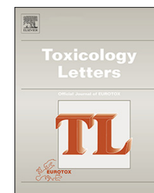
Toxicology Letters 290 (2018) 29–35

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet



The CAR agonist TCPOBOP inhibits lipogenesis and promotes fibrosis in the mammary gland of adolescent female mice

Pengfei Xu[a,b,1], Fan Hong[a,1], Jing Wang[a,c], Shu Dai[a], Jialin Wang[a], Yonggong Zhai[a,b,*]

[a] Beijing Key Laboratory of Gene Resource and Molecular Development, College of Life Sciences, Beijing Normal University, Beijing, China
[b] Key Laboratory for Cell Proliferation and Regulation Biology of State Education Ministry, College of Life Sciences, Beijing Normal University, Beijing, China
[c] Department of Biology Science and Technology, Baotou Teacher's College, Baotou, China

A R T I C L E I N F O

Keywords:
TCPOBOP
CAR
Lipogenesis
Fibrosis
Adolescent
Mammary gland

A B S T R A C T

Constitutive androstane receptor (CAR) is a nuclear receptor that not only regulates drug-metabolizing enzymes but also influences energy metabolism. TC, 1, 4-bis [2-(3, 5-dichloropyridyloxy)] benzene (TCPOBOP) has been shown to inhibit lipogenesis in the liver and adipose tissues. The mammary gland is mainly composed of fat pads and duct systems in adolescent female mice. Here, activation of CAR by TC reduces the mammary gland weight, blocks lipid accumulation by inhibiting lipogenesis and gluconeogenesis, and accelerates collagen formation and fibrosis in the mammary fat pad of adolescent female mice. This information provides a reference for CAR activation, which may affect mammary gland development in adolescent females.

1. Introduction

Constitutive androstane receptor (CAR), also known as nuclear receptor subfamily 1, group I, member 3 (NR1I3), is a sensor and detoxifier of both endobiotic and xenobiotic substances (Baes et al., 1994; Yan et al., 2015). According to multiple published studies, this receptor plays roles in regulating drug-metabolizing enzymes, transporters and energy metabolism. CAR activation up-regulates the expression of target genes of the phenobarbital response element (PBRE) to prevent hepatotoxicity by hydroxylating, conjugating, and eliminating potentially harmful molecules (Wei et al., 2000; Gao and Xie, 2012; Yan and Xie, 2016). Recently, CAR has been reported to be a potential therapeutic target for the treatment of several metabolic diseases, including atherosclerosis (Sberna et al., 2011a; Sberna et al., 2011b), obesity (Gao et al., 2009), fatty liver disease (Dong et al., 2009) and diabetes (Dong et al., 2009; Gao et al., 2009), by balancing endogenous homeostasis of components such as cholesterol, bile acids, bilirubin and glucose.

TC, 1, 4-Bis [2-(3, 5-dichloropyridyloxy)] benzene, (TCPOBOP), which was initially isolated as a pesticide contaminant, is the most potent mouse CAR agonist and is well known to induce liver hypertrophy and hyperplasia in mice (Tzameli et al., 2000; Wei et al., 2000). TC also activates the human Pregnane X receptor (PXR) and is the most potent known member of the phenobarbital-like class of cytochrome P450 (CYP)-inducing agents (Smith et al., 1993). TC-induced CAR activation has been shown to improve insulin sensitivity, inhibit fat accumulation and ameliorate hepatic steatosis in both diet-induced obese and leptin-deficient (ob/ob) mice (Dong et al., 2009; Gao et al., 2009), activated CAR also reversed cholesterol transport in low-density lipoprotein receptor-deficient ($Ldlr^{-/-}$) and apolipoprotein E-deficient ($ApoE^{-/-}$) mice (Sberna et al., 2011a; Sberna et al., 2011b).

Treatment with TC induces CAR activation and influences lipid biosynthesis by targeting downstream lipogenic genes in the liver and adipose tissue (Yan et al., 2015). The mammary gland is an exocrine gland in mammals and is composed of fat pads formed by adipocytes and ductal systems (Brisken and Ataca, 2015). However, no experimental studies have evaluated the effects of TC on lipid metabolism in the mammary gland. Therefore, researchers have not determined whether TC-mediated CAR activation influences the development of the mammary gland. The aim of the present study was to investigate the effects of TC-induced CAR activation on lipid metabolism and fibrosis in the mammary gland of adolescent female mice. TC, the CAR agonist, reduced the mammary gland weight, decreased fat accumulation by

Abbreviations: ACC, acetyl-CoA carboxylase; α-SMA, alpha-smooth muscle actin; CAR, constitutive androstane receptor; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6pase, glucose-6-phosphatase; Gon-WAT, gonadal white adipose tissue; H&E, hematoxylin and eosin; IGF1, insulin-like growth factor 1; PBRE, phenobarbital response element; PAI-1, plasminogen activator inhibitor-1; PEPCK, phosphoenolpyruvate carboxykinase; PGC1α, peroxisome proliferator activated receptor gamma coactivator 1α; PPARα, peroxisome proliferator-activated receptor α; PXR, pregnane X receptor; SCD1, stearoyl-CoA desaturase 1; SREBP-1c, sterol regulatory element-binding transcription factor 1c; TC, TCPOBOP; XRE, xenobiotic response element

* Corresponding author at: College of Life Sciences, Beijing Normal University, No.19 Xinjiekou Wai Street, Beijing, 100875, China.
E-mail address: ygzhai@bnu.edu.cn (Y. Zhai).
[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.toxlet.2018.03.017
Received 30 November 2017; Received in revised form 9 March 2018; Accepted 13 March 2018
Available online 14 March 2018
0378-4274/ © 2018 Elsevier B.V. All rights reserved.

inhibiting lipogenesis and gluconeogenesis, and promoted collagen formation and fibrosis in the mammary glands of adolescent female mice.

2. Materials and methods

2.1. Materials

TCPOBOP (T1442) and Oil Red O were purchased from Sigma-Aldrich (St Louis, Missouri, USA). TCPOBOP was dissolved in DMSO initially, and dissolved in saline configured to 0.05 mg/mL when used. Three-week-old female C57BL/6J mice were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). BODIPY® 493/503 (D3922) was purchased from Thermo Fisher Scientific (Shanghai, China).

2.2. Design of animal experiments

Three-week-old female C57BL/6J mice were randomly assigned to four groups (n = 5), housed in a light- and climate- controlled room (12-h light/dark cycle) and fed normal chow diets. TC-treated mice received intraperitoneal injections of TCPOBOP (0.5 mg/kg) twice per week for 2 or 5 weeks, and the vehicle-treated mice were injected with DMSO. Mice were euthanized with CO^2 at the indicated times. The mammary gland (fourth pairs), liver, and gonadal white adipose tissue (Gon-WAT) were carefully collected and weighted after the mice were sacrificed, and the samples were stored at −80 °C until further analysis. The experimental procedures were performed according to the guidelines of Ethics and Animal Welfare Committee College of Life Science Beijing Normal University, which approved the study (Approval No. CLS-EAW-2015-006); these guidelines conform to the US National Institutes of Health guidelines.

2.3. Histological analysis

Mammary glands, adipose and liver tissues were fixed with 4% formaldehyde. For paraffin sectioning, tissues were embedded in paraffin, cut into 5 μm sections and stained with Hematoxylin and Eosin (H &E), Masson's trichrome, and Sirus red staining using standard procedures (Altamirano et al., 2017; Wang et al., 2017; Xu et al., 2017a). Adipocyte sizes were analyzed using Image Pro Plus software. For frozen sections, tissues were embedded in OCT compound and sectioned into 8 μm sections. Frozen liver sections were stained with Oil Red O using a previously described method to observe lipid droplets (Xu et al., 2016). For immunofluorescence staining, frozen sections of the mammary glands and adipose tissue were incubated with a Cy3-conjugated α-smooth muscle actin antibody (1:400; Sigma-Aldrich) and then stained with BODIPY.

2.4. Gene and protein expression analysis

Total RNA was extracted from tissue using an RNAprep Pure kit from Tiangen Biotech Co., Ltd. (Beijing, China). For the real-time PCR analysis, reverse transcription was performed with oligdT-18 primers and M-MLV transcriptase from Promega (Madison, USA). SYBR Green qPCR SuperMix (TransGen Biotech, Beijing, China) was used to perform the reactions on an ABI 7500 real-time PCR system, according to the manufacturer's instructions and our previous study (Xu et al., 2017b). The primer sequences used are listed in Table 1. Western blotting analysis was performed used the standard process. The membranes were incubated with primary antibodies against fatty acid synthase (FAS, 1:1000; Santa Cruz), stearoyl-CoA desaturase-1 (SCD-1, 1:1000; Santa Cruz), sterol regulatory element-binding transcription factor 1c (SREBP-1c, 1:1000; Santa Cruz) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:2000; Santa Cruz), followed by the appropriate secondary antibodies. The intensity of the protein bands was quantified using ImageJ. Data were normalized to GAPDH levels.

Table 1
Primer sequences used for real-time PCR.

Target gene	Sequence(5′-3′)	Product length (bp)
ACC	F: GGACAGACTGATCGCAGAGA R: TGGAGAGCCCCACACACA	75
CYP2b10	F: AAGGAGAAGTCCAACCAGCA R: CTCTGCAACATGGGGGTACT	147
FAS	F: CCCTTGATGAAGAGGGATCA R: ACTCCACAGGTGGGAACAAG	115
G6pase	F: TCTGCCCCAGGAATCAAAAAT R: TGGGCAAAATGGCAAGGA	77
PAI-1	F: GTCTTTCCGACCAAGAGCAG R: ATCACTTGGCCCATGAAGAG	208
PEPCK	F: AGGAGGAGTACGGGCAGTTG R: CTTCAGCTTGCGGATGACA	62
PGC1α	F: GGAGCCGTGACCACTGACA R: TGGTTTGCTGCATGGTTCTG	176
PPARα	F: ATGCCAGTACTGCCGTTTTC R: TTGCCCAGAGATTTGAGGTC	168
SCD1	F: CCGGAGACCCCTTAGATCGA R: TAGCCTGTAAAAGATTTCTGCAAACC	89
SREBP-1c	F: AACCAGAAGCTCAAGCAGGA R: TCATGCCCTCCATAGACACA	141
GAPDH	F: GTCGTGGATCTGACGTGCC R: TGCCTGCTTCACCACCTTCT	72

2.5. Biochemical analysis

Liver lipid concentrations were measured using the commercially kits, as previously described (Xu et al., 2016). The liver triglyceride assay kit (E1013) and total cholesterol assay kit (E1015) were obtained from Applygen Technologies Co., Ltd. (Beijing, China).

2.6. Statistical analysis

The results are presented as the means ± SEM. Statistical analyses were performed using SPSS version 20.0 (IBM Corp). Differences between groups were analyzed using Student's *t*-test. Statistical significance was set to $p < 0.05$.

3. Results

3.1. TC-induced CAR activation reduced the mammary gland weight

Three-week-old female C57BL/6J mice were treated with vehicle (DMSO) or the CAR agonist TC (0.5 mg/kg, twice a week) for 2 or 5 weeks to determine the effect of TC on the mammary gland (Fig. 1A). As shown in Fig. 1B, TC had almost no effect on body weight. As expected, the expression of CYP2b10, a CAR target gene, was robustly elevated in the TC-treated mice (Fig. S1). Consistent with the findings of a previous study using an obese male mouse model (Gao et al., 2009), TC-induced CAR activation markedly enlarged the liver and inhibited fat accumulation in Gon-WAT (Fig. 1D and E). The mammary gland (breast) is mainly composed of fat pads. Fig. 1C showed a representative pair of mammary glands from a mouse that had been treated with the drug for 5 weeks, at which time the difference in the mammary gland weight between the TC-treated group and vehicle group was completely represented by the tissue/body weight ratio (Fig. 1F).

3.2. TC reduced fat accumulation in the liver, mammary fat pad and Gon-WAT in vivo

Activation of the CAR nuclear receptor ameliorates fatty liver disease in ob/ob mice and HFD-fed mice (Dong et al., 2009). In our normal control diet-fed mice, Oil Red O staining and the liver lipid content analysis indicated that TC also effectively inhibited lipid accumulation

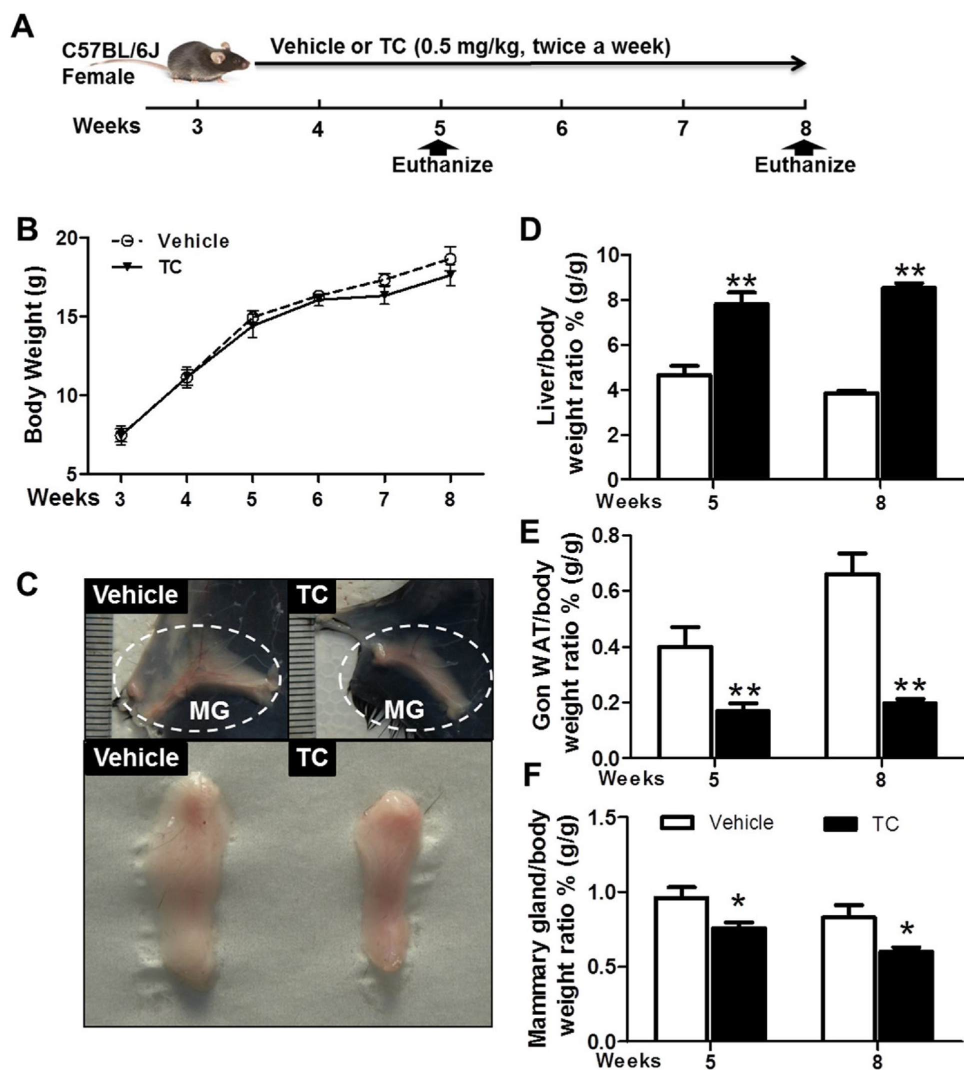


Fig. 1. TC treatment reduced the mammary gland weight in female mice. Three-week-old female C57BL/6J mice were treated with vehicle or TC (0.5 mg/kg, intraperitoneal injections, twice per week) for 2 or 5 weeks. (A), Schematic of the experimental procedures. (B), Growth curve. (C), Gross appearance of a representative pair of mammary gland from vehicle- and TC-treated mice at 8 weeks. Liver/body weight ratio (D), Gon-WAT/body weight ratio (E), mammary gland/body weight ratio (F) of female C57BL/6J mice at 5 and 8 weeks. n = 5 per group. The results are expressed as the means ± SEM. *, $p < 0.05$; **, $p < 0.01$, compared with the vehicle-treated group.

in the mouse liver (Fig. 2A–C). Next, we measured the sizes of adipocytes obtained from mammary fat pad and Gon-WAT sections using an automated imaging analysis (Fig. 2D). According to the analysis of the adipocyte size distribution, the TC treatment increased the number of smaller adipocytes in the two tissues (Fig. 2E). As shown in Fig. 2F, the mean adipocyte size in the mammary gland fat pad and Gon-WAT of the TC-treated mice was reduced to approximately half the size of that in the vehicle control mice.

3.3. TC suppressed the expression of genes encoding lipogenic enzymes

We profiled the expression of metabolic genes in vehicle- and TC-treated mice at 5 and 8 weeks to obtain an understanding of the mechanism by which TC inhibits fat accumulation. In the liver, TC decreased the expression of the lipogenic genes FAS, SCD-1 and SREBP-1c at 5 and 8 weeks and acetyl-CoA carboxylase (ACC) at 8 weeks (Fig. 3A). The expression of the FAS and SCD-1 genes was significantly reduced at both time points and the expression of the ACC and SREBP-1c was reduced at 8 weeks in the mammary gland of TC-treated mice (Fig. 3B). TC significantly decreased FAS expression in Gon-WAT at both time points and decreased ACC, SCD-1 and SREBP-1c expression in Gon-WAT at 8 weeks (Fig. 3C). We also detected the protein production in the mammary gland. It showed that TC treatment significantly decreased FAS and SCD-1 protein expression and had slightly reduction in SREBP-1c (Fig. 3D). The inhibition of lipogenesis was accompanied by a reduction in fat accumulation, as shown in Fig. 2.

3.4. TC suppressed the expression of genes encoding gluconeogenic enzymes

CAR and peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) are master regulators of gluconeogenesis (Gao et al., 2015; Moreau et al., 2008). In TC-treated mice, the hepatic expression of two important gluconeogenic enzymes, glucose-6-phosphatase (G6pase) and phosphoenolpyruvate carboxykinase (PEPCK), as well as the energy metabolism nuclear receptor peroxisome proliferator-activated receptor α (PPARα), and the transcriptional coactivator PGC1α were also significantly reduced at 5 and 8 weeks (Fig. 4A). The expression of these four genes was significantly reduced at 8 weeks, and the expression of G6pase and PPARα was decreased at 5 weeks in the mammary glands of TC-treated mice (Fig. 4B).

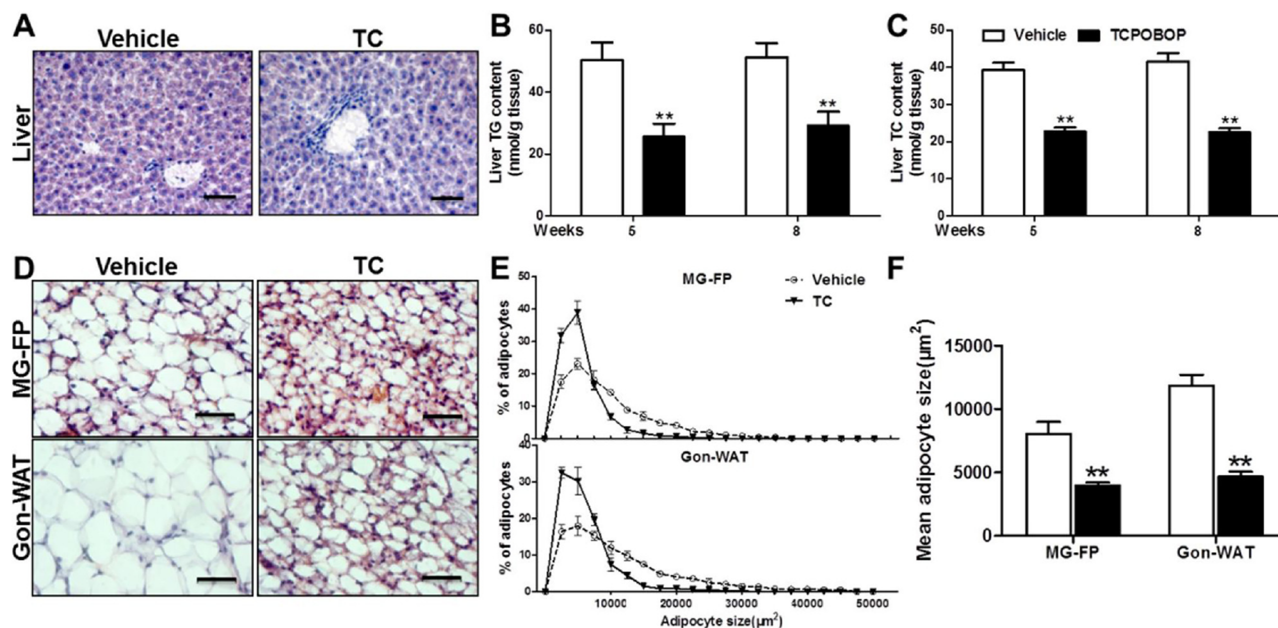


Fig. 2. TC inhibited fat accumulation in the liver, mammary gland fat pad and Gon-WAT in female mice. (A), Oil Red O staining of liver sections from female C57BL/6J mice treated with vehicle or TCPOBOP for 5 weeks. Triglyceride (B) and total cholesterol (C) contents in mouse liver 5 and 8 weeks after treatment. (D), H&E staining of the mammary gland fat pad (MG-FP) and Gon-WAT of vehicle- and TC-treated mice at 8 weeks. Adipocyte size distribution (E) and mean adipocyte size (F) in each group. n = 5 mice per group (scale bar: 50 μm). The results are expressed as the means ± SEM. **, $p < 0.01$, compared with the vehicle-treated group.

3.5. TC promoted collagen formation and fibrosis in the mammary gland and Gon-WAT

CAR activation has been shown to enhance the profibrotic effects on a model of experimental dermal fibrosis (Avouac et al., 2014). We speculated that TC-mediated CAR activation exerts profibrotic effects on the mammary gland and adipose tissue. As shown in the Masson's trichrome and Sirus red staining, the TC treatment promoted collagen formation in mice (Fig. 5A and B). Alpha-smooth muscle actin (α-SMA) is a commonly used marker of myofibroblast formation (Hinz, 2007). As shown in Fig. 5C, the TC treatment resulted in smaller lipid droplets and increased expression of the α-SMA protein in the mammary gland fat pad and Gon-WAT of adolescent female mice. PAI-1 appears to play a significant role in the progression to fibrosis (Ghosh and Vaughan, 2012). Here, the expression of PAI-1was slightly increased at 5 and 8 weeks in the mammary glands of TC-treated mice, although the difference was not statistically significant (Fig. S2).

4. Discussion

The mouse mammary gland is an organ that mainly comprises fat formed by adipocytes during puberty (Macias and Hinck, 2012). During this period, estrogen, growth hormone, and insulin-like growth factor 1 (IGF1) initiate branching morphogenesis to create a ductal tree that fills the fat pad (Macias and Hinck, 2012; Brisken and Ataca, 2015). Lipid biosynthesis plays an important role in mammary gland development in adolescent female mice. In the current study, we investigated the effects of TC-induced CAR activation on lipid metabolism in the mammary gland of adolescent female mice. TC reduced fat accumulation by suppressing the expression of lipogenic- and gluconeogenic-related genes and increased collagen formation and fibrosis in the mammary gland fat pad.

Initially, the toxicity of TC has been studied several decades ago. It showed that TC induced liver hyperplasia and hepatomegaly, stimulated DNA synthesis and promoted hepatocarcinogenesis in mice (Dragani et al., 1985). Intriguingly, TC was a remarkably potent species-selective inducer of CYP450 in mice but not in rats. *In vitro*, TC induced both nuclear localization and CYP2B10 expression in primary hepatocytes (Kelley et al., 1985). TC activation of CAR mediated specific xenobiotic induction of drug metabolism and enhanced acetaminophen-induced hepatotoxicity (Wei et al., 2000; Zhang et al., 2002). Treatment with TC attenuated steatohepatitis through induction of genes involved in fatty acid oxidation in mice fed a methionine/choline-deficient diet (Baskin-Bey et al., 2007). Recently, several studies have investigated the effect of CAR on lipid metabolism *in vivo*, and most of them treated with TC and simultaneously fed an HFD to induced obesity or fatty liver models in adult male wild type and ob/ob mice (Dong et al., 2009; Gao et al., 2009). We previously reported that activation of CAR inhibited the expression of LXR target genes and LXR ligand-induced lipogenesis (Zhai et al., 2010). TC also ameliorated preeclampsia and insulin resistance in high-fat diet-induced obese pregnant mice (Masuyama and Hiramatsu, 2012a, 2012b). The controversy between TC being a toxic substance and a useful therapeutic target for lipid metabolic diseases still exists. Here, we first used 3-week-old adolescent female mice and fed them a normal chow diet for 2 or 5 weeks. In our model, TC inhibited lipid accumulation in the liver and adipose tissues, indicating that lipogenesis was inhibited in female adolescent mice. Furthermore, the mammary gland weight was reduced by approximately 25%. Moreover, TC also inhibited lipogenesis and gluconeogenesis in the breast, particularly in mice that had been treated with TC for 5 weeks. These findings have enabled us to better understand female breast dysplasia during adolescence.

Fibrosis is defined as the formation of excessive deposits of extracellular matrix components in various tissues or organs during a reactive or repair process (Birbrair et al., 2014). Fibrosis plays a major role in adipose tissue dysfunction and has attracted increasing attention (Buechler et al., 2015; Sun et al., 2013). Few studies have examined fibrosis in mammary gland fat pads, but remodeling and stiffness in the mammary gland extracellular matrix during normal development is associated with tumor progression (Schedin and Keely, 2011). TC-induced CAR activation was recently shown to contribute to the pathological development of fibrosis in a mouse model of systemic sclerosis by regulating TGFβ signaling (Avouac et al., 2014). TC was initially isolated as a pesticide contaminant. Further studies are required to determine whether TC administration mediates collagen formation and fibrosis in the breast and is correlated with breast cancer.

Fig. 3. TC inhibited lipogenesis in the liver, mammary gland and Gon-WAT. The mRNA expression of genes involved in lipogenesis was measured in the liver (A), mammary gland (B), and Gon-WAT (C) using real-time PCR in mice that had been treated for 5 and 8 weeks. n = 5 per group. (D) The protein production and relative expression were measured by western blot in the mammary gland at 8 weeks. The results are expressed as the means ± SEM. *, $p < 0.05$; **, $p < 0.01$, compared with the vehicle-treated group.

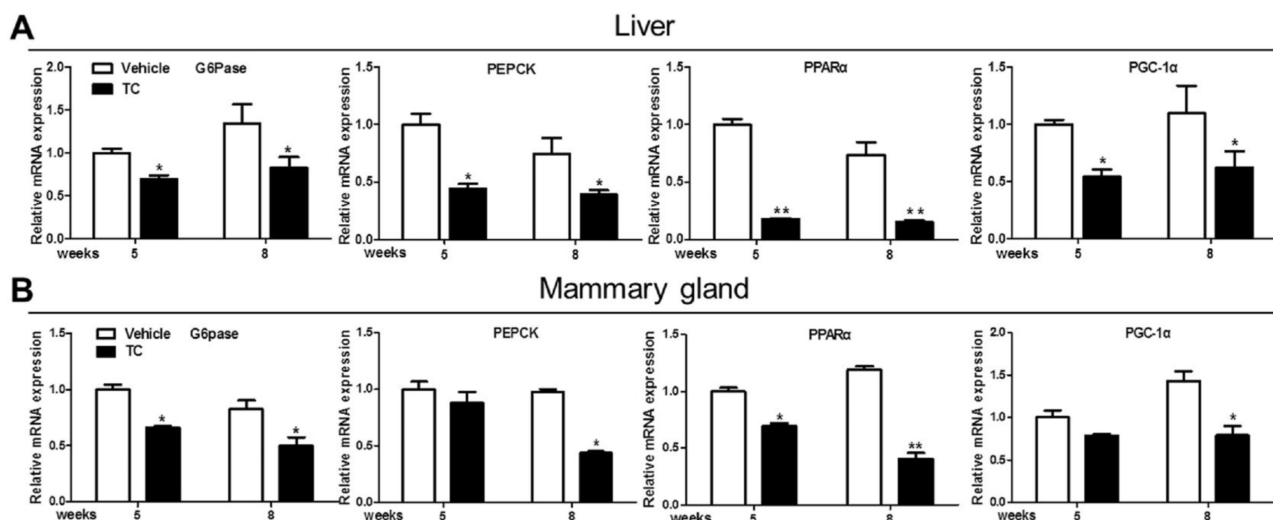


Fig. 4. TC inhibited gluconeogenesis in the liver and mammary gland. The mRNA expression of genes involved in gluconeogenesis was measured in the mouse Liver (A) and mammary gland (B) using real-time PCR 5 and 8 weeks after treatment. n = 5 per group. The results are expressed as the means ± SEM. *, $p < 0.05$; **, $p < 0.01$, compared with the vehicle-treated group.

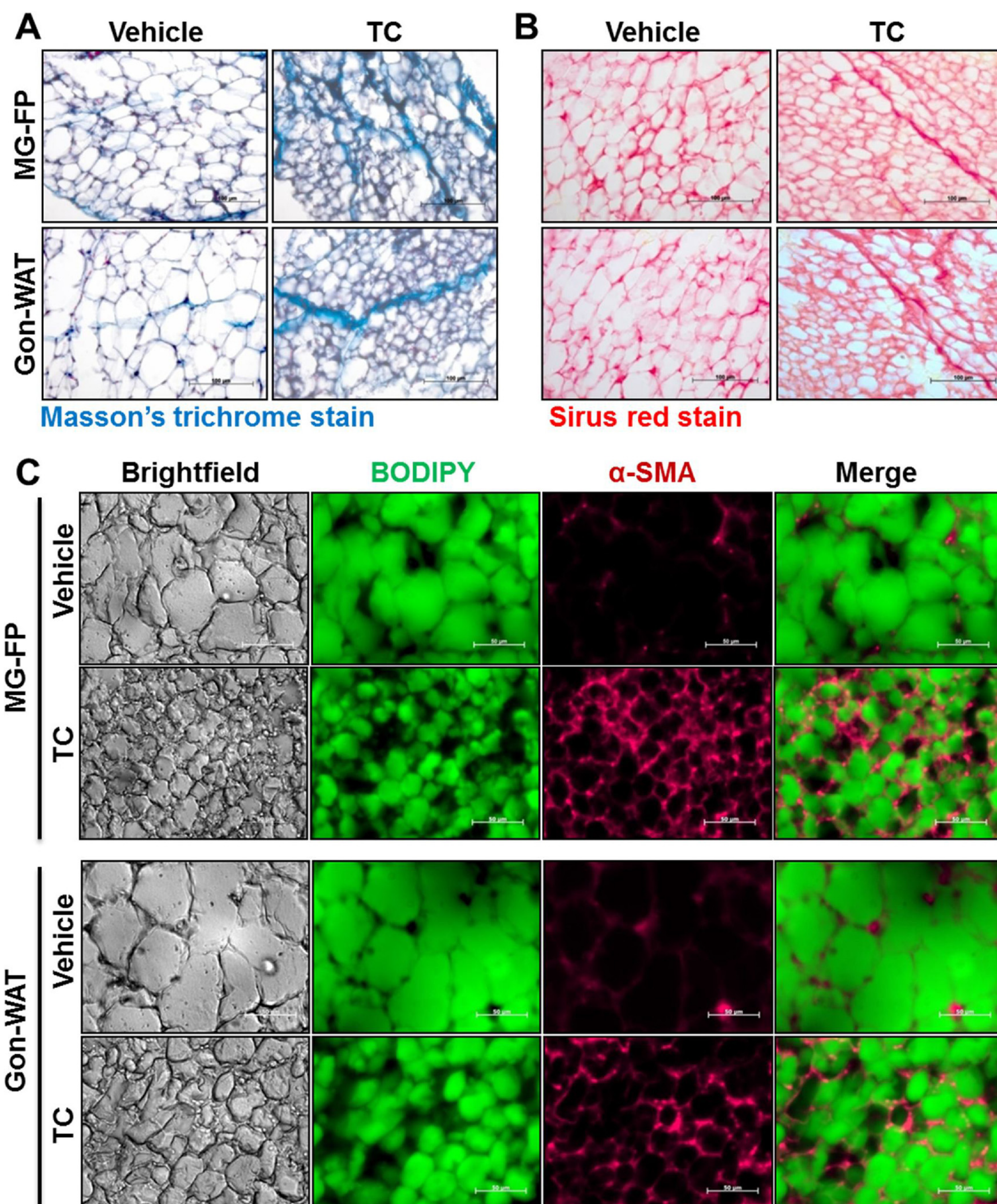


Fig. 5. TC promoted collagen formation and fibrosis in mammary gland fat pads and Gon-WAT in female mice. Masson's trichrome staining (A), Sirius red staining (B). (C), BODIPY and α-SMA immunofluorescence in the mammary gland fat pad (MG-FP) and Gon-WAT of vehicle- and TC-treated mice at 8 weeks. n = 5 mice per group. Scale bar: 100 μm (A and B), 50 μm (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In summary, our results have revealed an important metabolic function for TC-mediated CAR activation in abnormal breast development in adolescent female mice. Thus, the activation of CAR by some endobiotics or xenobiotics may influence mammary gland development in adolescent females.

Declaration of interest

None.

Funding

This work was supported by grants from National Natural Science Foundation of China (no. 31571164 and no. 31271207 to Y.G. Zhai) and Natural Science Foundation of Inner Mongolia (no. 2017BS0801 to J. Wang).

Conflicts of interest

The authors have declared that no competing interests exist. This project is supported by academic grants and there is no financial conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the

online version, at https://doi.org/10.1016/j.toxlet.2018.03.017.

References

Altamirano, G.A., Delconte, M.B., Gomez, A.L., Alarcon, R., Bosquiazzo, V.L., Luque, E.H., Munoz-de-Toro, M., Kass, L., 2017. Early postnatal exposure to endosulfan interferes with the normal development of the male rat mammary gland. Toxicol. Lett. 281, 102–109.

Avouac, J., Palumbo-Zerr, K., Ruzehaji, N., Tomcik, M., Zerr, P., Dees, C., Distler, A., Beyer, C., Schneider, H., Distler, O., Schett, G., Allanore, Y., Distler, J.H., 2014. The nuclear receptor constitutive androstane receptor/NR1I3 enhances the profibrotic effects of transforming growth factor beta and contributes to the development of experimental dermal fibrosis. Arthritis Rheumatol. 66, 3140–3150.

Baes, M., Gulick, T., Choi, H.S., Martinoli, M.G., Simha, D., Moore, D.D., 1994. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Mol. Cell. Biol. 14, 1544–1552.

Baskin-Bey, E.S., Anan, A., Isomoto, H., Bronk, S.F., Gores, G.J., 2007. Constitutive androstane receptor agonist, TCPOBOP, attenuates steatohepatitis in the methionine choline-deficient diet-fed mouse. World J. Gastroenterol. 13, 5635–5641.

Birbrair, A., Zhang, T., Files, D.C., Mannava, S., Smith, T., Wang, Z.M., Messi, M.L., Mintz, A., Delbono, O., 2014. Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner. Stem Cell Res. Ther. 5, 122.

Brisken, C., Ataca, D., 2015. Endocrine hormones and local signals during the development of the mouse mammary gland. Wiley Interdiscip. Rev. Dev. Biol. 4, 181–195.

Buechler, C., Krautbauer, S., Eisinger, K., 2015. Adipose tissue fibrosis. World J. Diabetes 6, 548–553.

Dong, B., Saha, P.K., Huang, W., Chen, W., Abu-Elheiga, L.A., Wakil, S.J., Stevens, R.D., Ilkayeva, O., Newgard, C.B., Chan, L., Moore, D.D., 2009. Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. Proc. Natl. Acad. Sci. U. S. A. 106, 18831–18836.

Dragani, T.A., Manenti, G., Galliani, G., Della Porta, G., 1985. Promoting effects of 1,4-bis [2-(3,5-dichloropyridyloxy)]benzene in mouse hepatocarcinogenesis. Carcinogenesis 6, 225–228.

Gao, J., Xie, W., 2012. Targeting xenobiotic receptors PXR and CAR for metabolic diseases. Trends Pharmacol. Sci. 33, 552–558.

Gao, J., He, J.H., Zhai, Y.G., Wada, T.R., Xie, W., 2009. The constitutive androstane receptor is an anti-obesity nuclear receptor that improves insulin sensitivity. J. Biol. Chem. 284, 25984–25992.

Gao, J., Yan, J., Xu, M., Ren, S., Xie, W., 2015. CAR suppresses hepatic gluconeogenesis by facilitating the ubiquitination and degradation of PGC1alpha. Mol. Endocrinol. 29, 1558–1570.

Ghosh, A.K., Vaughan, D.E., 2012. PAI-1 in tissue fibrosis. J. Cell. Physiol. 227, 493–507.

Hinz, B., 2007. Formation and function of the myofibroblast during tissue repair. J. Investig. Dermatol. 127, 526–537.

Kelley, M., Lambert, I., Merrill, J., Safe, S., 1985. 1, 4-Bis[2-(3, 5-dichloropyridyloxy)] benzene (TCPOBOP) and related compounds as inducers of hepatic monooxygenases. Structure-activity effects. Biochem. Pharmacol. 34, 3489–3494.

Macias, H., Hinck, L., 2012. Mammary gland development. Wiley Interdiscip. Rev. Dev. Biol. 1, 533–557.

Masuyama, H., Hiramatsu, Y., 2012a. Treatment with a constitutive androstane receptor ligand ameliorates the signs of preeclampsia in high-fat diet-induced obese pregnant mice. Mol. Cell. Endocrinol. 348, 120–127.

Masuyama, H., Hiramatsu, Y., 2012b. Treatment with constitutive androstane receptor ligand during pregnancy prevents insulin resistance in offspring from high-fat diet-induced obese pregnant mice. Am. J. Physiol. Endocrinol. Metab. 303, E293–300.

Moreau, A., Vilarem, M.J., Maurel, P., Pascussi, J.M., 2008. Xenoreceptors CAR and PXR activation and consequences on lipid metabolism, glucose homeostasis, and inflammatory response. Mol. Pharm. 5, 35–41.

Sberna, A.L., Assem, M., Gautier, T., Grober, J., Guiu, B., Jeannin, A., Pais de Barros, J.P., Athias, A., Lagrost, L., Masson, D., 2011a. Constitutive androstane receptor activation stimulates faecal bile acid excretion and reverse cholesterol transport in mice. J. Hepatol. 55, 154–161.

Sberna, A.L., Assem, M., Xiao, R., Ayers, S., Gautier, T., Guiu, B., Deckert, V., Chevriaux, A., Grober, J., Le Guern, N., Pais de Barros, J.P., Moore, D.D., Lagrost, L., Masson, D., 2011b. Constitutive androstane receptor activation decreases plasma apolipoprotein B-containing lipoproteins and atherosclerosis in low-density lipoprotein receptor-deficient mice. Arterioscler. Thromb. Vasc. Biol. 31, 2232–2239.

Schedin, P., Keely, P.J., 2011. Mammary gland ECM remodeling, stiffness, and mechanosignaling in normal development and tumor progression. Cold Spring Harb. Perspect. Biol. 3, a003228.

Smith, G., Henderson, C.J., Parker, M.G., White, R., Bars, R.G., Wolf, C.R., 1993. 1,4-Bis [2-(3,5-dichloropyridyloxy)]benzene, an extremely potent modulator of mouse hepatic cytochrome P-450 gene expression. Biochem. J. 289 (Pt. 3), 807–813.

Sun, K., Tordjman, J., Clement, K., Scherer, P.E., 2013. Fibrosis and adipose tissue dysfunction. Cell Metab. 18, 470–477.

Tzameli, I., Pissios, P., Schuetz, E.G., Moore, D.D., 2000. The xenobiotic compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene is an agonist ligand for the nuclear receptor CAR. Mol. Cell. Biol. 20, 2951–2958.

Wang, J., Xu, P., Xie, X., Li, J., Zhang, J., Wang, J., Hong, F., Li, J., Zhang, Y., Song, Y., Zheng, X., Zhai, Y., 2017. DBZ (Danshensu bingpian zhi), a novel natural compound derivative, attenuates atherosclerosis in apolipoprotein E-Deficient mice. J. Am. Heart Assoc. 6, e006297.

Wei, P., Zhang, J., Egan-Hafley, M., Liang, S., Moore, D.D., 2000. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. Nature 407, 920–923.

Xu, P.F., Dai, S., Wang, J., Zhang, J., Liu, J., Wang, F., Zhai, Y.G., 2016. Preventive obesity agent montmorillonite adsorbs dietary lipids and enhances lipid excretion from the digestive tract. Sci. Rep. 6, 19659.

Xu, P., Hong, F., Wang, J., Cong, Y., Dai, S., Wang, S., Wang, J., Jin, X., Wang, F., Liu, J., Zhai, Y., 2017a. Microbiome remodeling via the montmorillonite adsorption-excretion axis prevents obesity-related metabolic disorders. EBioMedicine 16, 251–261.

Xu, P., Wang, J., Hong, F., Wang, S., Jin, X., Xue, T., Jia, L., Zhai, Y., 2017b. Melatonin prevents obesity through modulation of gut microbiota in mice. J. Pineal Res. e12399.

Yan, J., Xie, W., 2016. A brief history of the discovery of PXR and CAR as xenobiotic receptors. Acta Pharm. Sin. B 6, 450–452.

Yan, J., Chen, B., Lu, J., Xie, W., 2015. Deciphering the roles of the constitutive androstane receptor in energy metabolism. Acta Pharmacol. Sin. 36, 62–70.

Zhai, Y., Wada, T., Zhang, B., Khadem, S., Ren, S., Kuruba, R., Li, S., Xie, W., 2010. A functional cross-talk between liver X receptor-alpha and constitutive androstane receptor links lipogenesis and xenobiotic responses. Mol. Pharmacol. 78, 666–674.

Zhang, J., Huang, W., Chua, S.S., Wei, P., Moore, D.D., 2002. Modulation of acetaminophen-induced hepatotoxicity by the xenobiotic receptor CAR. Science 298, 422–424.