeneously with anti-GST-P antibody and clearly distinguishable from the adjacent tissue in the TBDD-dosed rats of both sexes sacrificed on Day 36 after a single administration (Figure 1). The GST-P-positive foci were found in both 100 and 300 µg TBDD/kg-dosed rats of both sexes, but were not localized in any specific zone of the liver. Their incidence and number of GST-P-positive hepatocytes in the focus were increased dose-dependently (Table 1). More foci were found in females than in males, indicating a gender difference in the induction of the GST-P-positive foci.

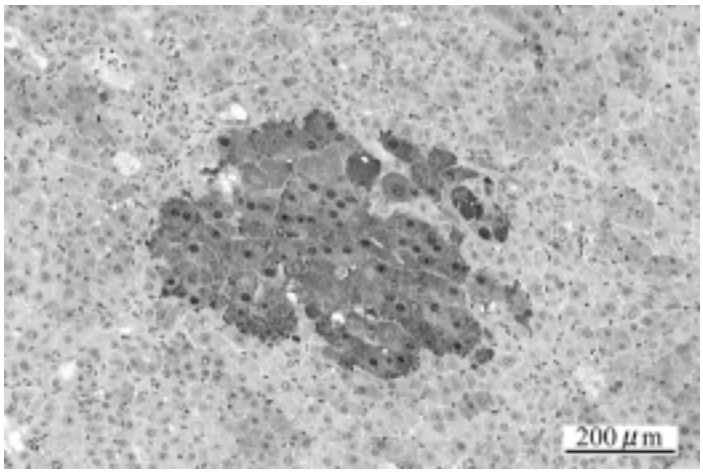

Figure 1.

A GST-P-positive focus composed of 58 hepatocytes in a male rat administered 300 µg TBDD/kg body weight and sacrificed on Day 36.

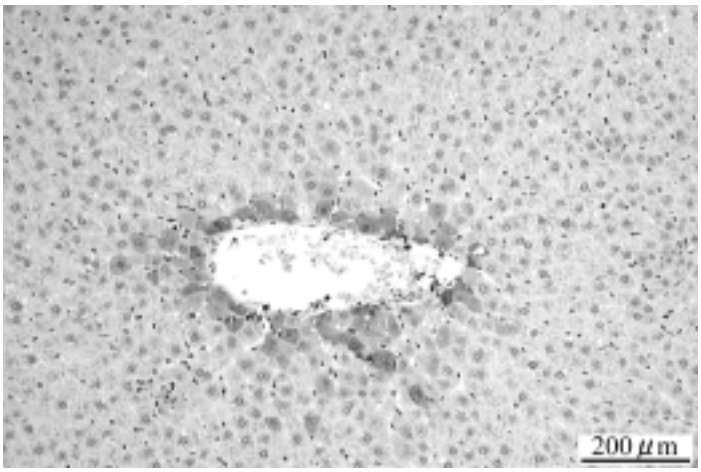

Figure 2.

A GST-P-positive area composed of the aggregated hepatocytes stained heterogeneously with anti-GST-P antibody in a male rat administered 100 µg TBDD/kg body weight and sacrificed on Day 36. The lesion was present predominantly in the centrilobular region, and its severity was scored as 1+, as shown in the footnote of Table 1.

Another type of aggregated hepatocytes stained heterogeneously with anti-GST-P antibody was also found in the TBDD-dosed rats of both sexes (Figure 2), and termed as a GST-P-positive area of hepatocytes. This type of the hepatic lesion was scored for its severity, depending on the percentage area occupied by the GST-P-positive area. Notably, the GST-P-positive area was present predominantly in the centrilobular region, and its incidences and severities were increased dose-dependently (Table 1). The GST-P-positive area was manifest at lower dose levels

for the TBDD-dosed females than the GST-P-positive foci. Females were more sensitive to this type of hepatic lesion than males, indicating a gender difference in induction of the enzyme-altered hepatocytes.

Table 1 Histopathological examination of the hepatocytes stained with anti-GST-P-antibody in the TBDD-dosed and control rats of both sexes sacrificed on Day 36 after the single administration.

Dose of TBDD (μg/kg)	No. Rats	GST-P-positive foci (No. of focus-bearing animals)					GST-P-positive area			
		No. of GST-P-positive hepatocytes in the focus					Grades of severity [a]			
		3-5	6-10	11-20	21-60	Total	–	1+	2+	3+
Male Rats										
0	5	0(0)	0(0)	0(0)	0(0)	0(0)	5	0	0	0
10	5	0(0)	0(0)	0(0)	0(0)	0(0)	5	0	0	0
30	5	0(0)	0(0)	0(0)	0(0)	0(0)	5	0	0	0
100	5	0(0)	0.2(1)	0.2(1)	0(0)	0.4(2)	0	5	0	0
300	5	0.4(1)	0.4(2)	0(0)	0.2(1)	1.0(3)	1	1	2	1
Female Rats										
0	5	0(0)	0(0)	0(0)	0(0)	0(0)	5	0	0	0
10	5	0(0)	0(0)	0(0)	0(0)	0(0)	3	2	0	0
30	5	0(0)	0(0)	0(0)	0(0)	0(0)	2	3	0	0
100	5	0.2(1)	0.2(1)	1.4(2)	1.6(2)	3.4(3)	1	0	4	0
300	2[b]	0(0)	1.5(2)	1.5(2)	0(0)	3.0(2)	1	0	1	0

a) 1+:Less than 10% of total liver area were occupied by the GST-P-positive area.
2+:10 to 40% of total liver area were occupied by the GST-P- positive area.
3+:40 to 80% of total liver area were occupied by the GST-P-positive area.
b) Three female rats died before the scheduled necropsy on Day 36.

Discussion

It has been demonstrated that a single intraperitoneal injection of a hepatocarcinogen, diethylnitrosamine (DEN), in rats induces the preneoplastic, GST-P-positive hepatocellular foci in the liver[9]. Moore et al.[7, 8] reported that the GST-P-positive foci are also induced in the liver at 48 hours and 3 days after a single administration of DEN to rats. The DEN-induced, GST-P-positive foci are thought to be derived clonally from the single "DEN-initiated" hepatocytes, and to develop to hepatocellular tumors. Consistent with morphological features of the DEN-initiated, GST-P-positive hepatocellular foci[7, 8], it was found in this study that a single oral administration of TBDD to rats induced foci composed of several to 60 GST-P-positive hepatocytes. It remains unclear whether TBDD acts directly on DNA, fixes the mutations and initiates the hepatocytes like DEN, since no *in vitro* or *in vivo* genotoxicity studies of TBDD have been reported to date. Early appearance and morphological features of the TBDD-induced GST-P-positive foci found in this study suggest that TBDD may be a hepatocarcinogen like 2,3,7,8-tetrachlorodibenzo-*p*-dioxins (TCDD). Indeed, the GST-P-positive, altered hepatocellular foci are known as a preneoplastic lesion that allows to predict hepatocellular tumors with high probability[9].

TBDD-induced hepatocarcinogenicity is likely to occur, since a chlorinated analogue of TBDD, TCDD, is classified as being carcinogenic to humans (Group 1) by IARC[13], and since there are some similarities in molecular structure and mode-of-actions for toxic effects including a mechanism involved in the Ah receptor between TBDD and TCDD[13]. However, in the absence of convincing evidence that TCDD is a mutagen or that it covalently binds to DNA and in the presence of sufficient evidence in experimental animals for hepatocarcinogenicity of TCDD[14], TCDD is thought not to be directly genotoxic and either may be causing mutations via an indirect mechanism and/or could be acting to promote the development of tumors from previously initiated cells[13]. In the two-stage model for hepatocarcinogenesis, TCDD induced the preneoplastic, GST-P-positive foci in the TCDD-dosed rats only after the initiation by DEN, whereas administration of TCDD alone in the non-initiated rat did not significantly increase the GST-P-positive foci as compared with the control group[15]. Acute and chronic administrations of TCDD marginally increased DNA synthesis and markedly

decreased apoptosis in the GST-P-positive liver foci of the DEN-initiated rat, while these changes were much less pronounced in normal hepatocytes of the rats treated with TCDD[16]. Repeated administration of TCDD in the DEN-initiated, previously partially hepatectomized rat induced enzyme-altered hepatocellular foci stained with glucose-6-phosphatase, canalicular ATPase and γ-glutamyl transpeptidase, whereas the incidence of the enzyme-altered foci was not increased after the repeated administration of TCDD alone in the partially hepatectomized and non-initiated rat[17]. These studies of the two-stage hepatocarcinogenesis model suggest that TCDD may act as a promotor for hepatocarcinogenesis. The early appearance and morphological features of GST-P-positive foci in the liver shortly after a single oral administration of TBDD without any initiating treatment will warrant further studies on carcinogenicity and mutagenicity of TBDD.

Another notable finding is the GST-P-positive area occupied by the aggregated hepatocytes stained positively but heterogeneously with anti-GST-P antibody, which is different from the GST-P-positive foci. These GST-P-positive areas were characterized by their predominant occurrence in the centrilobular region. In our previous study[6], marked morphological changes such as tigroid cytoplasmic basophilia and hepatocellular hypertrophy occurring primarily in the centrilobular region as well as increased hepatic levels of AHH, ECOD and EROD were observed after a single administration of TBDD in rats of both sexes. Thus, the GST-P-positive area was present coincidentally in the same centrilobular region where the marked morphological changes were observed. Primarily centrilobular localization of immunohistochemically stained CYP1A1 in fixed liver sections[18] and CYP1A1 associated enzyme activity measured as EROD activity[19] was also reported in the DEN-initiated, TCDD-dosed rat. These results suggest that TBDD may produce the enzyme-altered hepatocytes in the centrilobular region, which are different from the clonally proliferating, GST-P-positive hepatocytes that morphologically form a focus in the liver.

Acknowledgment
The present study was financially supported by the Health and Labour Sciences Research Grant of Japan, Research on Occupational Safety and Health (Grant-in-Aid No. H14-Labour-26).

References

1. Buser H-R. *Environ Sci Technol* 1986; 20:404.
2. Ivens IA, Löser E, Rinke M, Schmidt U, Neupert M. *Toxicology* 1992; 73:53.
3. Birnbaum LS, Morrissey RE, Harris MW. *Toxicol Appl Pharmacol* 1991; 107:141.
4. Mason G, Zacharewski T, Denomme MA, Safe L, Safe S. *Toxicology* 1987; 44:245.
5. Yamamoto S, Nagano K, Senoh H, Takeuchi T, Matsumoto M, Ohbayashi H, Noguchi T, Yamazaki K, Arito H, Matsushima T. *Environ Health Preventive Med* 2006; 11:136.
6. Ohbayashi H, Sasaki T, Matsumoto M, Noguchi T, Yamazaki K, Aiso S, Nagano K, Arito H, Yamamoto S. *J Toxicol Sci* 2007; 32:47.
7. Moore MA, Nakagawa K, Satoh K, Ishikawa T, Sato K. *Carcinogenesis* 1987; 8:483.
8. Moore MA, Nakagawa K, Ishikawa T. *Jpn J Cancer Res (Gann)* 1988; 79:187.
9. Ito N, Tsuda H, Tatematsu M, Inoue T, Tagawa Y, Aoki T, Uwagawa S, Kagawa M, Ogiso T, Masui T, Imaida K, Fukushima S, Asamoto M. *Carcinogenesis* 1988; 9:387.
10. Sato K, Kitahara A, Satoh K, Ishikawa T, Tatematsu M, Ito N. *Gann* 1984; 75:199.
11. Satoh K, Kitahara A, Soma Y, Inaba Y, Hatayama I, Sato K. *Proc Natl Acad Sci USA* 1985; 82:3964.
12. Tatematsu M, Mera Y, Ito N, Satoh K, Sato K. *Carcinogenesis* 1985; 6:1621.
13. International Agency for Research on Cancer (IARC). *In: IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 69*, IARC (ed.), Lyon, 1997.
14. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CN, Barnard SD, Hummel RA, Humiston CG. *Toxicol Appl Pharmacol* 1978; 46:279.
15. Maronpot RR, Foley JF, Takahashi K, Goldsworthy T, Clark G, Tritscher A, Portier C, Lucier G. *Environ Health Perspect* 1993; 101:634.
16. Stinchcombe S, Buchmann A, Bock KW, Schwarz M. *Carcinogenesis* 1995; 16:1271.
17. Pitot HC, Goldsworthy T, Campbell HA, Poland A. *Cancer Res* 1980; 40:3616.
18. Tritscher AM, Goldstein JA, Portier CJ, McCoy Z, Clark GC, Lucier GW. *Cancer Res* 1992; 52:3436.
19. Tritscher AM, Clark GC, Sewall C, Sills RC, Maronpot R, Lucier GW. *Carcinogenesis* 1995; 16:2807.