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Furazolidone induces the activity of microsomal enzymes that metabolize furazolidone in chickens

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Abreviations: FZ,furazolidone; PB,Phenobarbital; β-NF, β-naphthoflavone; APN. Aminopyrine; APND, aminopyrine N-demethylase; DPI, diphenylene iodonium chloride; DMSO,2. Materials and Methods

Abstract

The nitrofuran antibacterial agent furazolidone (FZ) is still used in veterinary medicine in some countries in the Middle and Far East. The present study aimed to show the effect of FZ on the activity of microsomal enzymes that metabolize FZ, and to identify the enzyme that contributes to FZ metabolism in chickens. Wistar rats and White Leghorn chickens were administered FZ once a day for 4 consecutive days. FZ metabolism was accelerated by FZ administration in chickens, but not in rats. The elevation of FZ metabolism coincided with the induction of NADPH cytochrome P450 reductase (CPR) activity in chickens, but such induction was not observed in rats. FZ metabolizing activities were inhibited in the presence of a CPR inhibitor (diphenylene iodonium chloride) but not by the addition of archetypal cytochrome P450 inhibitors (CO or *n*-octylamine). The preset study concluded that FZ accelerated its own metabolism in the chicken by induction of the activity of CPR.

Key words: chicken; cytochrome P450; Furazolidone; liver; NADPH cytochrome P450 reductase

1. Introduction

The nitrofuran antimicrobial drug N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone (furazolidone; FZ) has been used for >40 years for the treatment of certain bacterial and protozoal infections in humans and animals (e.g., broilers) [1]. The use of FZ in food-producing animals has been forbidden in European Union (EU) countries, the USA, Japan as well as many other countries owing to its mutagenic [2] and carcinogenic activities [3, 4]. However, FZ is still in use in some countries of the Middle and Far East as a feed additive for livestock [1, 5] and also used for gastrointestinal infectious diseases in human [6, 7]. In particular, domestic poultry is target animals for this antimicrobial drug.

Nitrofuran derivatives (including FZ) were reduced at the nitro group in the initial step of their biotransformation, and continued to undergo further reduction of the nitro group whereas other parts of these compounds might be metabolized in many different ways [16, 21, 29].

These derivatives (including FZ) are reduced in the alimentary tract and liver; this results in the formation of reduced metabolites that are reactive intermediates accompanying free-radical reactions that lead to formation of superoxide anion radicals and lipid peroxidation via redox cycling process [15, 16, 17]. It has been suggested that the nitro-reductive intermediates of nitrofuran derivatives are genotoxic [2, 18, 19]. The metabolite, which binds to protein covalently, is formed during the reductive metabolism of FZ in the livers of rats and pigs [20, 21], as well as in the livers of turkeys and chickens [17, 22]. It has also been suggested that the reductive metabolites of FZ bind to proteins as well as glutathione and react with DNA, consequently relating

to the cytotoxicity that is exerted by several different mechanisms in Caco-2, HEp-2, and V79 cell lines [21]. FZ is therefore considered to have manifold bioactivities through initial nitro reduction and subsequent metabolic activations *in vivo*.

The metabolic rate of FZ might be facilitated by multiple doses of FZ in animals. Vroomen et al. [23] indicated that a decrease in the concentration of non-metabolized FZ in the urine of pigs was observed 2 days after FZ medication, and suggested that specific enzymes that metabolize FZ might have been induced. However, in chicken, one of main animal species to be treated with this drug, the effect of FZ on its metabolism little have been investigated.

This study showed the effects of FZ on its metabolic rate in chickens, and identified the main enzyme responsible for the increase of FZ metabolic rate in this species. This study revealed that FZ metabolic rate was increased by FZ treatment in chicken, and the fascilited activity of NADPH cytochrome P450 reductase (CPR) was responsible for the increase of the FZ metabolic rate.

2. Material and Methods

2.1 Chemicals

Nicotinamide adenine dinucleotide (NADPH), glucose-6-phospohate (G6P), and glucose-6-phosphate dehydrogenase (G6PDH) were obtained from Oriental Yeast Company Limited (Tokyo, Japan). FZ was from Ueno Fine Chemical Industry Company Limited (Osaka, Japan), whereas *n*-octylamine, β-naphthoflavone (β-NF), and phenobarbital sodium (PB) were from Wako Pure Chemical Industries, Limited (Osaka, Japan). Nitrofurantoin, cytochrome c, and CPR were from Sigma–Aldrich (St Louis, MO, USA), and diphenylene iodonium chloride (DPI) was from Dojin Chemical Laboratory (Kumamoto, Japan). All other chemicals were of analytical grade.

Before use, drugs were prepared as follows: FZ was suspended in 2% aqueous acacia solution, and PB dissolved in 0.9% saline solution. Acetone was dissolved in 0.9% saline for a 20% aqueous solution. β-NF(16mg/ml) was dissolved in corn oil.

2.2 Animal treatment

Treatment of all animals was undertaken according to the policies of the International Animal Care and Use Committee of Hokkaido University (Sapporo, Japan).

Female Wistar rats (age, 6 weeks) were obtained from Japan SLC, Incorporated (Hamamatsu, Japan). They were housed in steel cages and fed a pellet diet (Nihon Nosan Kogyo Company, Yokohama, Japan) with water *ad libitum*. Female White Leghorn chickens (age, 2 months) were obtained from Hokkaido Central Chicken Farm (Hokkaido, Japan). They were housed in steel cages and fed a standard diet (Nihon Nosan Kogyo) and water *ad libitum*. Animals were maintained at 23°C in a 12-h

dark/light cycle (starting at 07:00 h).

Rats and chickens were divided into six groups of three. Each animal was administered FZ (high dose, 125 mg/kg/day; low dose, 62.5 mg/kg/day, by crop tube for 4 days), PB (80 mg/kg/day, intraperitoneally for 3 days), β-NF (80 mg/kg/day, intraperitoneally for 3 days), or acetone (5 mL/kg/day, by crop tube for 4 days) once a day. Control animals received 2% acacia solution (5ml/kg /day, by crop tube, once a day, for 4 days).

2.3 Preparation of liver microsomal fractions

Rats and chickens were killed with carbon dioxide 24 h after the last dose and their livers removed. Liver microsomes were prepared according to the method of Omura and Sato [35]. Liver samples were homogenized with three volumes of ice-cold 1.15% KCl. The homogenate was centrifuged at 9,000 × *g* at 4°C for 20 min. The supernatant was centrifuged twice at 105,000 × *g* at 4°C for 70 min each. The pellet was resuspended in 0.1 M potassium phosphate buffer (pH 7.4), frozen in liquid nitrogen, and stored at–80° C until use. The microsome protein concentration was determined by the method of Lowry et al. [36].

2.4 Metabolism (disappearance) of furazolidone

The metabolism (rate of substrate disappearance) of FZ was measured according to modified methods of Sanwald et al. [37] and Yoshida and Kondo [38]. The reaction mixture consisted of 100 μM FZ (dissolved in dimethyl sulfoxide (DMSO)), 5 mM $MgCl_2$, 1 mM ethylenediamine tetra-acetic acid (EDTA), and 1 mg microsomal protein (or 2.5 μg of purified CPR in the inhibition study) in 1 mL of 0.1 M $K_2P_2O_7$ (pH 7.4) buffer. The mixture was pre-incubated for 10 min at 37°C. The reaction was started by

the addition of 10 μL G6PDH (200 U/mL) and 10 μL 50 mM NADPH mixture (in the case of CPR, 20 μL 50 mM NADPH). After 10 min, the reaction was terminated by the addition of 4 mL ethyl acetate. After the addition of 6.72 nmol nitrofurantoin to the mixture as an internal standard and mixing well for 1 min, the organic phase was separated by centrifugation at 1,200 × *g* for 5 min. After evaporation of the organic phase, the residue was dissolved in the mobile phase. The sample was analyzed using a high-performance liquid chromatography (HPLC) system comprising a Shimadzu LC-6A pump, SPD-6A detector, and C-R6A recording data processor (Shimadzu Seisakusho Limited, Kyoto Japan). HPLC was carried out using a Wakosil-2-5C18G column (150 mm × 4.6 mm I.D.; Wako), a mobile phase of acetonitrile:DW of 40:60, and a wavelength of 358 nm. In the inhibition study of FZ metabolism, *n*-octylamine dissolved in DMSO was added to the incubation mixture at a final concentration of 0.1 M as a CYP inhibitor, and DPI (in DMSO) was added to the incubation mixture at a final concentration of 0.2 M as a flavin enzyme inhibitor. Measurement of FZ metabolism are repeated three times.

2.5 CPR

The activity of CPR was assayed according to the method of Omura and Takesue [39]. The rate of cytochrome c reduction was followed at 415 nm using a spectrophotometer (Hitachi-U-300, Hitachi, Tokyo, Japan). Reductase activity was quantified using an extinction coefficient of 19.6 mM/cm. CPR dependent activity was independently repeated three times.

2.6 Effect of a CYP inhibitor on aminopyrine N-dimethylase (APND) activity

APND activity was determined by measuring the rate of formaldehyde formation according to the methods of Cooper and Brodie [40], and Nash [41]. The reaction mixture consisted of 1 mM aminopyrine, 4 mM $MgCl_2$, 10 mM G6P, and 1 mg microsomal protein in 1 mL of 0.1 M $K_2P_2O_7$ (pH 7.4) buffer. The mixture was pre-incubated for 10 min at 37°C. The reaction was started by the addition of 10 μL G6PDH (200 U/mL) and 10 μL 50 mM NADPH mixture (in the case of CPR, 20 μL 50 mM NADPH). After 10 min, the reaction was terminated by the addition of 0.8 mL of 10% $ZnSO_4$. The rate of formaldehyde formation was followed by spectrophotometric means at 415 nm. In the inhibition study of APND, *n*-octylamine was added to the incubation mixture at a final concentration of 0.1 M.

2.7 Statistical analyses

Data are mean ± SD. Comparisons between two groups were carried out using the Student's *t*-test. Multiple comparisons were also undertaken using the Student's *t*-test, followed by adjustment of *p* values using the Bonferroni correction. $P<0.05$ was considered significant.

3. Results

3.1 Effects of FZ and CYP inducers on FZ metabolism

Figure 1 shows the effects of administration of FZ or CYP inducers on FZ metabolism in the liver microsomes of rats and chickens. No significant change in FZ-metabolizing activity was observed in the FZ-treatment groups of the rat (Fig. 1A). PB- and acetone-treated rat groups showed slight (but significant) increases in FZ metabolism activity. In contrast to the effect of FZ treatment in rats, FZ administration to chickens resulted in large and significant increases in FZ-metabolizing activity, i.e., 2.1-fold in the 125 mg/kg FZ group and 1.6-fold in the 62.5 mg/kg FZ group, as compared with that of the control group (Fig. 1B). The chicken group administered acetone also showed a significant increase in FZ metabolism. However, a significant alteration of FZ metabolism was not observed in PB- and β-NF-treated chickens.

3.2 Effects of FZ and CYP inducers on P450 reductase-dependent activity

Figure 2 shows the CPR in the liver microsomes of rats and chickens. In rats, no significant change in CPR activity was observed in FZ- and β-NF-treatment groups, whereas the PB- and acetone-treated groups showed significant increases in this activity (Fig. 2A). In contrast, administration of 125 mg/kg FZ resulted in an increase in CPR activity in chickens (Fig. 2B). Acetone administration also significantly increased the enzymatic activity of CPR whereas, surprisingly, treatment with PB as well as β-NF had no effect on CPR activity in chickens.

3.3 Effects of a non-selective CYP inhibitor on FZ metabolism in chickens

In chickens, the relationship between FZ metabolism activity and the CYP-dependent reaction was examined using the CYP inhibitor *n*-octylamine, which is a non-specific inhibitor of CYP reactions (Fig. 3). FZ metabolic rates which were increased significantly by treatment with 125 mg/kg FZ and by acetone were not inhibited by *n*-octylamine (Fig. 3A). APN was examined as a typical substrate of the CYP-dependent reaction. APND activities were inhibited significantly by *n*-octylamine treatment (Fig. 3B). The APND activity that was increased by treatment with β-NF or acetone was also significantly inhibited in the presence of the CYP inhibitor.

3.4 Effects of a specific P450 reductase inhibitor on FZ metabolism in the liver microsomes of chickens

The effect of DPI (typical CRP inhibitor) was examined using chicken liver microsomes (Fig. 4). FZ metabolism in chicken liver microsomes was significantly inhibited by DPI addition, but not by the addition of *n*-octylamine or CO. Direct evidence that CPR can metabolize FZ has been not reported, so we tested this hypothesis. We confirmed that FZ could be reduced by pure CPR from rabbit liver induced with PB, and also showed that the FZ reduction was significantly inhibited by DPI (Fig. 5).

4. Discussion

In the current study, we fixed the dosage of FZ (62.5 and 125 mg/kg/day) to correspond to the dose used in a previous study [42] which used FZ at a therapeutic level of 400 ppm in chickens.

A significant change in the activity to metabolize FZ in the hepatic microsomes of rats treated with 125 mg/kg/day FZ was not observed, whereas archetypal inducers of CYP enzymes, PB or acetone, increased the metabolic rate of FZ (Fig. 1). In contrast to the rat, FZ treatment increased FZ metabolism in chickens by about 1.6- to 2-fold compared with that of the control, and FZ metabolic rate was also increased by acetone. CPR was increased by PB and acetone in rats, but no change was observed by FZ and β-NF (Fig. 2). In chickens, FZ treatment significantly increased the level of CPR in the liver, suggesting that this may be the cause of the increase in FZ metabolism in FZ-treated chicken. These results may explain the result of Vroomen et al. who observed that the elimination of FZ in pig blood was facilitated by successive FZ treatment [23]. Also, these effects of FZ correspond to the results of Bartlet et al. [27]. They observed that sleeping time induced by pentobarbital was decreased by FZ treatment in chickens.

Acetone, PB and β-NF are archetypal inducers of CYP. Acetone is perceived as an inducer of CYP2E1 in rats [43] and for CYP2H1, CYP2H2, and CYP2E in chickens [44]. PB is thought to be an inducer of a large subset of CYP genes and CPR, in mammals and/or non-mammals [45]. In birds, however, the evidence for PB-type induction is contradictory to that observed in mammals [46]. As to the induction of CPR by PB treatment in chicks, Pilch and Combs [48] demonstrated that PB administration (100 mg/kg/day for 6 days) by crop tube increased the enzyme activity as well as the

activities of APND and aniline hydroxylase. Gupta et al. [49] demonstrated that injection of PB (80 mg/kg, i.p., for 4 days) induced the activities of some CYP-related activities in adult hen livers, but did not refer to CPR. Additionally, in Japanese quails, injection of PB (70 mg or 150 mg/kg/day, i.p., for 5 days) resulted in a significant increase in CYP contents, but no change in the level of CPR [50].

In the present study, the lack of effect of PB treatment on CPR activity in the chicken may be related to the bird-specific sensitivity of microsomal enzymes (including CPR) to PB [50]. Unlike humans or rats, little is known about the effect of β-NF and acetone on CPR in chickens, although β-NF and hexachlorobiphenyl (which is a potent inducer of CYP1 and CYP2B) significantly induced CPR activity in the Japanese quail. The inability to significantly induce the activity of CPR by treatment with β-NF in chickens could be attributed to inter-species differences [50].

Treatment with high doses of FZ induced FZ metabolism as well as significant CPR activity in the chicken (Figures 1 and 2). Similar tendencies were observed in chickens treated with acetone, and in rats treated with PB or β-NF. These results suggest that the increase in the metabolic rate of FZ may be associated with CPR activity in chicken liver microsomes. However, previous reports suggested that the increase in the metabolic rate of FZ was attributable to CYPs [34, 51]. Actually, we observed two-fold elevation of CYP2C subfamily protein expression in liver of FZ treated chickens (data not shown). Therefore, we confirmed the contribution of CYP to FZ metabolism using a typical CYP inhibitor (Fig. 3). The metabolizing activity of FZ was not inhibited by the non-specific CYP inhibitor *n*-octylamine. In the reference experiment, APND was significantly inhibited by this non-specific CYP inhibitor. Aminopyrine is catalyzed by CYPs in the chicken liver [49, 52]. Therefore, we concluded that CYP might not

contribute to the metabolizing activity of FZ that was induced by treatment with FZ (125 mg/kg/day). We then determined the effect of a typical inhibitor of CPR on the metabolism of FZ using the flavoprotein enzyme inhibitor DPI (Fig. 4). In contrast to the result of CYP inhibition with *n*-octylamine or CO, DPI significantly inhibited FZ metabolism. Additionally, this study used a FZ metabolism assay using recombinant CPR from rabbit livers to confirm that this type of enzyme can directly carry out the reduction of FZ, and the FZ metabolism was also inhibited significantly by DPI. This result corresponds to that of Abraham et al. [18] and Vroomen et al. [20]. They suggested that CPR was responsible for the reductive metabolism of FZ, and that CYP was hardly involved in the metabolism of FZ in rat livers.

In conclusion, successive treatments of FZ given orally resulted in a significant, dose-dependent increase in the metabolic rate of FZ in the chicken, whereas no effect was observed in the rat. Additionally, FZ treatment resulted in an increase in the activity of CPR that coincided with an increase in the metabolic rate of FZ in the chicken. This inductive effect of FZ on drug metabolizing enzymes in chicken was investigated in a successive study. The result revealed that FZ treatment induced some CYP-related activities, and some CYP apoprotein.

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References

[1] B.H. Ali, Pharmacological, therapeutic and toxicological properties of furazolidone: Some recent research, Vet. Res. Commun. 23 (1999) 343-360.

[2] D.R. McCalla, Mutagenicity of nitrofuran derivatives, Environ. Mut. 5 (1983) 745-765.

[3] B.H. Ali, Pharmacology and toxicity of furazolidone in man and animals: Some recent research, Gen. Pharmacol. 20 (1989) 557-563.

[4] Food and Drug Administration, Part V. New Animal Drugs. Furazolidone (NF-180), Fedeal Register 41 (1976) 19906-19921.

[5] X.Z. Hu, Y. Xu, A. Yediler, Determinations of residual furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in fish feeds by HPLC-UV and LC-MS/MS, respectively, J. Agric. Food Chem. 55 (2007) 144-1149.

[6] F. DiMario, F.G. Cavallaro, C. Scarpignato, Rescue' therapies for the management of Helicobacter pylori infection, Dig. Dis. 24 (2006) 113-130.

[7] A. Morgner, J. Lebenz, S. Miehlke, Effective regimens for the treatment of Helicobacter pylori infection, Expert Opin. Investig. Drugs 15 (2006) 995-1016.

[8] L.A.P. Hoogenboom, M.C.J. Berghmans, T.H.G. Polman, R. Parker, I.C. Shaw, Depletion of protein-bound furazolidone metabolites containing the 3-amino-2-oxazolidinone side-chain from liver, kidney and muscle tissues from pigs, Food Addit. Contam. 9 (1992) 623-630.

[9] L.A.P. Hoogenboom, G.D. Sonne, K. van Bruchem, I.C.Enninga, J.A. van Rhijin, H. Heskamp, M.B.M. Huveneers-Oorsprong, J.C.M. van der Hoeven, H.A. Kuiper, Absorption of a mutagenic metabolite released from protein-bound residues of

furazolidone, Environ. Toxicol. Pharmacol. 11 (2002) 273-287.

[10] A.M. Timperio, H.A. Kuiper, L. Zolla. Identification of a furazolidone metabolite responsible for the inhibition of amino oxidases, Xenobiotica 33 (2003) 153-167.

[11] L. Zolla, A.M. Timperio, Involvement of active oxygen species in protein and oligonucleotide degradation induced by nitrofurans, Biochem. Cell Biol. 83 (2005) 166-175.

[12] S. Chumaneem, S. Sutthivaiyakit, P. Sutthivaiyakit, New reagent for trace determination of protein-bound metabolites of nitrofurans in shrimp using liquid chromatography with diod array detecter, J. Agric. Food Chem. 57 (2009) 1752-1759.

[13] M.I. Lopez, M.F. Feldlaufer, A.D. Williams, P.S. Chu, Determination and confirmation of nitrofuran residues in honey using LC-MS/MS, J. Agric. Food Chem. 55 (2007) 1103-1108.

[14] E. Verdon, P. Couder, P. Sanders, Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoin, nifursol) in poultry muscle tissue through the detection of their five major metabolites (AOZ, AMOZ, SEM, AHD, DNSAH) by liquid chromatography by coupled to electrospray tandem mass spectrometry, Anal. Chim. Acta 586 (2007) 336-347.

[15] F.J. Peterson, G.F. Combs, J.L. Jr., Holtzman, R.P. Mason, Metabolic activation of oxygen by nitrofurantoin in the young chick, Toxicol. Appl. Pharmacol. 65 (1983) 162-169.

[16] L. Rossi, I. De Angelis, J.Z. Pedersen, E. Marchese, A. Stammati, G. Rotilio, F. Zucco, N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidinone activation by the human intestinal cell line Caco-2 monitored through noninvasive electron spin resonance

spectroscopy, Mol. Pharmacol. 49 (1996) 547-555.

[17] W.E. Stroo, S.W. Schaffer, Furazolidone-enhanced production of free radicals by avian cardiac and hepatic microsomal membranes, Toxicol. Appl. Pharmacol. 98 (1989) 81-86.

[18] R.T. Abraham, J.E. Knapp, M.B. Minningh, L.K. Wong, M.A. Zemaitis, A.D. Alvin, Reductive metabolism of furazolidone by Escherichia coli and rat liver in vitro. Drug Metab. Dispos. 12 (1984) 732-741.

[19] J. Gajewska, M. Szczypka, B. Tudek, T. Szymczyk, Studies on the effect of ascorbic acid and selenium on the genotoxicity of nitrofurans: nitrofurazone and furazolidone, Mut. Res. 232 (1990) 191-197.

[20] B.H. Ali, M.O.M. Tanila, A.K. Bashir, The effect of furazolidone and furaltadone on drug metabolism in rats. Eur. J. Drug Metab. Pharmacokinet. 21 (1996) 327-332.

[21] I. DeAngelis, L. Rossi, J.Z. Pedersen, A.L. Vignoli, O. Vincentini, L.A.P. Hoogenboom, T.H.G. Polman, A. Stammati, F. Zucco, Metabolism of furazolidone: alternative pathways and modes of toxicity in different cell lines, Xenobiotica 29 (1999) 1157-1169.

[22] R.J. McCracken, J.A. van Rhijin, D.G. Kennedy, The occurrence of nitrofuran metabolites in the tissues of chickens exposed to very low dietary concentrations of the nitrofurans, Food Addit. Contam. 22 (2005) 567-572.

[23] L.H.M. Vroomen, M.C.J. Berghmans, P. Hekman, L.A.P. Hoogenboom, H.A. Kuiper, The elimination of furazolidone and its open-chain cyano-derivative from adult swine," Xenobiotica 17 (1987)1427-1435.

[24] M. Fukuhara, E. Takabatake, The effects of nitrofuran derivatives on hepatic microsomal mixed-function oxidase activity in rats, Toxicol. Appl. Pharmacol. 42

(1977) 571-581.

[25] I. Rahden-Staron, H. Czeczot, M. Szumilo, Induction of rat liver cytochrome P450 isoenzymes CYP1A and CYP2B by different fungicides, nitrofurans, and quercetin, Mut. Res. 489 (2001) 57-66.

[26] N. Sasaki, Effect of furazolidone on duration of righting reflex loss induced with hexobarbital and zoxazolamine in the rat, J. Vet. Med. Sci. 56 (1994) 667-670.

[27] A.L. Bartlet, S. Harvey, H. Klandorf, Contrasting effects of nitrofurans on plasma corticosterone in chickens following administration as a bolus or diet additive, J. Vet. Pharmacol. Ther. 13 (1990) 261-269.

[28] K. Tatsumi, H. Nakabeppu, Y. Takahashi, S. Kitamura, Metabolism in Vivo of furazolidone: Evidence for formation of open-chain carboxylic acid and α-ketoglutaric acid from the nitrofuran in rats, Arch. Biochem. Biophys. 234 (1984) 112-116.

[29] L.H. Vroomen, J.P. Groten, K. van Muiswinkel, A. van Velduizen, P.J. van Bladeren, Identification of reactive intermediate of furazolidone formed by swine liver microsomes, Chem. Biol. Interact. 64 (1987) 167-179.

[30] L.H.M. Vroomen, B. van Ommen, P.J. van Bladeren, Quantitative studies of the metabolism of furazolidone by rat liver microsomes, Toxicol. in Vitro 1 (1987) 97-104.

[31] K. Tatsumi, T. Ou, H. Yamada, H. Yoshimura, H. Koga, T. Horiuchi, T. Isolation and identification of the metabolite of furazolidone, J. Pharmacobio-dyn 1 (1978) 256-261.

[32] K. Tatsumi, H. Yamada, H. Yoshimura Y. Kawazoe, Metabolism of furazolidone by milk xanthine oxidase and rat liver 9000g supernatant: Formation of a unique nitrofuran metabolite and an aminofuran derivative, Arch. Biochem. Biophys. 208 (1981) 167-174.

[33] J. Pourahmad, S. Khan P.J. Obrien, Lysosomal oxidative stress toxicity induced by

nitrofurantoin redoxcycling in hepatocytes, Adv. Environ. Med. Biol. 500 (2001) 261-265.

[34] A.J. Streeter, T.R. Krueger, B.A. Hoener, Oxidative metabolites of 5-nitrofurans. Pharmacol. 36 (1988) 283-288.

[35] T. Omura, R. Sato, The carbon monooxygenase-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature, J. Biol. Chem. 239 (1964) 2370-2378.

[36] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265-275.

[37] P. Sanwald, E.A. Blankson, B.D. Dulery, J. Schoun, N.D. Huebert, J. Dow, Isocratic high-performance liquid chromatographic method for the separation of testosterone metabolites, J. Chromatogr. B Biomed. Sci. Appl. 672 (1995) 207-215

[38] K. Yoshida, F. and Kondo, Liquid chromatographic determination of furazolidone in swine serum and avian egg, J. AOAC. Int. 78 (1995) 1126-1129.

[39] T. Omura, S. and Takesue, A new method for simultaneous purification of cytochrome b5 and NADPH-cytochrome c reductase from rat liver microsomes, J. Biochem. 67 (1970) 249-257.

[40] J.R. Cooper, B.B. Brodie, The enzymatic metabolism of hexobarbita, J. Pharmacol. Exp Ther. 114 (1955) 409-417.

[41] T. Nash, The calorimetric estimation of formaldehyde by means of the Hantzsch reaction, J. Biol. Chem. 55 (1953) 416-422.

[42] B.H. Ali, A.L. Bartlet, Inhibition of monoamine oxidase by furazolidone in the chicken and the influence of the alimentary flora thereon, Br. J. Pharmacol. 71 (1980) 219-224.

[43] J.B. Schenkman, H. Greim, Cytochrome P450 Handbook of Experimental Pharmacology 105, Springer-Verlag., Berlin Heidelberg, 1993.

[44 J.F. Sinclair, S. Wood, L. Lambrecht, N. Gorman, L. Mende-Mueller, L. Smith, J. Hunt, P. and Sinclair, Isolation of four forms of acetone-induced cytochrome P-450 in chicken liver by h.p.l.c. and their enzymic characterization, Biochem. J. 269 (1990) 85-91.

[45] D.J. Waxman, L. Azaroff, Phenobarbital induction of cytochrome P-450 gene expression, Biochem. J. 281 (1992) 577-592.

[46] M.J.J. Ronis, C.H. Walker, The microsomal monooxygenases of birds, Rev. Biochem. Toxicol. 10 (1989) 301-384.

[47 H.V. Goriya, A. Kalia, S.K. Bhavsar, C.G. Joshi, D.N. Ranle, A.M. Thaker, Comparative evaluation of phenobarbital-induced CYP3A and CYP2H1 gene expression by quantitative RT-PCR in bamtam, bamtamized White Leghorn and White Leghorn chicks, J. Vet. Sci. 6 (2005) 279-285.

[48] S.M. Pilch, G.F. Combs, Effects of dietary vitamin E and selenium on the mixed-function oxygenase system of male and female chicks, Comp. Biochem. Physiol. C. 69 (1981) 331-335.

[49] R.P. Gupta, D.M. Lapadula, M.B. Abou-Donia, Purification and characterization of cytochrome P450 isoenzymes from Phenobarbital-induced adult hen liver, Biochem. Physiol. C. 96 (1990) 163-176.

[50] H.M. Carpenter, D.E. William, D.R. Buhler, A comparison of the effects of hexachlorobenzene, β-naphthoflavone, and Phenobarbital on cytochrome P450 and mixed-function oxidases in Japanese quail, J. Toxicol. Environ. Health 15 (1985) 93-108.

[51] A.R. Goeptar, E.J. Groot, H. Scheerens, J.N.M. Commandeur, N.P.E. Vermeulen, Cytotoxicity of mitomycin c and adriamycin in freshly isolated rat hepatocytes: The role of cytochrome P450, Cancer Res. 54 (1994) 2411-2418.

[52] R.P. Gupta, D.M. Lapadula, M.B. Abou-Donia, Purification and characterization of cytochrome P-450 isozymes from β-naphthoflavone-induced adult hen liver, Arch. Biochem. Biophys. 282 (1990) 170-182.

FIGURE LEGENDS

Figure 1. Effects of FZ, PB, β-NF, and acetone on the activity to metabolize FZ in hepatic microsomes.

The activity to metabolize FZ in the hepatic microsomes of animals treated with FZ (62.5 or 125 mg/kg/day), PB (80 mg/kg/day), β-NF (80 mg/kg/day), acetone (5 mL/kg in 20%) and 2% acacia solution (5 mL/kg) to control animals. FZ metabolizing activity was measured by HPLC-UV. (A) FZ-metabolism in rats. (B) FZ metabolism in chickens. Each column represents the mean of 3 animals carried out in duplicate, and the range bars indicate the SD. The asterisk indicates a significant difference from the control animal (*$p<0.05$, **$p<0.01$).

Figure 2. Effects of FZ, PB, β-NF, and acetone on CPR activity in hepatic microsomes.

Hepatic microsomes were prepared from animals killed 24 h after the last treatment (FZ, PB, β-NF, or acetone). CPR-dependent activities were measured by spectrophotometric means by observing the change in absorption maxima of reduced cytochrome c at 550 nm. Each column represents the mean of 3 animals carried out in duplicate, and the range bars indicate the SD. The asterisk indicates a significant difference from the control animal (*$p<0.05$, **$p<0.01$).

Figure 3. Effects of CYP and a flavin enzyme inhibitor on the metabolism of FZ or aminopyrine in chicken liver microsomes.

The activity to metabolize furazolidone or aminopyrine with CYP inhibitor (*n*-octylamine) in the hepatic microsomes of chickens treated with FZ, PB, β-NF, or

acetone. FZ-metabolizing activity was measured by HPLC-UV. Each value represents mean ± SD for three animals. (A) FZ metabolism in chicken liver microsomes with or without a CYP inhibitor. (B) Activity of APND with or without a CYP inhibitor in the hepatic microsomes of chickens. Each column represents the mean of 3 animals carried out in duplicate, and the range bars indicate SD. The asterisk indicates a significant difference from the value without the inhibitor (*$p<0.05$).

Figure 4. Change in the results of FZ metabolism with CYP inhibitors and a flavoprotein enzyme inhibitor in the hepatic microsomes of chickens.

FZ metabolism in microsomal fraction was detected after co-incubation with various CYP and CPR inhibitors. Each column represents the mean of 3 experiments, and the range bars indicate the SD. The asterisk (*) indicates a significant difference from the control value ($p<0.05$).

Figure 5. FZ metabolism by rabbit recombinant P450 reductase, and its inhibition by a flavoprotein inhibitor.

Recombinant CPR (2.5 μg) prepared from rabbit liver induced with phenobarbital were used in the incubation mixture for the assay to measure the FZ metabolic rate. Each column represents the value of one experiment carried out in duplicate.

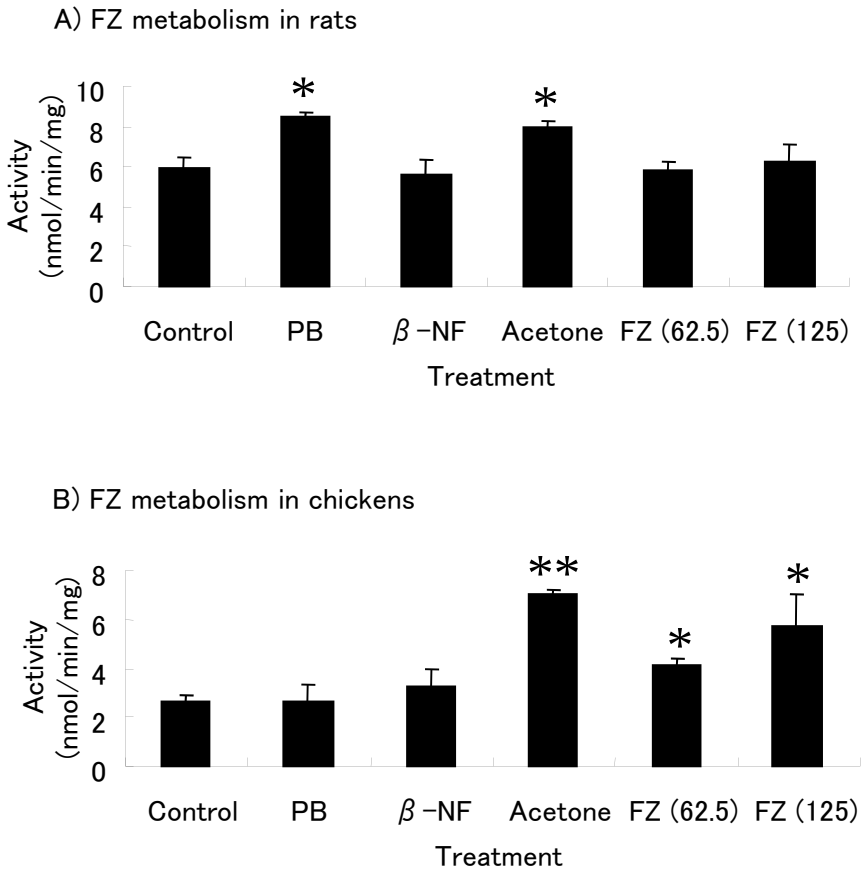
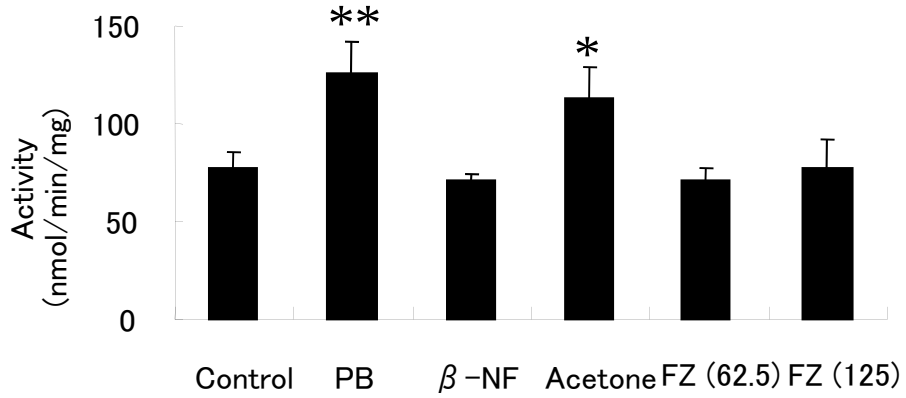

A) FZ metabolism in rats
Activity (nmol/min/mg)
10
8
6
4
2
0
*
*
Control
PB
β-NF
Acetone
FZ (62.5)
FZ (125)
Treatment
B) FZ metabolism in chickens
Activity (nmol/min/mg)
8
6
4
2
0
**
*
*
Control
PB
β-NF
Acetone
FZ (62.5)
FZ (125)
Treatment

Figure 1

A) CPR activity in rats



B) CPR activity in chickens

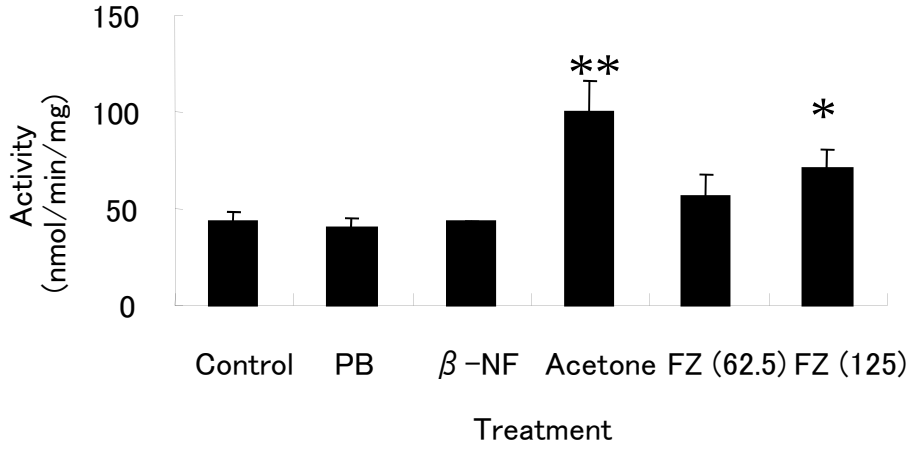
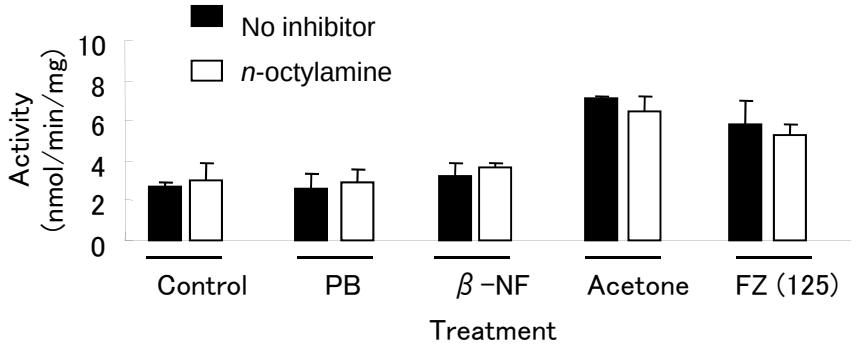


Figure 2

A) FZ metabolism with a CYP inhibitor in chickens



B) APND metabolism with a CYP inhibitor in chickens

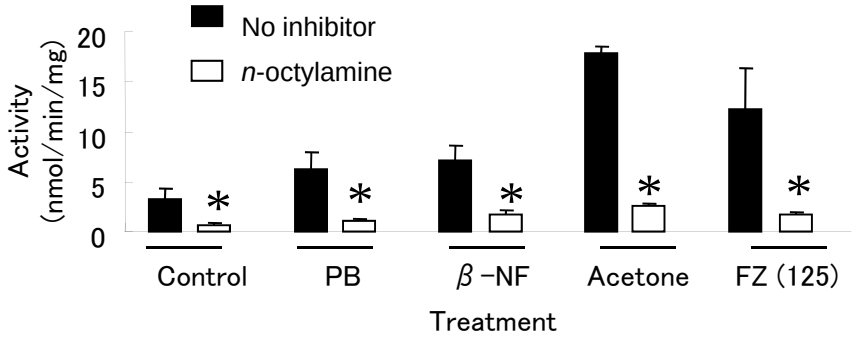


Figure 3

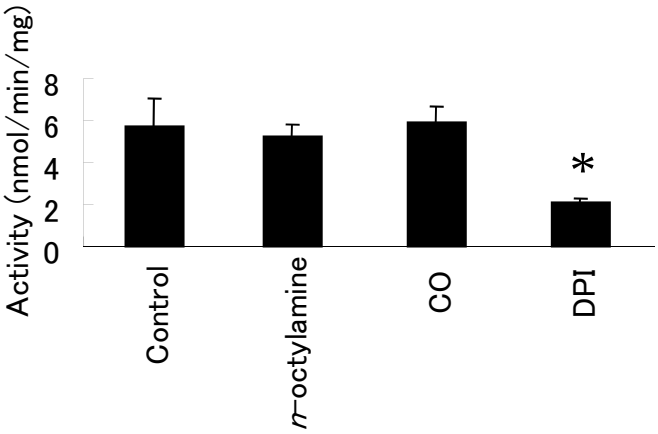


Figure 4

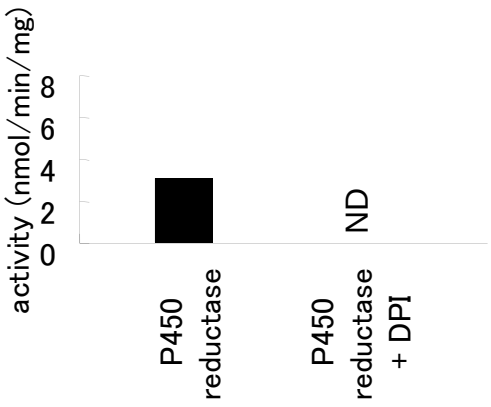

activity (nmol/min/mg)
8
6
4
2
0
P450 reductase
P450 reductase + DPI
ND

Figure 5