TCDD-induced chick cardiotoxicity is abolished by a selective cyclooxygenase-2 (COX-2) inhibitor NS398

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Abstract

Halogenated aromatic hydrocarbons (HAHs), including 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), are known to cause severe heart defects in avian species. However, the mechanism of TCDD-induced chick cardiovascular toxicity is unclear. In this study, we investigated cyclooxygenase-2 (COX-2) as a possible mechanism of TCDD-induced cardiotoxicity. Fertile chicken eggs were injected with TCDD and a COX-2 selective inhibitor, NS398, and we investigated chick heart failure on day 10. We found that the chick heart to body weight ratio and atrial natriuretic factor (ANF) mRNA expression were increased but this increase was abolished with treatment of NS398. In addition, the morphological abnormality of an enlarged ventricle resulting from TCDD exposure was also abolished with co-treatment of TCDD and NS398. Our results suggested that TCDD-induced chick heart defects are mediated via the nongenomic pathway and that they do not require the genomic pathway.

Keywords atrial natriuretic factor, cardiac malformation, chick embryo, cyclooxygenase-2 inhibitor, nongenomic pathway, TCDD

Introduction

TCDD (2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin) is an environmental pollutant, known to cause various types of toxicities, such as teratogenicity, immunotoxicity, and reproductive toxicity (Bird et al. 1983; Larson et al. 1995; Walker et al. 1997; Peden-Adams et al. 1998; Blankenship et al. 2003). Cardiac abnormalities are one of the most severe teratogenicities in avian species. A mass mortality called "chick edema disease" has resulted in millions of chickens being killed with TCDD and halogenated aromatic hydrocarbon (HAH) contaminated feed (Schmittle et al. 1958; Higginbotham et al. 1968).

Although it is known that the heart is a common target of TCDD during fetal development in fish, avians, and mammals, specific characteristics of heart defects vary among these individual models (Kopf et al. 2009). A common finding in all of these models is a reduction in myocyte proliferation, and consequently, a change in heart size with developmental exposure of TCDD (Jones et al., 2009; Antkiewicz et al. 2005; Thackaberry et al. 2005). In fish and avians, but not mammals, cardioteratogenicity of TCDD is associated with mortality (Kopf et al. 2009). In avian species, it is reported that an increase in heart weight, myosin protein content, atrial natriuretic factor (ANF) mRNA expression, and ventricular cavity size, and a reduction in cardiac β-adrenergic receptor responsiveness are induced by embryonic exposure to TCDD (Walker et al. 1997; Walker et al. 2000; Heid et al. 2001; Jones et al. 2009; Kopf et al. 2009). These TCDD-induced cardiovascular diseases are pointed out to have relevance to expression of the arylhydrocarbon receptor (AHR) (Walker et al. 1997).

AHR is a basic-helix-loop-helix/PAS (Per-Arnt/AhR-Sim) family protein and a transcription factor activated by ligand binding. Ligand activated AHR is translocated to the nucleus and forms a heterodimer with AHR nuclear translocator (ARNT). This heterodimer

binds to XRE, and then transcription of AHR target genes is activated. Walker et al. (1997) reported that AHR and ARNT proteins are expressed in cardiac myocytes during chick cardiogenesis. It has been suggested that TCDD exposure deprives the cells of AHR and ARNT to form AHR/ARNT dimers, and that this interrupts normal cellular functions requiring AHR or ARNT (Walker et al. 1997). For example, AHR/ARNT dimerization sequesters the ARNT/hypoxia inducible factor (HIF-1α) signaling pathway, which is involved in coronary angiogenesis (Gradin et al. 1996; Chan et al. 1999; Heid et al. 2001). Additionally, in chicken embryos, cytochrome P450 (CYP) 1A4 and CYP1A5 are induced by means of TCDD in cardiomyocytes (Jones et al. 2009). It has been suggested that this increased CYP1A5 activates the arachidonic acid metabolic pathway by production of epoxyeicosatrienoic acids (EETs), and that this excessive production of arachidonic acids metabolites causes cardiotoxicity (Toraason et al. 1995; Jones et al. 2009; Roman et al. 2006).

In addition to the classical action pathway requiring ARNT, known as the "genomic pathway", recent investigations have focused on inflammatory responses mediating nongenomic pathways (Matsumura 2009). Ligand binding-AHR activation causes a rapid increase in intracellular Ca^{2+} concentration, and it activates cytosolic phospholipase A2 (cPLA2). COX-2 expression is then induced, and this results in an inflammatory response (Matsumura 2009). In fish, it is reported that cardiovascular toxicity can be prevented by the selective COX-2 inhibitors NS398 and SC236 (Teraoka et al. 2008; Dong et al. 2010a). It was also found that TCDD-induced morphological defects in the embryonic heart, accompanied by edema formation, are caused by up-regulation of COX-2 (Dong et al. 2010a). COX-2 is reported to play a pivotal role in mediating TCDD-induced inflammatory responses that are mediated by AHR through a nongenomic signaling route (Matsumura 2009). This nongenomic signaling pathway is clearly distinguishable from the genomic pathway, or CYP1A pathway, as it does not

require ARNT. This genomic pathway is inadequate to fully explain the various types of toxicities caused by TCDD, but some of them can be explained by the nongenomic route (Li et al. 2008; Matsumura 2009; Dong et al. 2010b). Therefore, COX-2 is regarded as a critical factor for the nongenomic toxicological pathway of TCDD.

To clarify the mechanism that causes avian heart defects via the nongenomic pathway, we investigated the effect of COX-2 inhibition on TCDD cardiotoxicity in chicken embryo, using a COX-2 specific inhibitor NS398 (Futaki et al. 1994).

Materials and methods

Animals and Treatments

White Leghorn fertile chicken eggs were obtained from Hokuren (Hokkaido, Japan) and were stored at 4°C after being laid. Sixteen eggs for each group (1: TCDD, 2: TCDD + NS398, 3: NS398, 4: control) were randomly selected and following treatments were performed. A total of 50 pmol TCDD (ULTRA Scientific, RI, USA) was dissolved in 5 μl of corn oil and injected into the yolk prior to incubation as previously described (Mclaughlin et al. 1963, Walker et al. 1997, Heid et al. 2001). Additionally, eggs were injected into air cells with 10 μg NS398 (Cayman Chemical, MI, USA) dissolved in 12.5 μl of 50% dimethylsulfoxide (DMSO; DMSO: phosphate buffered saline (PBS) =1:1). An equivalent volume of vehicle was injected to the control eggs. We confirmed that ten-fold higher amounts (100 μg) of NS398 had no effect on body weight, heart weight, heart/body weight, cardiac toxicity, or lethal rate (data not shown). Eggs were sealed with wood glue and incubated at 37.5°C, with 60% humidity for 10 days (D10). On D10, embryos and hearts were rinsed in PBS, blotted on filter paper, and their wet weights were measured. Liver and heart were obtained and immediately frozen in liquid nitrogen and stored at –80°C until total RNA was isolated by TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA). All experiments using animals were performed according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee.

RNA purification and cDNA synthesis

Hepatic and cardiac RNA were purified with a TURBO DNA-free Kit (Applied Biosystems,

Bedford, MA, USA), and reverse-transcribed to cDNA primed by oligo(dT). The cDNA was synthesized as follows: 2 μg total RNA and 2.5 pmol oligo(dT) primer were incubated in a total volume of 3.5 μl DEPC water at 70°C for 10 min. This mixture was then made up to 10 μl with 2 μl of (5x) RT-buffer, 8 μl of 10mM dNTP, and 0.5 μl of reverse transcriptase (Toyobo Co. Ltd., Osaka, Japan). The mixture was incubated at 42°C for 50 min and 90°C for 5 min.

Quantitative real-time RT-PCR

Quantitative real-time RT-PCR was undertaken using the Step One Plus Real-Time PCR System (Applied Biosystems) and reagents for Thunderbird SYBR qPCR Mix (Toyobo). The primers in this experiment are listed in Table 1. The content of the mixture for PCR was 1 × Master Mix, 0.3 μM of each primer, 200 ng of cDNA, and 1 × ROX reference dye. The total volume of the reaction mixture was kept constant at 10 μl by the addition of RNase-free water. The reaction was carried out as follows: 95°C for 10 min, 40 cycles of 95°C for 10 s, and annealing and extension for 60 s; data collection was performed at the step of annealing and extension. For vascular endothelial growth factor (VEGF-A), angiotensin II type 1 receptor (AT_1), angiotensin II type 2 receptor (AT_2), and angiotensin converting enzyme (ACE), 3 step PCR was performed as follows: 95°C for 10 min, 40 cycles of 95°C for 15 s, annealing for 30 s, and extension at 72°C for 60 s; data collection was performed at the step of extension. The temperatures of annealing and extension were 56°C for beta-myosin heavy chain (β-MHC), 60°C for VEGF-A, 63°C for AT_1, AT_2, and ACE, and 53°C for the rest of the primers. Duplicate measurements were taken.

D10 chick heart aspect ratio

Hearts of D10 chick embryos were removed and photographs were taken. The horizontal length was measured from the line of the maximal length of the heart, passing through the peak of the left ventricle. A vertical line was drawn through the apex of heart, vertical to the horizontal line. The aspect ratio was obtained by dividing the horizontal length by the vertical length.

Statistical analysis

Data were analyzed using Dunnett's test with a significance level of $p < 0.05$ using JMP software (version 7.0; SAS Institute, Cary, NC, USA). Values are expressed as mean ± standard error (SE).

Results

CYP1A5 mRNA expression in the liver and heart

As suitable biomarkers of TCDD exposure, hepatic and cardiac CYP1A5 mRNA expressions were quantified. In liver, CYP1A5 was significantly induced in TCDD-exposed chicks ($p<0.05$), regardless of whether they were also exposed to NS398 (Fig. 1A). CYP1A5 mRNA expression was 15.6-fold higher in TCDD exposed chicks compared with that in control chicks, and 15.1-fold higher in TCDD + NS398 exposed chicks compared with that in chicks only exposed to NS398. Injection of NS398 did not change CYP1A5 expression (1.1 fold) compared to control group. Cardiac CYP1A5 expression was not significantly different among the groups, and it showed less induction than that in the liver (Fig. 1B).

Embryo lethality

The number of chicks with arrested development before D10 was the highest in the TCDD-treated group, followed by the TCDD + NS398-treated group (Table 2). The procedure to inject the corn oil to the yolk, and DMSO in PBS to the air cell is suspected of causing the high mortality. The NS398-treated and control groups were similar in development, with the lowest number of chicks with arrested development before D10. The lethal rate of the TCDD-treated group was 1.34-fold higher than that in the TCDD + NS398-treated group, and 1.83-fold higher than that in the NS398-treated and control groups.

Heart and body weight

Heart and body wet weights of chick embryos were measured, and body weight was significantly decreased with TCDD treatment on D10 (Table 2). Both TCDD-treated groups, with or without NS398, were reduced in body weight by 78-84%. In contrast, heart weight to body weight was significantly increased in the TCDD-treated group compared to that in the control group ($p<0.05$, Table 2). There was no difference in the heart weight to body weight ratio among the TCDD + NS398-treated, NS398-treated, and control groups. There was a trend for heart wet weight to be increased in the TCDD group (Table 2).

COX-2 mRNA expression in the liver and heart

There were no significant differences in COX-2 mRNA expression in either the liver or heart among the groups (Fig. 2).

ANF, SERCA2, and β-MHC mRNA expression in the heart

To evaluate grades of heart failure in each group, we quantified mRNA expression of the following cardiac stress markers, ANF, sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase type2 (SERCA2), and β-MHC. TCDD injection without NS398 exhibited a marked up-regulation of ANF mRNA expression (Fig. 3), but this elevation was completely abolished with TCDD + NS398. From the result, it is indicated that heart disease was more severe in this group compared with the other groups. There were no differences in SERCA2 and β-MHC mRNA expression among the groups (Fig. 4).

VEGF-A mRNA expression in the heart

Cardiac VEGF-A mRNA expression was significantly up-regulated with TCDD treatment (Fig. 5). VEGF-A mRNA expression in the TCDD-treated group was 2.7-fold higher than that in the control group, and it was similar to that in the TCDD + NS398-treated group.

AT_1, AT_2, and ACE mRNA expression

To investigate the involvement of renin-angiotensin system to the up-regulation of ANF, mRNA expression of AT_1, AT_2, and ACE were examined. No significant difference was observed in these genes among the differently treated groups (Fig. 6).

Cardiac malformation

From a morphological perspective, the TCDD-treated hearts had enlargement of the left ventricle, whereas the other groups appeared similar with no abnormalities (Fig. 7). Although the severity of ventricular enlargement differed, all chick hearts treated with TCDD exhibited such a defect.

The heart aspect ratio represents the value gained from the horizontal length divided by the vertical length of the heart; therefore, a high value indicates the possibility of ventricular enlargement. The heart aspect ratio was the highest in the TCDD-treated group, followed by the TCDD + NS398-treated group, but this was not significant (data not shown).

Discussion

Dioxins, such as TCDD, are known to cause heart defects in various animal species. In particular, this cardiotoxicity can be fatal in avian species (Kopf et al. 2009). However, the mechanism that causes this toxicity is still unclear.

Accumulating evidence shows that COX-2 mediates the TCDD-induced nongenomic signaling pathway, which results in inflammatory responses (Matsumura 2009). This specific pathway is different from the well known genomic pathway that induces CYP1A, CYP1B, and AHRR, as this nongenomic pathway does not require ARNT. COX-2 is also reported to be induced with the binding of the AHR to XREs at the COX-2 promoter region (Degner et al. 2007; Degner et al. 2009). However, the nongenomic pathway is workable without involvement of ARNT or AHR translocation into nucleus. *In vitro* study revealed that activation of nongenomic pathway with TCDD exposure could be observed even under the condition of lack of ARNT using siRNA (Dong et al. 2009; Li et al. 2010). Additionally, this nongenomic pathway was activated even in the mice which possess AHR lacking the capability of translocating into the nucleus (Dong et al. 2010b; Li et al. 2010). The mechanism of TCDD cardiovascular toxicity in fish has been gradually determined, and it has been demonstrated that the nongenomic pathway is involved in TCDD-induced heart failure (Teraoka et al. 2008; Dong et al. 2010a).

In the present study, we showed that COX-2 is involved in the pathogenic mechanism of heart development using the COX-2 inhibitor NS398. Although COX-2 appears to be required for TCDD cardiovascular toxicity in both fish and birds, toxicities are known to vary among fish, birds, and mammals (Kopf et al. 2009). For example, TCDD causes an increase in heart weight, myosin protein content (Walker et al. 2000), the incidence of arrhythmias

(Sommar et al. 2005), and apoptosis in the heart (Ivnitski et al. 2001), and it can depress cardiac β-adrenergic receptor responsiveness (Walker et al. 2000; Sommar et al. 2005) and myocyte proliferation (Ivnitski et al. 2001) in avian species, but not in fish. Additionally, the mechanisms of cardiovascular toxicity are unknown, even in fish. It is well known that TCDD exposure in yolk increases the wet weight of D10 chick embryo hearts (Walker et al. 1997; Walker et al. 2000; Heid et al. 2001; Head et al. 2010), but heart size is diminished with TCDD treatment in fish. It is unknown whether these different toxicities among species are provoked via the same pathway.

In chickens, it has been reported that ANF mRNA expression is increased with TCDD on D10 and D12, but not on D8 (Walker et al. 2000). In this study, ANF was used as a marker of severity of heart failure on D10, as this protein has increased expression in animal models of myocardial infarction, heart failure, and hypertrophy (Perrella et al. 1992; Kawakami et al. 1996; Cameron et al. 2000; Walker et al. 2000; Cameron et al. 2003). ANF is known to be highly expressed in the fetal heart, and it is secreted as it develops in the adult (Cameron et al. 2000). ANF inhibits proliferation of vascular smooth muscle cells (Abell et al. 1989) and induces apoptosis of cardiac myocytes (Wu et al. 1997), accordingly regulating cardiovascular cell growth and proliferation. The current study demonstrated that only ANF mRNA expression levels changed with TCDD treatment, while the other cardiac muscle stress markers, SERCA2 and β-MHC, were unchanged (Figs. 3 and 4). It has been reported that ANF mRNA expression is increased in more severe heart defects, such as a reduction in cardiac output and overt heart failure, and that this increase does not depend on the presence of hypertrophy. On the other hand, SERCA2 is down-regulated in cardiac hypertrophy models, and up-regulation of β-MHC expression has been found in hypertrophy models with normal cardiac function (Dorn et al. 2003). Therefore, ANF is often used as a marker of cardiac pathology or stress, rather than a

marker for hypertrophy, which the other two markers (SERCA2 and β-MHC) are used for. Taken together, our findings that expression of these two proteins did not change with TCDD or NS398 treatment suggest that heart defects caused by TCDD are not always accompanied by cardiac hypertrophy, and that they might be associated with impaired cardiac and cardiomyocyte function. It is reported that the activation of renin-angiotensin system induces the cardiac hypertrophy, and particularly, the angiotensin II (Ang II) is known to take part in cardiac remodeling, growth, and apoptosis (Mazzolai et al. 1998; De Mello et al. 2000). ANF expression, which was induced by TCDD treatment in this study, was also reported to be up-regulated with Ang II (Sadoshima et al. 1993a). In addition to it, some of the inflammatory responses seen in nongenomic pathway, including the activation of PKC and up-regulation of intracellular Ca^{2+} concentration, are induced by Ang II via AT_1 (Sadoshima et al. 1993b; Matsumura 2009). Therefore, the increase in expression of AT_1 is regarded to be associated with left ventricular hypertrophy (Iijima et al. 1998). In the present study, changes in AT_1 mRNA expression could not be observed with TCDD or NS398 treatment. AT_2 was also investigated as it is reported to be down-regulated with the PKC activation, and Ca^{2+} release, and found no significant difference among groups. ACE, whose induction has been reported in left ventricular hypertrophy (Schunkert et al. 1990; Passier et al. 1995; Pieruzzi et al. 1995), did not change significantly in the current study, though it tended to be induced with TCDD treatment.

In the present study, CYP1A5 mRNA expression, heart to body weight ratio, and ANF mRNA expression were significantly increased with TCDD treatment (Figs. 1 and 3, Table 2). However, ANF induction and the increase in heart wet weight were abolished with co-treatment of the selective COX-2 inhibitor NS398, whereas induction of CYP1A5 did not change with COX-2 inhibition. These results indicate that heart failure shown by ANF up-regulation or heart weight gain is caused via a pathway requiring COX-2 (nongenomic pathway). Because NS398

did not affect CYP1A5 mRNA expression (Fig. 1), heart failure, which disappeared with NS398 treatment, was clearly dissociated from the CYP1A5 induction pathway, or the genomic pathway. This was similar to a previous study in fish that where TCDD caused developmental toxicity independent from the CYP1A induction mechanism (Carney et al. 2004). Additionally, the lethal rate of developing chicks was highest in the TCDD-treated group, and it was reduced with COX-2 inhibition (Table 2). Since there was no change between the NS398 treated and control groups in the lethal rate, it is possible that exposure of NS398 reduces the lethal toxicity of TCDD.

COX-2 mRNA expression did not change with treatment of TCDD or NS398 in our study (Fig. 2), though COX-2 mRNA up-regulation by TCDD exposure is reported in another study in early developmental medaka embryos (Dong et al. 2010a). The time-dependent change of COX-2 mRNA expression with or without of TCDD would be needed to be studied.

Moreover, we reached the same conclusion that the TCDD toxicity is abolished with co-treatment of NS398 from the morphological study. Hearts treated with only TCDD showed an enlarged ventricle, and this morphological abnormality was not observed in TCDD + NS398 exposed hearts (Fig. 7). Furthermore, the chick heart horizontal to vertical length ratio tended to be reduced with NS398 exposure (data not shown).

During development, VEGF is reported to stimulate the creation of new blood vessels. Among the VEGF family members, VEGF-A is indispensable during embryonic development, and it is known to take part in a variety of functions, including proliferation and migration of vascular endothelial cells. Ivnitski‐Steele et al. (2003) suggested that one of the causes of TCDD-induced heart defects is a decrease in VEGF-A expression at the stage of coronary vascular development, and that this decrease results in a reduction in coronary vascular outgrowth. In this study, mRNA expression of VEGF-A was induced with TCDD exposure (Fig.

5). We speculate that this up-regulation of VEGF-A was not caused via the nongenomic pathway because induction was also observed in the TCDD + NS398 group. It is reported that the AHR/ARNT heterodimer binds to estrogen response elements (EREs), with mediation of the estrogen receptor (ER), and activates transcription of VEGF-A (Ohtake et al. 2003). This pathway of VEGF-A induction is very different from the nongenomic pathway. Therefore, this suggests that TCDD-induced heart abnormalities are not associated with the expression of VEGF-A.

We found in both TCDD exposed groups significantly reduced chick body weights compared to the non-TCDD-treated groups (Table 2). However, this reduction in weight remained the same with co-treatment of NS398. This trend is very similar to that of CYP1A5 induction with TCDD. Therefore, it is expected that a reduction in body weight or fetal growth suppression is not related to the nongenomic pathway. There are some reports in mammals that have shown that a lethal reduction in body weight, called wasting syndrome, is due to the effects of CYP1A (Fletcher et al. 2001; Uno et al. 2004). Given these, the body weight reduction in the TCDD treated groups in our study could have been caused via the genomic pathway.

In conclusion, it is suggested that TCDD causes heart failure by COX-2 activation in avian species. This phenomenon is similar to that of fish, while some of the toxicity characteristics are very different among animal species. In summary, TCDD-induced chick heart defects are caused via the nongenomic pathway, and these cardiovascular toxic effects are separate from the genomic pathway.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Primers used for quantitative PCR analysis

	Forward primer (5' to 3')	Reverse primer (5' to 3')
β-actin	TGGGTATGGAGTCCTGTGGTA	CGGATATCCACATCGCACTT
CYP1A5	CCAGACCTTCGACAAGAACA	GATCTTCTCGTTTGGGATCT
COX-2	CCCAGCACTTCACTCATCAA	TTTCCATCCTTGCGAAGTCT
ANF	GACCTGCAAGAGCCTCAAAC	GGATGCTAGCTTGGGTTCAG
SERCA2	AAGGGCGTGCAATTTACAAC	ACCCATAACAGCTGGACAGG
β-MHC	CGGGATGCACTCTTGGTAAT	TCCTTCAGATGCCCAAATTC
VEGF-A	ATGAACTTTCTGCTCACTTGG	CCGTCTCGGTTTTTCACATC
ACE	CGCTGGGGCACTCCTGGAGTATT	GGACCATGACTGGGCCCACAT
AT_1	CTGTTTCAGGAAGGCACAGTTAC	TGTCATATCGAAAGCCACAGAC
AT_2	CCCTGATACGAGCACAGGAG	GCACTTGCTTGATCTCAGCCCC

Table 2 Lethal rate, body weight, and heart weight of D10 chick embryos

	TCDD	TCDD + NS398	NS398	Control
Sample number	5 / 16	8 / 16	10 / 16	10 / 16
Lethal rate (%)	68.8	50	37.5	37.5
Body weight (mg)	1802 ± 57*	1728 ± 49*	2110 ± 83	2182 ± 61
Heart weight (mg)	19.9 ± 2.5	15.5 ± 1.6	18.5 ± 1.1	16.7 ± 1.0
Heart weight / body weight ratio (%)	1.10 ± 0.11*	0.89 ± 0.08	0.87 ± 0.03	0.77 ± 0.04

Fertile chicken eggs were injected with TCDD or NS398 on D0 and incubated for 10 days. The sample number indicates the number of samples developed to D10/total number of the injected eggs. Wet weights of embryo body weights and heart weights were obtained from D10 chick embryos. The results are expressed as the mean ± SE. *indicates a significant difference compared with the control group (Dunnett's test; $p<0.05$).

Figure Legends

Fig. 1

CYP1A5 mRNA expression of D10 chick A) liver and B) heart was analyzed using quantitative real-time RT-PCR. Data were normalized to the expression of β-actin in each organ. The results are shown as mean ± SE (n=3-4). *indicates a significant difference from the control group (Dunnett's test; $p<0.05$).

Fig. 2

COX-2 mRNA expression of D10 chick A) liver and B) heart was analyzed using quantitative real-time RT-PCR. Data were normalized to the expression of β-actin in each organ. The results are shown as mean ± SE (n=3-4).

Fig. 3

ANF mRNA of D10 chick heart was analyzed using quantitative real-time RT-PCR. Data were normalized to the expression of β-actin in each organ. The results are shown as mean ± SE (n=3-4). *indicates a significant difference from the control group (Dunnett's test; $p<0.05$).

Fig. 4

A) SERCA2 and B) β-MHC mRNA expression of D10 chick heart was analyzed using quantitative real-time RT-PCR. Data were normalized to the expression of β-actin in each organ. The results are shown as mean ± SE (n=3-4).

Fig. 5

VEGF-A mRNA expression of D10 chick hearts was analyzed using quantitative real-time RT-PCR. Data were normalized to the expression of β-actin in each organ. The results are shown as mean ± SE (n=3-4). *indicates a significant difference from the control group (Dunnett's test; $p<0.05$).

Fig. 6

A) ACE, B) AT_1, and C) AT_2 mRNA expression of D10 chick hearts were analyzed using quantitative real-time RT-PCR. Data were normalized to the expression of β-actin in each organ. The results are shown as mean ± SE (n=3-4). *indicates a significant difference from the control group (Dunnett's test; $p<0.05$).

Fig. 7

Hearts of D10 chicken embryos treated with either A) TCDD, B) TCDD + NS398, C) NS398, or D) non-treated. Left ventricular enlargement is shown by the arrow. Bars = 1.5 mm

Fig. 1

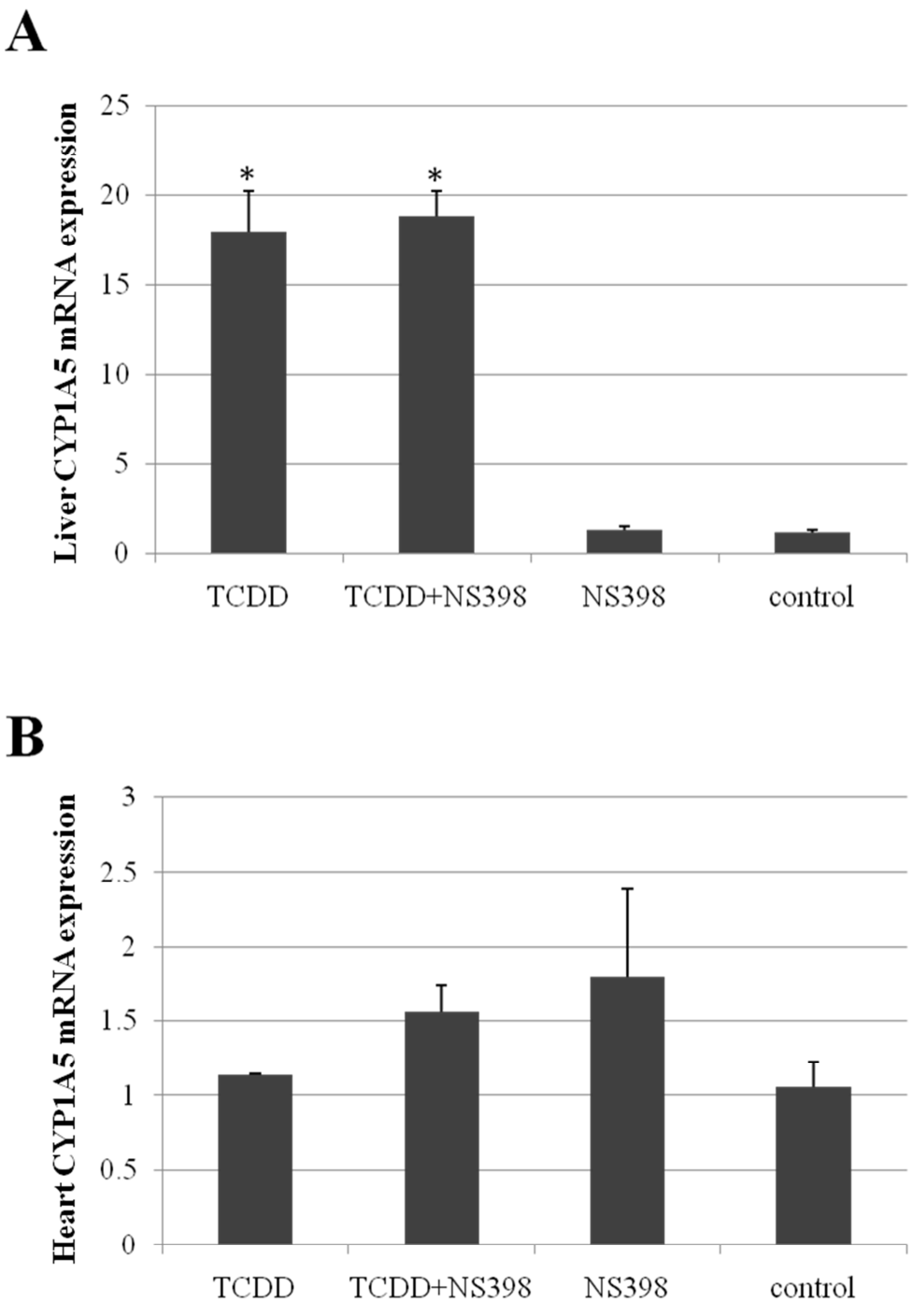
A
Liver CYP1A5 mRNA expression
25
20
15
10
5
0
*
*
TCDD
TCDD+NS398
NS398
control
B
Heart CYP1A5 mRNA expression
3
2.5
2
1.5
1
0.5
0
TCDD
TCDD+NS398
NS398
control

Fig. 2

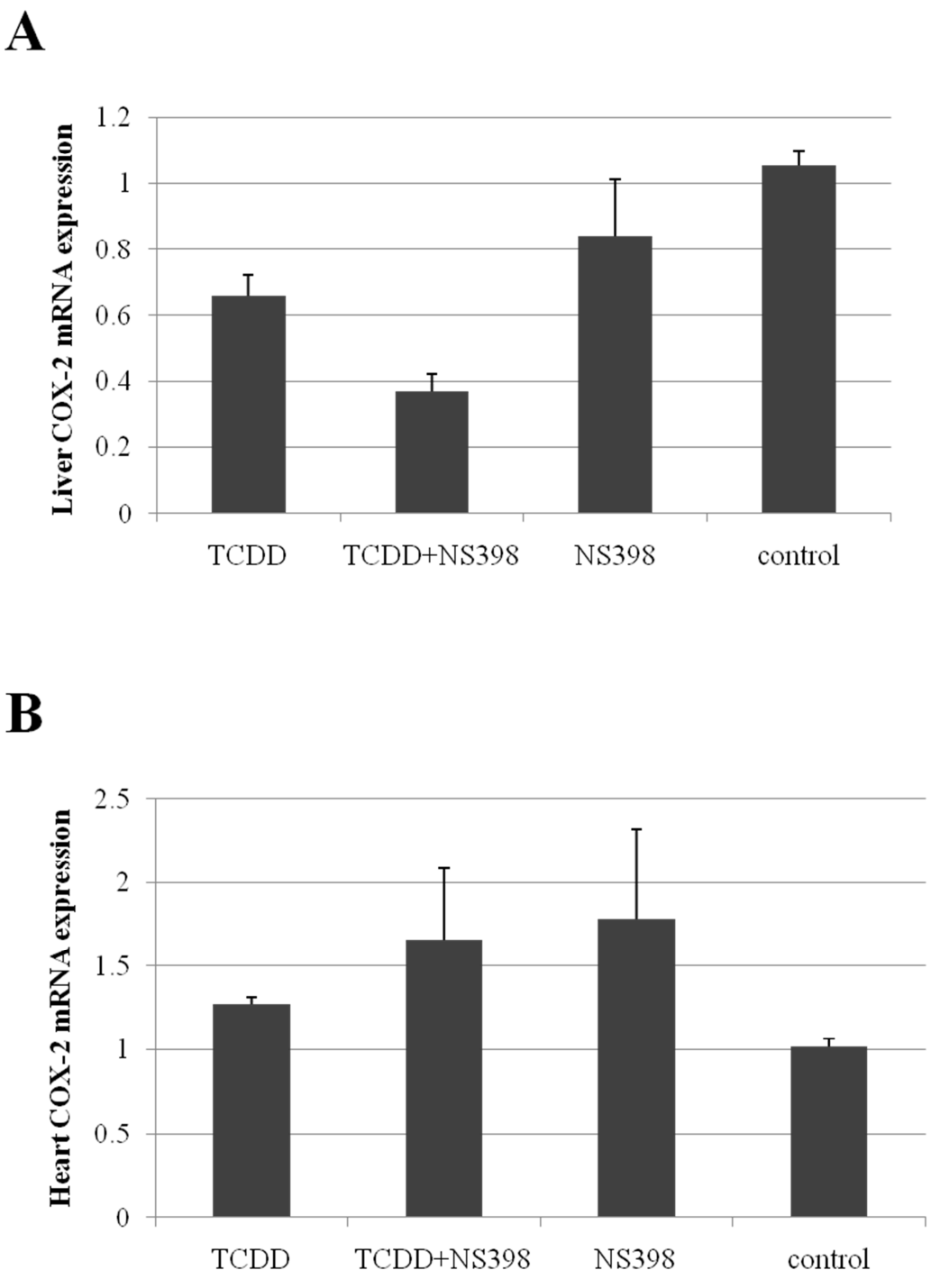
A
Liver COX-2 mRNA expression
1.2
1
0.8
0.6
0.4
0.2
0
TCDD
TCDD+NS398
NS398
control
B
Heart COX-2 mRNA expression
2.5
2
1.5
1
0.5
0
TCDD
TCDD+NS398
NS398
control

Fig. 3

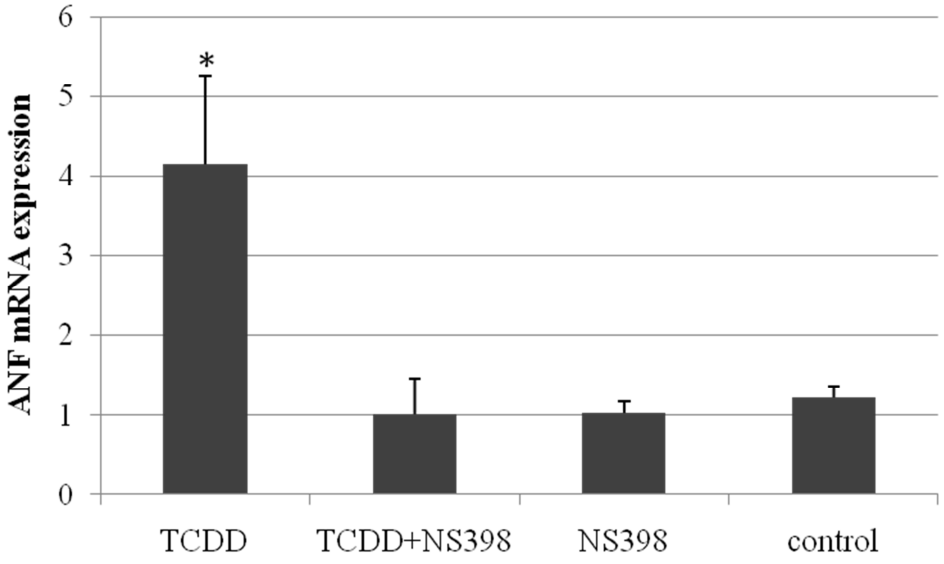

Fig. 4

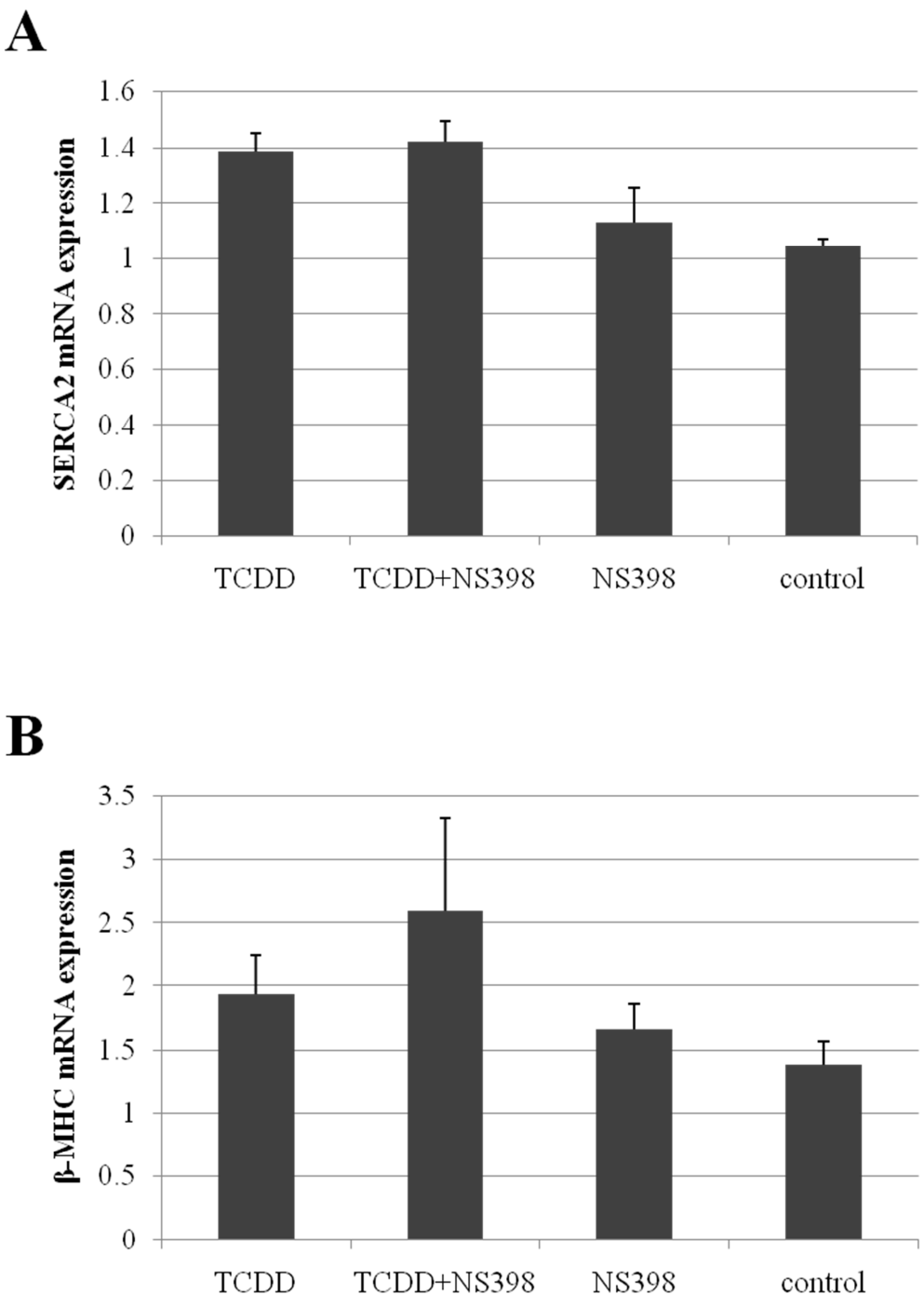
A
SERCA2 mRNA expression
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
TCDD
TCDD+NS398
NS398
control
B
β-MHC mRNA expression
3.5
3
2.5
2
1.5
1
0.5
0
TCDD
TCDD+NS398
NS398
control

Fig. 5

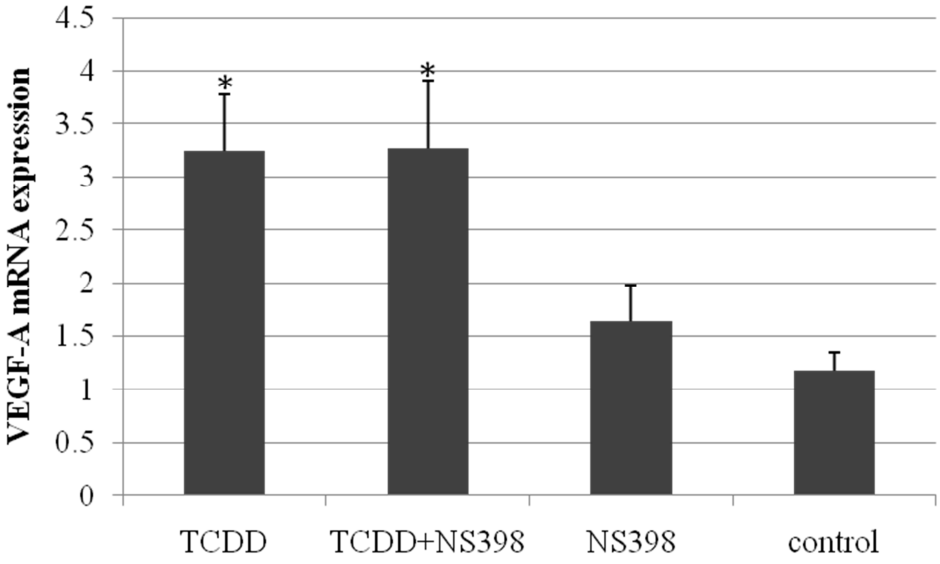
VEGF-A mRNA expression
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
TCDD
TCDD+NS398
NS398
control

Fig. 6

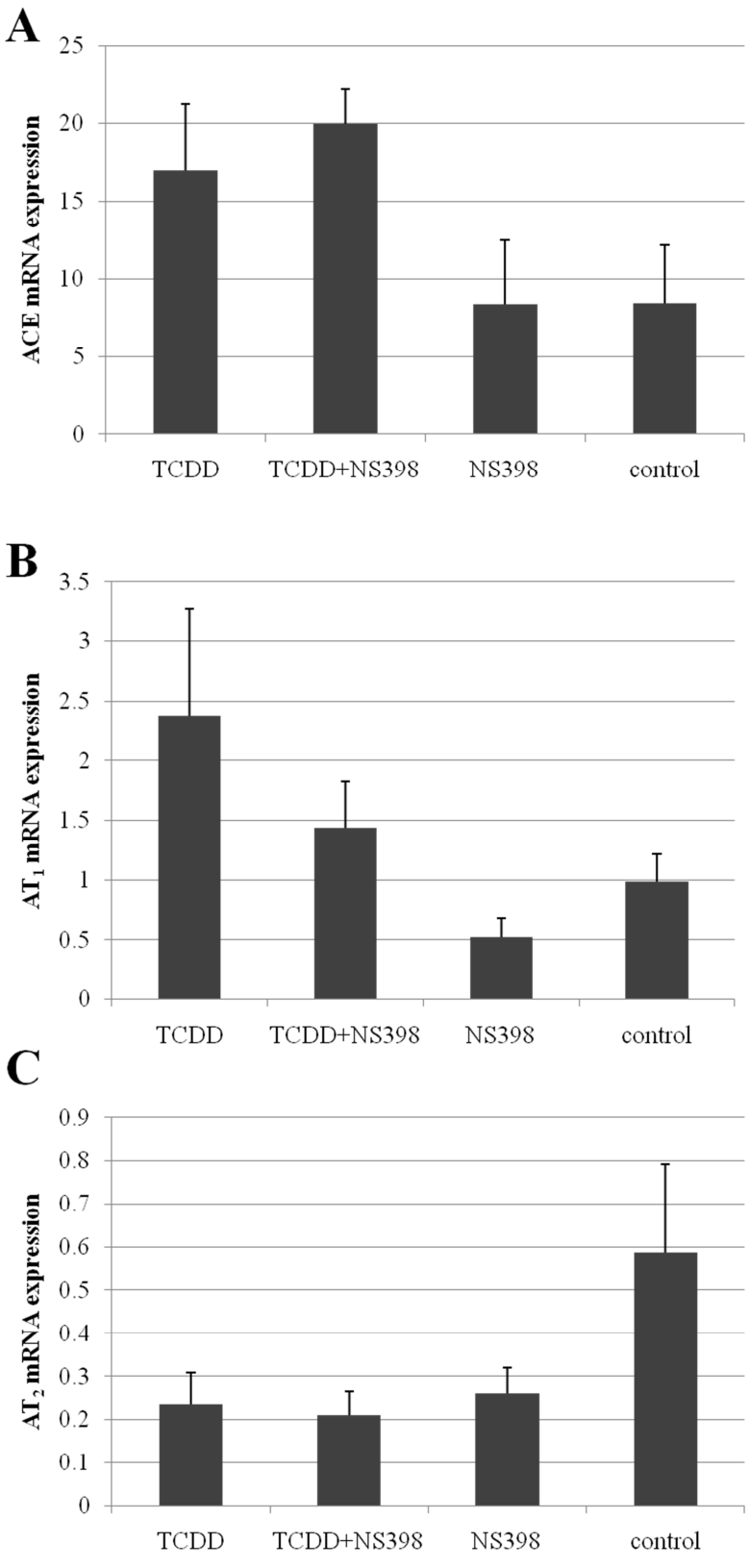
A
ACE mRNA expression
25
20
15
10
5
0
TCDD
TCDD+NS398
NS398
control
B
AT1 mRNA expression
3.5
3
2.5
2
1.5
1
0.5
0
TCDD
TCDD+NS398
NS398
control
C
AT2 mRNA expression
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
TCDD
TCDD+NS398
NS398
control

Fig. 7