Carbonyl Reduction of Mequindox by Chicken and Porcine Cytosol and Cloned Carbonyl Reductase

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ABSTRACT:

Mequindox (MEQ) is a novel synthetic quinoxaline 1,4-dioxides derivative, which is widely used as a veterinary drug and animal feed additive. However, the metabolic mechanism of MEQ is rarely reported. The N-oxide reduction mechanism of MEQ was reported in our previous work. In this article, the toxicity and the reduction of the carbonyl of MEQ were studied. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assays demonstrated that the carbonyl-reduced MEQ, 2-isothioanol MEQ was much less toxic than MEQ. High-performance liquid chromatography analysis showed that the cytosol extracts of chicken and pig livers were able to reduce MEQ to 2-isothioanol MEQ and the reaction was NADPH-dependent. Further study via enzyme-inhibitory experiment revealed that carbonyl reductase 1 (CBR1) participated in this metabolism. The enzyme activity analysis showed that both chicken CBR1 (cCBR1) and porcine CBR1 (pCBR1) were capable of catalyzing the carbonyl reduction of MEQ and its N-oxide reductive metabolite, 1-deoxymequindox. By comparison of the kinetic constants, we observed that the activity of cCBR1 was higher than pCBR1 to MEQ and the standard substrate of CBR1, menadione. On the other hand, both CBR1s exhibited higher activity to 1-deoxymequindox than MEQ. Mutation analysis suggested that the difference of amino acid at position 141/142 may be one possible reason that caused the activity difference between cCBR1 and pCBR1. Thus far, CBR1 was first reported to participate in the carbonyl reduction of MEQ. Our results will be helpful to recognize the metabolic pathways of quinoxaline drugs deeply and to provide a theoretical basis for controlling the negative effects of these drugs.

Introduction

Mequindox (MEQ), namely 3-methyl-2-acetyl-1,4-dioxyquinoxaline (C₁₁H₁₀N₂O₃), is a novel synthetic quinoxaline derivative, developed by Lanzhou Institute of Animal Husbandry and Veterinary Drugs, Chinese Academy of Agricultural Sciences (Lanzhou, China). As a new member of the quinoxaline-1,4-dioxides, MEQ has a strong inhibitory effect against several Gram-positive and Gram-negative bacteria, such as Escherichia coli, Salmonella sp., and Pasteurella sp. through inhibiting bacterial DNA synthesis (Ihsan et al., 2010). Because of its antimicrobial and growth-promoting effects (Vicente et al., 2009), MEQ has been widely used in animal breeding and as a veterinary medication to treat livestock diseases including swine dysentery and piglet white diarrhea since its discovery in the 1980s.

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ABBREVIATIONS: MEQ, mequindox; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; HPLC, high-performance liquid chromatography; IPTG, isopropyl-β-D-thiogalactoside; CBR1, carbonyl reductase 1; cCBR1, chicken carbonyl reductase 1; pCBR1, porcine carbonyl reductase 1; MEN, menadione; QUE, quercetin; IPTG, isopropyl β-D-thiogalactoside.
thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and menadione (MEN) were responsible for the reduction. Cells were treated with various concentrations (0–200 μg/ml) of MEQ and 2-isooethanol MEQ for 48 h. Cell viability was assessed using the MTT assay, and the viability of MEQ- or 2-isooethanol MEQ-treated cells was shown as a percentage of the control cells. Each point represents the mean ± S.D. of three duplicated experiments.

In this study, we tested whether the liver cytosol extracts of two important livestock species, pigs and chickens, were able to reduce the carbonyl of MEQ, and we identified the possible main enzymes that were responsible for the reduction.

**Materials and Methods**

**Chemicals.** MEQ (mol. wt. 218.21, purity 99.8%), N1-deoxymequindox, 2-isooethanol MEQ, and 2-isooethanol N1-deoxymequindox were synthesized at the Institute of Veterinary Pharmaceuticals (Wuhan, China). 3-(4,5-Dimethyl-2-isoethanol MEQ, and 2-isoethanol were purchased from Roche Chemical Co. (Guangzhou, China). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Fisher Scientific (Guangzhou, China). Water was purified using a Milli-Q system (Millipore, Billerica, MA). Isopropyl β-D-thiogalactoside (IPTG) was obtained from Gen-View Scientific, Inc. (Wellington, FL). All primers were synthesized in Shanghai Invitrogen Biotechnology Co., Ltd., Guangzhou Office (Guangzhou, China). All other chemicals and reagents were of the highest analytical grade available.

**Animals.** Danish Landrace × Yorkshire × Duroc cross-breed pigs (4–5 months old) were purchased from the College of Veterinary Medicine at South China Agricultural University. Chickens (2 months old) were purchased from the Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangzhou, China). Both pigs and chickens were fed with commercial standard diet and were allowed access to water ad libitum to ensure the absence of therapeutic or illicit treatments before slaughtering.

**Preparation of Pig and Chicken Liver Cytosol Extracts.** Cytosol extracts were prepared following the procedures described previously (Liu et al., 2008). The obtained cytosol suspension was stored at −80°C until used. Cytosol protein concentrations were estimated by the Bradford (1976) method using bovine serum albumin as the protein concentration reference standard.

**Cloning of Chicken CBR1 and Porcine CBR1.** Total RNA of pig and chicken livers were isolated using TRIzol reagent (Invitrogen) and were transcribed to cDNA through reverse transcription-polymerase chain reaction. The cDNAs were stored at −80°C until used. Primers for construction were designed using the NCBI database.

**Expression and Purification of CBR1s.** The cCBR1 and pCBR1 constructs were transfected into E. coli BL21 (DE3)-competent cells. Cells were cultured with 0.6 mM IPTG for 3 h at ASPET Journals on June 22, 2017 dmd.aspetjournals.org Downloaded from
were plated on Luria-Bertani broth agar supplemented with kanamycin (30 mg/l) for selection. First, colonies were randomly picked and cultured at 37°C in 2 ml of Luria-Bertani broth with kanamycin (30 mg/l), and then the culture was expanded to 100 ml. Finally, cultures were expanded to 1 liter at a volume rate of 1:100 and were grown at 37°C until OD600 of 0.5 to 0.7 was reached. IPTG was added at a final concentration of 0.8 mM, and cells were allowed to continue to grow for another 4 h. The *E. coli* cells were harvested by centrifugation (6000 rpm for 10 min at 4°C; Beckman Coulter Commercial Enterprise (China) Co., Ltd., Guangzhou, China). The precipitate was suspended and lysed in buffer containing 50 mM potassium phosphate, 10 mM imidazole, 300 mM NaCl, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. Lysates were supplemented with lysozyme (1 mg/ml) and were incubated for 1 h at 4°C. Subsequently, lysates were sonicated (200 W, 10 s, 30) and centrifuged for 30 min at 4°C (25,000 g). The 6×His-tagged proteins were purified using nickel-nitrilotriacetic acid affinity chromatography. The cCBR1 and pCBR1 proteins were eluted in buffer containing 50 mM potassium phosphate, pH 7.4, 300 mM NaCl, and 100 mM imidazole. Proteins were extensively dialyzed for 12 h in 50 mM potassium phosphate buffer containing 10% glycerol at 4°C with two buffer changes. After dialysis, proteins were aliquoted and stored at −80°C until used. We used Western blotting to detect the recombinant proteins. In brief, membrane was first incubated with a polyclonal His-tag monoclonal antibody (1:1000 dilution; Novagen, Madison, WI), followed by incubation with a secondary rabbit anti-mouse IgG antibody conjugated with horseradish peroxidase (1:3000 dilution; Invitrogen). Biotinylated protein ladder (Cell Signaling Technology, Danvers, MA) was used for detecting the molecular mass of the proteins.

**Assay Measuring Carbonyl Reduction of Liver Cytosol Extracts.** The incubation mixture contained 50 mM potassium phosphate buffer (pH 7.4), 200 μM NADPH, cytosol proteins, and one of the following substrates: 50 μM MEQ, 50 μM 1-deoxymequindox. The total volume of the reaction system was 1 ml. The boiled cytosol extracts were used as a negative control of the proteins, and the NADPH was added or not to determine whether the reaction is NADPH-dependent. The enzyme activity inhibitor of CBR1, 10 μM quercetin (QUE) (Forrest and Gonzalez, 2000), was added or not to test whether the carbonyl reductive activity of the liver cytosol extracts was attributed to CBR1. All mixtures were incubated at 37°C for 30 min in a water bath. The reaction was stopped by adding 2 ml of ethyl acetate, which was also used to extract target chemicals from the incubation mixture. The collected liquid in the organic phase was evaporated and dried under high-purity nitrogen. The residues were dissolved in 100 μl of methanol and then were subjected to HPLC for analysis.

**Enzyme Kinetic Analysis of cCBR1 and pCBR1.** The enzyme activities of the purified CBR1s were measured by following the initial rate of NADPH oxidation at 340 nm at 37°C using a WFX UV-2802PCS Spectrophotometer (UNICO, Shanghai, China). The assay mixtures contained 50 mM potassium phosphate buffer (pH 7.4), 200 μM NADPH, MEN at various concentrations (10–320 μM), and enzyme in a total volume of 1.0 ml. The enzyme concentration

![Fig. 2. The molecular structures of MEQ and its metabolites, two other quinoxaline drugs, and MEN.](image)

![Fig. 3. Overexpression and purification of recombinant chicken (A) and pig (B) CBR1 in *E. coli* BL21 (DE3). The purity of the enzyme is shown in 10% SDS-polyacrylamide gel electrophoresis. Lane 1, the molecular mass ladder. Lane 2, cell lysate from uninduced BL21 (DE3) cells. Lane 3, cell lysate from 0.8 mM IPTG (4 h)-induced BL21 (DE3) cells. Lane 4, supernatant after ultracentrifugation; lane 5, cell debris after ultracentrifugation. Lane 6, purified recombinant CBR1, eluted by the buffer containing 50 mM potassium phosphate, 300 mM NaCl, and 100 mM imidazole.](image)
was optimized for each enzyme and substrate concentration to ensure that measured rates were in the linear range of the enzyme kinetic curve. The reaction was initiated by the addition of NADPH to the assay mixture. Blanks without enzyme were routinely included. The substrates were dissolved in absolute ethanol. The final concentration of the solvent in the assay mixture did not exceed 4% (v/v). The assay mixtures contained 50 mM potassium phosphate buffer (pH 7.4), 200 μM NADPH at 37°C for 30 min. The reaction was stopped by adding 2 ml of ethyl acetate. The chromatograph was operated at a flow rate of 1 ml/min at room temperature, with UV detection at 305 nm. Quantitative analysis of reductive products was performed using the peak area.

**Mutagenesis, Expression, Purification, and Activity Analysis of Variant Enzymes.** The mutations including cCBR1 (V141M), cCBR1 (V141E), pCBR1 (E142M), and pCBR1 (E142V) were introduced using the site-directed mutagenesis method. Primers are displayed in Supplemental Table 1. The methods of expression and purification of variant enzymes were the same as those for wild-type CBR1s. In addition, the activities of the purified variant enzymes were measured by following the initial rate of NADPH oxidation at 340 nm at 37°C. The assay mixtures contained 50 mM potassium phosphate buffer (pH 7.4), 200 μM NADPH, 100 μM MEN, and enzyme in a total volume of 1.0 ml.

**Results**

**Toxicity of the Carbonyl Group of MEQ.** The carbonyl of xenobiotic carbonyl compounds was considered to be the toxic group (Oppermann and Maser, 2000; Matsunaga et al., 2006). To determine the toxic effect of the carbonyl of MEQ, the toxicity of MEQ and the carbonyl-reduced MEQ, 2-isothiocyanate MEQ, to the HepG2 cells was tested by MTT assay. Consistent with previous reports on MEQ toxicity (Liu et al., 2012), the cell survival rates of HepG2 cells were inversely correlated to increasing concentrations of MEQ after 48-h treatment. The IC50 of MEQ was 58.71 μg/ml (Fig. 1). As expected, 2-isothiocyanate MEQ was much less toxic compared to HepG2 cells (Fig. 1). The cell survival rates of 2-isothiocyanate MEQ were higher than those of MEQ at all used concentrations, in which the IC50 was 260.44 μg/ml. Thus, these results indicated that the carbonyl of MEQ was one of the toxic groups of MEQ and the reduction of which is important for its detoxification.

**Carbonyl Reductive Activity of Chicken and Pig Liver Cytosol Extracts to the MEQ.** To test whether MEQ may be metabolized through carbonyl reduction in pig and chicken livers, the MEQ was incubated with chicken and pig liver cytosol, and the metabolites were detected by HPLC. As shown in Table 1, the metabolites of MEQ, 2-isothiocyanate MEQ and 2-isothiocyanate 1-deoxymequindox (Fig. 2) were detected after incubation with chicken and pig cytosol extracts. When cytosol was incubated with MEQ only, the liver cytosol extracts showed limited activities toward MEQ (2.93 ± 0.44 pmol·min⁻¹·mg⁻¹ for chicken and 4.06 ± 0.68 pmol·min⁻¹·mg⁻¹ for pig). When the cofactor NADPH was added to the incubation system, they
showed much higher activities toward MEQ (36.58 ± 5.16 pmol·min⁻¹·mg⁻¹ for chicken and 22.54 ± 8.55 pmol·min⁻¹·mg⁻¹ for pig), which indicated that enzymes involved in this process are NADPH-dependent.

The CBR1 of many species, including chicken and pig, were previously proved to have carbonyl reductive activity toward many endogenous and xenobiotic carbonyl compounds. To determine whether CBR1 may be involved in the carbonyl reduction of MEQ, we used QUE, the potent inhibitor of CBR1, to inhibit the activities whether CBR1 may be involved in the carbonyl reduction of MEQ, endogenous and xenobiotic carbonyl compounds. To determine previously proved to have carbonyl reductive activity toward many

In addition, the purified CBR1s were confirmed by Western blot analysis, which showed the molecular mass of the His-tagged cCBR1 and pCBR1 was approximately 31 and 33 kDa, respectively (Fig. 4), which was close to the calculated molecular mass (31.59 for cCBR1 and 33.15 kDa for pCBR1).

**Kinetic Characterization of the Enzymatic Activities of cCBR1 and pCBR1 with MEN.** To determine whether the purified CBR1s were active, and, if so, to determine the kinetic properties of the enzymes, we used MEN as a standard substrate to conduct Michaelis-Menten kinetic studies, because of its specificity for CBR1 enzyme (Gonzalez-Covarrubias et al., 2007). In the presence of MEN, the $V_{\text{max}}$ of cCBR1 and pCBR1 was $8074.00 ± 346.00$ nmol and $279.30 ± 5.14$ nmol NADPH consumed/min/mg, respectively, and the $K_m$ of which were $76.46 ± 8.63$ and $19.92 ± 1.45$ µM, respectively (Fig. 5; Table 2). These results demonstrated that the purified cCBR1 and pCBR1 were enzymatically active and the cCBR1 had a higher catalytic efficiency than the pCBR1 to the standard substrate, MEN.

**Kinetic Characterization of the Enzymatic Activities of cCBR1 and pCBR1 with MEQ and 1-Deoxymequindox.** To confirm whether CBR1s can catalyze the carbonyl reductive reaction of MEQ and its N-oxide reductive metabolite, 1-deoxymequindox, the formation of the major alcohol metabolites after the incubation of MEQ and 1-deoxymequindox with purified CBR1s was measured in vitro. The MEQ, 1-deoxymequindox, and their reductive products 2-isoethanol MEQ and 2-isoethanol 1-deoxymequindox were used as standards and were observed to be eluted at approximately 9.6, 14.3, 6.3, and 9.8 min, respectively (Fig. 6). After the incubation of the cCBR1 and pCBR1 with MEQ, a single new chromatographic peak generated, which was identified as 2-isoethanol 1-deoxymequindox (Fig. 6, C and D). As a control, there were no detectable peaks at the 2-isoethanol MEQ and 2-isoethanol 1-deoxymequindox retention times in the absence of the protein.

**TABLE 2**

<table>
<thead>
<tr>
<th>Kinetic Parameter</th>
<th>cCBR1</th>
<th>pCBR1</th>
</tr>
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<tbody>
<tr>
<td>$V_{\text{max}}$, nmol·min⁻¹·mg⁻¹</td>
<td>8074.00 ± 346.00</td>
<td>279.30 ± 5.14</td>
</tr>
<tr>
<td>$K_m$, µM</td>
<td>76.46 ± 8.63</td>
<td>19.92 ± 1.45</td>
</tr>
<tr>
<td>$k_{cat}$, min⁻¹</td>
<td>250.20</td>
<td>9.00</td>
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<tr>
<td>$k_{cat}/K_m$, min⁻¹·µM⁻¹</td>
<td>3.27</td>
<td>0.45</td>
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</table>

* $K_m$ calculated from $M_i$, 31,000 (cCBR1) and $M_i$, 33,000 (pCBR1).
To further characterize the CBR1s, Michaelis-Menten kinetic properties were studied, using both MEQ and 1-deoxymequindox as substrates, separately. With MEQ as substrate, the $V_{\text{max}}$ and $K_m$ of cCBR1 were 1.21 ± 0.03 nmol · min⁻¹ · mg⁻¹ and 156.00 ± 12.47 μM, respectively (Fig. 7A; Table 3). Otherwise, the pCBR1 showed significantly low $V_{\text{max}}$ (0.49 ± 0.03 nmol · min⁻¹ · mg⁻¹) and high $K_m$ (469.80 ± 54.76 μM) compared with cCBR1 (Fig. 7A; Table 3). In addition, we observed that cCBR1 and pCBR1 exhibited higher activity to 1-deoxymequindox than MEQ, as shown by the significantly low $K_m$ and high $V_{\text{max}}$. With 1-deoxymequindox as substrate, the $V_{\text{max}}$ and $K_m$ of cCBR1 were 5.27 ± 0.32 nmol · min⁻¹ · mg⁻¹ and 105.00 ± 22.43 μM, respectively (Fig. 7B; Table 3). It is noteworthy that the pCBR1 showed $V_{\text{max}}$ (5.26 ± 0.25 nmol · min⁻¹ · mg⁻¹) and $K_m$ (116.10 ± 19.27 μM) values that were similar to those of cCBR1 (Fig. 7B; Table 3). Eadie-Hofstee plots for cCBR1 and pCBR1 verified linearity at the same concentration of MEQ and 1-deoxymequindox used to conduct the assays ($r^2 > 0.9$ for all plots). Taken together, through enzymatic kinetic characterization, both cCBR1 and pCBR1 showed the carbonyl reduction activity to MEQ and 1-deoxymequindox, and the activity of cCBR1 is higher than pCBR1 to MEQ; however, cCBR1 and pCBR1 showed similar activity toward 1-deoxymequindox and higher activity when MEQ was used as the substrate.

**Activity Analysis of Variant Enzymes.** To analyze the activity difference between cCBR1 and pCBR1, the amino acid sequences were aligned with hCBR1 and hCBR3. The alignment showed that most of the amino acid sequences in the substrate-binding sites and catalytic sites are similar within cCBR1, pCBR1, hCBR1, and hCBR3 (Fig. 8). However, it was worth noting that the amino acid at position 141/142, which was proven to affect the activity of CBR significantly, was different between cCBR1 and pCBR1 (Fig. 8). To test whether the amino acids at position 141/142 affect the activity of cCBR1 and pCBR1 significantly, the activity of variant enzymes at position 141 of cCBR1 and 142 of pCBR1 to MEN was analyzed. As shown in Table 4, the velocity of cCBR1 (V141M) (1097.33 ± 15.14 nmol NADPH consumed/min·mg protein⁻¹) was similar to that of the wild type (1018.67 ± 26.63 nmol·min⁻¹·mg protein⁻¹), but the cCBR1 (V141E) (825.33 ± 10.07 nmol·min⁻¹·mg protein⁻¹) was much lower. Meanwhile, the velocity of pCBR1 (E142M) (15.00 ± 0.47 nmol·min⁻¹·mg protein⁻¹) and pCBR1 (E142V) (11.00 ± 0.94 nmol·min⁻¹·mg protein⁻¹) were both higher than that of the wild type of pCBR1 (8.50 ± 0.24 nmol·min⁻¹·mg protein⁻¹).

**Discussion**

The main metabolites, detected after exposure to MEQ in vivo, indicated that MEQ might be mainly metabolized through three pathways including N-oxide reduction, carbonyl reduction, and methyl hydroxylation (Liu et al., 2010). Our previous work proved that aldehyde oxidase was involved in N-oxide reduction, carbonyl reduction, and methyl hydroxylation (Liu et al., 2010). Meanwhile, we observed that aldehyde oxidase could be metabolized by carbonyl reduction, and the chicken and pig liver cytosolic CBRs, cCBR1 and pCBR1, participated in the reaction. Comparative investigation of the metabolism of MEQ and 1-deoxymequindox by cCBR1 and pCBR1 showed that the carbonyl reduction ability of cCBR1 was higher than that of pCBR1, and the CYP3A family of cytochrome P450 monooxidases participated in the hydroxylation at the methyl of the side chains (Liu et al., 2011). The present study demonstrated that MEQ could be metabolized by carbonyl reduction, and the chicken and pig liver cytosolic CBRs, cCBR1 and pCBR1, participated in the reaction. Comparative investigation of the metabolism of MEQ and 1-deoxymequindox by cCBR1 and pCBR1 showed that the carbonyl reduction ability of cCBR1 was higher than that of pCBR1, and the CBR1s preferred to catalyze the carbonyl reduction of the N-oxide-reduced MEQ, 1-deoxymequindox.

High dose of MEQ was observed to lead to cytotoxicity and organ toxicity in liver and spleen (Wang et al., 2011), kidney (Huang et al., 2010), testis (Ihsan et al., 2011), and adrenal gland (Huang et al., 2009) in animals. The N-oxide reductive metabolism of MEQ can lead to the increasing of the intracellular reactive oxygen species, which cause damage to lipids, sugars, amino acid chains, and DNA (Liu et al., 2003; Ellis, 2007). Meanwhile, chemically, the reactive carbonyl group can interact with the

**TABLE 3**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Kinetic Parameter</th>
<th>cCBR1</th>
<th>pCBR1</th>
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<tr>
<td></td>
<td>$V_{\text{max}}$ nmol · min⁻¹ · mg protein⁻¹</td>
<td>1.21 ± 0.03</td>
<td>0.49 ± 0.03</td>
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<td></td>
<td>$K_{m}$ μM</td>
<td>156.00 ± 12.47</td>
<td>469.80 ± 54.76</td>
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<td>Mequindox</td>
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<td>0.0162</td>
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<td>1-Deoxymequindox</td>
<td>$V_{\text{max}}$ nmol · min⁻¹ · mg protein⁻¹</td>
<td>5.27 ± 0.32</td>
<td>5.26 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>$K_{m}$ μM</td>
<td>105.00 ± 22.43</td>
<td>116.10 ± 19.27</td>
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<tr>
<td></td>
<td>$k_{\text{cat}}/K_{m}$ min⁻¹ · μM⁻¹</td>
<td>0.1634</td>
<td>0.1736</td>
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$k_{\text{cat}}$ calculated from $M_r$ 31,000 (cCBR1) and $M_r$ 33,000 (pCBR1).
nucleophilic centers of proteins and nucleic acids (Oppermann and Maser, 2000; Matsunaga et al., 2006), which can cause cytotoxicity, genotoxicity, apoptosis, and the perturbations in signaling pathways (Forrest and Gonzalez, 2000). Likewise, the carbonyl-reduced MEQ, 2-isooethanol oxime, was also shown to be less toxic than MEQ itself in this study (Fig. 1). Therefore, the carbonyl reduction is rather important for the detoxification of MEQ. Furthermore, the chicken and pig liver cytosol extracts were found to be capable of catalyzing the carbonyl reduction of MEQ (Table 1). MEQ shares structural property similar to other members in the quinoxaline family (Fig. 2), but it is still unknown which enzymes are involved in the carbonyl reduction of them. However, whether other tissues of chicken and pig have the ability of carbonyl reduction to MEQ remains to be further investigated.

To combat the negative effects brought about by carbonyl compounds, organisms have involved several biotransformation enzyme systems, one of them being the CBRs (Oppermann and Maser, 2000; Matsunaga et al., 2006; Oppermann, 2007). CBR1 has been reported to metabolize many endogenous and xenobiotic carbonyl compounds to their corresponding alcohol. In the study, CBR1 was also found to be capable of metabolizing MEQ and its N-oxide reductive product, 1-deoxymequindox (Fig. 7). In many cases, the formation of a hydroxyl group makes the substrate more hydrophilic and provides products that can be conjugated, e.g., via glucuronidation of sulfation, thus facilitating excretion (Maser, 2000; Matsunaga et al., 2006; Oppermann, 2007). CBR1 may be easily bound and catalyzed by CBR1, which indicated the catalytic cleft of CBR1 than MEQ. In hence, 1-deoxymequindox may be more easily reduced than MEQ, which suggested that the catalytic activity difference between cCBR1 and pCBR1. However, the other amino acids that affect the activity remain to be further investigated.

In conclusion, this study revealed that the carbonyl group of MEQ is toxic and identified the enzymes that participated in the carbonyl reduction of MEQ in chicken and pig liver cytosols, CBR1s. In addition, CBR1 was able to catalyze the carbonyl reduction of its N-oxide reductive metabolite, 1-deoxymequindox, with a significantly higher catalytic activity compared with MEQ, which suggested that the carbonyl of 1-deoxymequindox may be more easily reduced than that of MEQ.

**TABLE 4**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>NADPH Oxidation</th>
<th>nmol·min⁻¹·mg protein⁻¹</th>
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<tr>
<td>cCBR1</td>
<td>Wild type</td>
<td>1018.67 ± 26.63</td>
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<tr>
<td></td>
<td>V141M</td>
<td>1097.33 ± 15.14</td>
</tr>
<tr>
<td></td>
<td>V141E</td>
<td>825.33 ± 10.07**</td>
</tr>
<tr>
<td>pCBR1</td>
<td>Wild type</td>
<td>8.50 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>E142M</td>
<td>15.00 ± 0.47*</td>
</tr>
<tr>
<td></td>
<td>E142V</td>
<td>11.00 ± 0.94</td>
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* P < 0.05.
** P < 0.01.
References


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