**NOTE** *Toxicology*

# Repeated Treatment with Furazolidone Induces Multiple Cytochrome P450-Related Activities in Chicken Liver, but Not in Rat Liver

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(Received 17 December 2012/Accepted 4 June 2013/Published online in J-STAGE 18 June 2013)

ABSTRACT. The nitrofuran antimicrobial agent, furazolidone (FZ), is still used in veterinary medicine in some countries in the Middle and Far Eastern countries. The present study aimed to investigate the effects of successive bolus doses of FZ and its metabolite 3-amino-2-oxazolidinone (AOZ) on cytochrome P450 (CYP)-related activities in the livers of rats and chickens. Female Wistar rats and white Leghorn chickens were orally administered FZ once a day for 4 consecutive days. FZ-treated chickens showed an increase in multiple CYP-related activities, however, rats treated with FZ did not show these changes. In chickens, treatment with FZ also induced production of microsomal CYP2C6-like apoprotein. The present study demonstrated that FZ caused a multiple-type induction of CYP-related activities in chickens, but not in rats.

KEY WORDS: antimicrobial substance, chicken, cytochrome P450, drug metabolism.

doi: 10.1292/jvms.12-0531; *J. Vet. Med. Sci.* 75(11): 1497–1502, 2013

The nitrofuran antimicrobial drug, N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone (furazolidone, FZ), has been used for more than forty years to treat certain bacterial and protozoal infections in humans and animals [3]. The use of FZ in food-producing animals has been forbidden in European Union countries, the U.S.A. and Japan as well as many other countries owing to its mutagenic and carcinogenic activities [1, 3]. However, FZ remains as an antibacterial and antiprotozoal feed additive for poultry, cattle and farmed fish in some Middle and Far Eastern countries [3, 14]. It is also used to treat infectious diseases in humans, especially for eradication of *Helicobacter pylori* [24]. Therefore, further findings concerning the pharmacological and toxicological properties of this drug can be anticipated [28].

A considerable number of studies have reported the undesirable and toxicological effects of FZ. One of the major side effects of FZ is its effect on drug-metabolizing enzymes. Alterations in drug-metabolizing enzyme activity induced by FZ may influence the pharmacological or toxicological action of some drugs and pollutants [27].

In the rat, successive administration of FZ in the diet has been shown to result in increased cytochrome P450 (CYP) content, and depending on the substrate used, an increase or decrease of CYP-related activities [11]. Successive oral administration of FZ was also reported to cause induction of hepatic CYP1A1 isozymes [32]. In addition, successive bolus doses of FZ in rats were shown to decrease the metabolic rate of two kinds of drugs in *in vivo* and increase the duration of barbital anesthesia [3]. In contrast, there are only a small number of conflicting reports on the effect of FZ on drug-metabolizing enzymes in chickens, one of the common animals treated with FZ. Treatment with FZ (0.04%, for 10 days) in feed caused a decrease in the duration of barbital anesthesia, but had no such effect when administered as a bolus dose of 200 mg/kg FZ [5]. Recently, we have demonstrated that FZ treatment in chickens induced facilitation of its metabolic rate that was dependent on increased activity of NADPH cytochrome P450 reductase in the liver [31].

FZ is generally reduced at the nitro group at the initial step of its biotransformation and then metabolized successively into metabolites containing a 3-amino-2-oxazolidinone (AOZ) side-chain, which bind covalently to proteins [3, 38]. AOZ inhibits monoamine oxidase (MAO) activity [35] and may be metabolized into irreversible MAO-inhibitors, 2-hydroxy ethyl hydrazine (HEH) in rats [34]. Although some MAO inhibitors suppress several CYP-related catalytic actions in human [26] and rat [9], there is little investigation of the effect of AOZ and HEH on microsomal CYP-dependent actions in chickens.

The aim of this study was to investigate the effect of successive bolus doses of FZ and its metabolites, AOZ and HEH, on CYP-related activities in rat and chicken livers. The current study demonstrated that chickens treated with FZ had an increase in CYP-related activities and also enhanced induction of CYP2C6-like apoprotein.

*The reagents were obtained as follows*: Nicotinamide adeninedinucleotide (NADPH), glucose 6-phospohate (G6P) and glucose 6-phosphate dehydrogenase (G6PDH) from Oriental Yeast Co. Limited (Tokyo, Japan); furazolidone (N-(5-nitro-2-furfurylidene)- 3-amino-2-oxazolidone,

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Table 1. Effects of FZ, AOZ, HEH and 2% acacia solutions on the hepatic microsomal CYP content and CYP-related activities in rats and chickens

| Animal | Treatment | CYP content (nmol/mg microsomal protein) | HXOH (nmol/mg/min) | PROD (pmol/mg/min) | APND (nmol/mg/min) | EROD (nmol/mg/min) | MROD (nmol/mg/min) | PNPH (nmol/mg/min) |
|---|---|---|---|---|---|---|---|---|
| Rat | Control | 0.67 ± 0.11 | 2.92 ± 0.46 | 28.7 ± 10.8 | 1.38 ± 0.18 | 0.54 ± 0.09 | 0.22 ± 0.03 | 8.11 ± 3.98 |
| | FZ (62.5) | 0.71 ± 0.06 | 2.17 ± 0.56 | 24.3 ± 7.5 | 1.53 ± 0.12 | 0.58 ± 0.13 | 0.21 ± 0.06 | 7.68 ± 0.37 |
| | FZ (125) | 0.80 ± 0.18 | 2.02 ± 0.55 | 31.7 ± 9.9 | 1.95 ± 0.53 | 0.72 ± 0.26 | 0.28 ± 0.10 | 10.41 ± 3.06 |
| | AOZ | 0.72 ± 0.06 | 2.04 ± 0.48 | 41.9 ± 13.3 | 1.57 ± 0.33 | 0.65 ± 0.15 | 0.29 ± 0.04 | 10.53 ± 4.28 |
| | HEH | 0.66 ± 0.12 | 2.12 ± 0.73 | 22.9 ± 9.8 | 1.50 ± 0.33 | 0.47 ± 0.12 | 0.17 ± 0.05 | 10.08 ± 1.85* |
| Chicken | Control | 0.20 ± 0.01 | 3.32 ± 0.64 | 1.9 ± 3.3 | 3.15 ± 1.26 | 0.20 ± 0.02 | 0.32 ± 0.12 | 0.57 ± 0.29 |
| | FZ (62.5) | 0.44 ± 0.16 | 4.57 ± 2.13 | 11.6 ± 14.6 | 7.72 ± 2.23* | 0.54 ± 0.23 | 0.57 ± 0.21 | 1.64 ± 0.56* |
| | FZ (125) | 0.65 ± 0.04* | 9.99 ± 3.95* | 11.4 ± 14.6 | 12.29 ± 3.99* | 0.64 ± 0.10* | 0.77 ± 0.24* | 2.71 ± 0.94* |
| | AOZ | 0.28 ± 0.06 | 3.73 ± 1.88 | 10.2 ± 13.5 | 4.09 ± 0.35 | 0.32 ± 0.18 | 0.41 ± 0.04 | 1.11 ± 0.15* |
| | HEH | 0.32 ± 0.02 | 4.54 ± 1.92 | 20.9 ± 17.8 | 6.15 ± 0.34 | 0.51 ± 0.23 | 0.53 ± 0.09 | 1.71 ± 0.50* |

Effects of FZ, AOZ, HEH and 2% acacia solution for control on hepatic microsomal CYP content and CYP-related activities in rat and chicken. Animals were treated with FZ (62.5 or 125 mg/kg/day given orally for four days), AOZ (57 mg/kg/day given intraperitoneally for 4 days), HEH (42 mg/kg/day given intraperitoneally for 4 days) and 2% acacia solution (5 m*l*/kg/day given orally for 4 days), respectively. Each value represents as activity of the mean ± SD carried out in duplicate for 3 animals. Each value represents the mean ± SD of duplicate experiments carried out in 3 animals. Asterisks indicate a significant difference from control animals (Dunnett's test, $P<0.05$).

FZ) and 3-amino-2-oxazolidinone (AOZ) from Ueno Fine Chemical Industry Co. Limited (Osaka, Japan); 2-hydroxyethyl hydrazine (HEH), 7-ethoxyresorufin, 7-methoxyresorufin and 7-pentoxyresorufin from Sigma-Aldrich (St. Louis, MO, U.S.A.); 4-nitrocatechol from Wako Pure Chemical Industries, Limited (Osaka, Japan); p-nitrophenol from Kanto Kagaku Co. Limited (Tokyo, Japan); and hexobarbital from Nagase & Co. Limited (Itami, Japan). Polyclonal anti-rat CYP1A1, 2B1, 2E1, 3A2 and 2C6 antibodies were obtained from Daiichi Pure Chemicals Co. Limited (Tokyo, Japan) and horseradish peroxidase-labeled anti-goat IgG from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, U.S.A.). All other chemicals and solvents used were of the highest quality commercially available. Before treatment, furazolidone was suspended in 2% aqueous acacia solution, whereas AOZ and HEH were dissolved in 0.9% saline solution.

Treatment of all the animals was undertaken according to the policies of the International Animal Care and Use Committee of Hokkaido University (Sapporo, Japan). Female Wistar rats, aged 6 weeks, were obtained from Japan SLC, Inc. (Hamamatsu, Japan), housed in steel cages and fed a pellet diet (Nihon Nosan Kogyo Co., Yokohama, Japan) with water *ad libitum.* Female white Leghorn chickens, aged 2 months, were obtained from Hokkaido Central Chicken Farm (Yubari, Japan), and housed in steel cages and fed a standard diet (Nihon Nosan Kogyo Co.) and water *ad libitum*. All the animals were maintained at 23°C in a 12-hr dark/light cycle starting at 07:00 hr. In this study, the FZ dosages and animals followed those described in the previous report [31]. The following treatment groups each consisted of 3 rats and 3 chickens: FZ (125 mg/kg/day given orally for 4 days), FZ (62.5 mg/kg/day given orally for 4 days), AOZ (57 mg/kg/day equivalent to FZ 125 mg/kg/day given intraperitoneally for 4 days), HEH (42 mg/kg/day equivalent to FZ 125 mg/kg given intraperitoneally for 4 days) and 2% aqueous acacia solution (5 m*l*/kg/day given orally for 4 days) as a control. The respective drugs were administered to the animals once a day.

The animals treated with the respective drugs were euthanized by decapitation under deep anesthesia with carbon dioxide 24 hr after the last administration of the drug. Liver microsomes were prepared according to the method of Omura and Sato [25] and the procedure of the preparation followed those described in a previous report [31]. The protein concentration of the microsome fraction was determined by the method of Lowry *et al*. [21] using a spectrophotometer (Hitachi U-3000, Hitachi Ltd., Tokyo, Japan). Total hepatic microsomal CYP content was measured according to the method of Omura and Sato [25].

*Hexobarbital hydroxylation*: The incubation and extraction procedures described by Farrel and Correia [10] were used to measure hexobarbital hydroxylase (HXOH) activity. After 15 min of incubation, the remaining hexobarbital was extracted with 4 m*l* heptan containing 1.5% isoamyl alcohol and then transferred to 1 m*l* of 0.8 M $K_2P_2O_7$ buffer (pH 11). The amount of remaining hexobarbital in the aqueous phase was measured as the difference in absorbance between 280 nm and 245 nm using the spectrophotometer..

*Alkoxyresorufin O-dealkylation*: Microsomal *O*-dealkylation of 7-ethoxyresorufin (EROD), 7-methoxyresorufin (MROD) and 7-pentoxyresorufin (PROD) were measured according to the method of Clark *et al*. [7]. After 10 min of incubation, formation of resorufin in methanol phase was measured using a spectrophotofluorometer (EP777, JASCO Corporation, Tokyo, Japan) at 528 nm excitation and 590 nm emission wavelengths.

*Aminopyrine N-demethylation*: Aminopyrine N-demethylase (APND) activity was determined by measuring the rate of formaldehyde formation according to the methods of Cooper and Brodie [8] and Nash [23]. After 10 min of incubation, the rate of formaldehyde formation in the supernatant solution was determined spectrophotometrically at 415 nm.

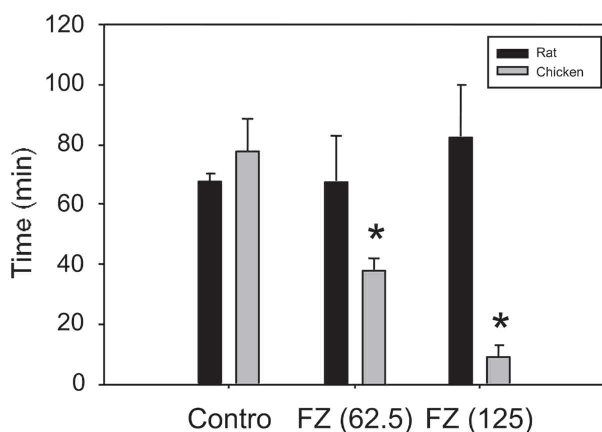


Fig. 1. Effect of FZ and 2% aqueous acacia solution on sleep time induced by hexobarbital in rats and chickens. Effect of FZ and 2% aqueous acacia solution on hexobarbital sleep time in rats (black column) and chickens (gray column). Twenty-four hours after the last dose, the duration of righting reflex loss induced by hexobarbital (100 mg/kg given intraperitoneally) was measured as sleep time. The animals were treated with FZ (62.5 or 125 mg/kg/day given orally by stomach or crop tube for 4 days) and 2% aqueous acacia solution (5 m*l*/kg/day given orally by stomach or crop tube for 4 days) as a control. Each column represents the mean of three animals, and the range bars indicate SD. The asterisks indicate a significant difference from controls (Dunnett's test, $P<0.05$).

*p-Nitrophenol hydroxylation*: The activity of p-nitrophenol hydroxylase (PNPH) was determined by measuring of p-nitrocatechol, according to the methods of Koop [17] and Sinclair *et al*. [33]. After 10 min of incubation, p-nitrocatechol transformed from p-nitrophenol in the supernatant was determined spectrophotometrically at 415 nm.

*Measurement of sleep time*: Twenty-four hours after the last administration of the drugs, the rats and chickens treated with FZ (125 mg/kg), FZ (62.5 mg/kg) or acacia solution received an intraperitoneal injection of hexobarbital (100 mg/kg). Sleep time in each animal was assessed as the duration of righting reflex loss induced by hexobarbital.

*Western blot analysis*: The analysis was performed on the hepatic microsomal protein obtained from the chickens treated with FZ (125 mg/kg) or 2% aqueous acasia solution as a control. Microsomal protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the methods of Laemmli [18] and Towbin *et al*. [36] and then transferred to a nitrocellulose membrane (Toyo Roshi Kaisha Ltd., Tokyo, Japan). Anti-rat CYP1A1, 2B1, 2C6, 2E1 and 3A2 antibodies were used to detect each CYP isoform. The membrane was immunostained using diaminobenzidine, and the staining patterns were analyzed using NIH Image v. 1.63 [20].

CYP content and related activities in rats and chickens with FZ, AOZ and HEH treatment are shown in Table 1. CYP content was slightly increased (but not significant statistically) in the FZ-treated rats. This result is consistent with a previous report [30]. In chickens, treatment with 62.5 mg/kg or 125 mg/kg of FZ resulted in a 2-fold and 3-fold increase in CYP content respectively, compared with controls. This increase in CYP content occurred in a dose-dependent manner.

FZ-treated rats showed no alteration in the CYP-related activities investigated in this study. These activities included CYP2B-related HXOH, CYP2B1-related PROD, CYP2D1-related APND, CYP1A-related EROD, MROD and CYP2E1-related PNPH [12]. These data are in contrast to the findings of Fukuhara and Takabatake [11] who demonstrated that rats fed FZ at a dose of 600 ppm for 7 days had a significant increase in aniline hydroxylase (CYP 2E1-related) activity and significant decrease in APND activity. This discrepancy may have been caused by the different method of FZ-dosing used in that study. Our results were also inconsistent with those of a previous study that showed FZ treatment in rats caused induction of CYP1A1 estimated using the Western blot analysis [32]. In contrast, treatment of chickens with 125 mg/kg of FZ was associated with a significant increase in the CYP-related activities (i.e., HXOH, APND, EROD, MROD and PNPH), except for PROD (Table 1). In addition, UDP-glucuronyl transferase activity also increased 2.3-fold (data not shown). These results partly corresponded to those of Bartlet *et al*. [5].

We also investigated the metabolites of FZ, AOZ and HEH in order to clarify the effect on CYP content and CYP-related activity (Table 1). In rats, no alterations in CYP content and CYP-related activities were produced by AOZ treatment. Treatment with HEH caused no change in CYP content and CYP-related activities, with the exception of PNPH. In chickens, AOZ and also HEH treatments caused a slight increase in CYP content, but no alteration in CYP-related activity with the exception of PNPH. This finding indicates that AOZ may not contribute to the augmentation of the multiple-types CYP-related activities induced by FZ in chickens. Treatment with HEH in both animals resulted in a significant increase in PNPH activity, and this finding was consistent with a previous report by Akin and Norred [4] who demonstrated that a hydrazine derivative increased aniline hydroxylase activity. Although HEH increased PNPH activity, AOZ had no such effect in rat. This result indicates that AOZ is not biotransformed to HEH in rats, in contrast to the suggestion of Stern *et al*. [34], but consistent with the findings of Timperio *et al*. [35].

Figure 1 shows the effects of administration of FZ on sleep time in the animals. Although it was not significant, FZ-treated rats showed a tendency of increased sleep time. This finding is inconsistent with previous studies that reported a significant increase in sleep time with FZ at multiple doses of between 100 – 400 mg/kg [3, 30]. This discrepancy may be attributable to the dose and frequency of FZ administration. In contrast to rats, FZ-treated chickens showed a significant decrease in sleep time. Our finding is consistent with the results of Bartlet *et al*. [5] who demonstrated that 400 ppm of FZ in feed provided for 10 days also caused a significant decrease in sleep time in the chickens. In the current study, chickens treated with 125 mg/g of FZ had a decrease in sleep time associated with a significant increase in HXOH activity, whereas those given 62.5 mg/kg of FZ showed a similar

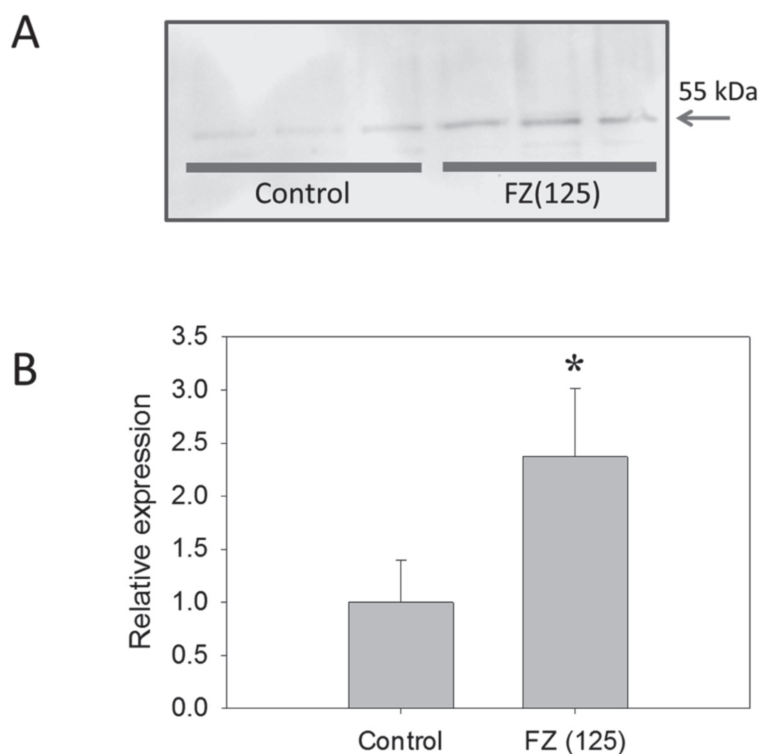


Fig. 2. Western blotting analyses in chicken liver microsomes. Hepatic microsomes were obtained from chickens treated with FZ (125 mg/kg/day for 4 days) and 2% acacia solution. Microsomal protein was separated by SDS-PAGE and transferred to a nitrocellulose membrane. The results of staining were analyzed using NIH Image v.163. A). Anti-rat CYP 2C6 antibody reacted with microsomal apoprotein. The arrow indicates the position of CYP2C6 isoforms. B) Expression level of CYP 2C6 apoprotein. Each column represents the mean of three animals, and the range bars indicate SD. The asterisk indicates a significant difference from controls (Student's *t*-test, $P<0.05$).

decrease in sleep time, but no significant increase in HXOH activity. The decrease in sleep time may therefore be attributable to an increase in HXOH activity, in combination with a significant increase in relative liver weight and /or NADPH P450 reductase activity [31]. Figure 2 shows the result of the Western blot analysis of hepatic microsomes obtained from chickens treated with either FZ (125 mg/kg) or 2% acacia solution (control). Western blot analysis was used to determine whether the increase in CYP-related activities in chickens treated with 125 mg/kg FZ was dependent on induction of hepatic microsomal CYP apoproteins. CYP apoproteins derived from the treated groups reacted with anti-rat CYP2B1, CYP2E1 and CYP2C6 antibody, and among them, CYP2C6 apoproteins were significantly induced by FZ treatment. We failed to detect CYP1A and CYP3A cross-reacted apoproteins; it may be due to their low expression levels and low amino acid homologies between the chicken and the rat.

Johnston *et al*. [15] showed that induction of various CYP, such as CYP2C6, CYP1A1 and CYP4A1 apoproteins after treatment with the pesticide, prochloraz, in red-legged partridges. Given these, we conclude that FZ treatment in chickens causes induction of multiple-types CYP and their related activities.

Our study demonstrated that there were species differences between rats and chickens regarding the effects of FZ on drug-metabolizing enzymes. A number of diverse factors that may cause species differences in the effect of CYP inducers on drug-metabolizing enzymes have been investigated [6]. Basically, variability in the effect of FZ on CYP induction between species depends on differences in the induction mechanism, which in birds include xenobiotic-sensing nuclear receptors of chicken X receptor, which might have a broader substrate spectrum than those of mammalian receptors of pregnane X receptor and constitutive androstane receptor for detoxification [13]. Differences in the type of isozyme induced by FZ may also contribute to species variability.

It was reported that lipid peroxidation of hepatic microsomes decreased CYP content in rats [19], and FZ causes lipid peroxidation of hepatic microsomes in both chickens [29] and rats [2]. Studies have shown that FZ causes greater lipid peroxidation of hepatic microsomes in rats compared

with chickens, as the effectiveness of the antioxidant system is superior in chickens [16, 22]. Accordingly, FZ may cause a greater decrease in the amount of CYP content in rats than that in chickens. As a consequence, the differences in hepatic CYP content between rats and chickens treated with FZ may be magnified by this reduction.

In conclusion, the current study demonstrated that successive treatments of FZ given orally to chickens resulted in a significant increase in hepatic CYP content and multiple CYP-related activities. FZ treatment also induced CYP2C6-like apoprotein in hepatic microsomes in chickens. AOZ administration at an equimolar dose to FZ (125 mg/kg) caused no alteration in CYP-related activities in both rats and chickens with the exception of PNPH activity. This study confirmed that FZ treatment in chicken causes induction of drug-metabolizing enzymes observed previously in rats and pigs [32, 37]. FZ may therefore induces multiple CYPs in humans and numerous animal species. Compounds, including FZ, which induce multiple CYP isozymes, have various toxic actions in animals, such as increasing the toxic effect of some drugs and pollutants [27]. It is therefore important that more attention is paid to the usage of FZ.

ACKNOWLEDGMENT. This study was supported in part by Grant-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, which were awarded to S. Fujita (No. 19208028) and M. Ishizuka (No. 24248056).

REFERENCES


1. Ali, B. H. 1989. Pharmacology and toxicity of furazolidone in man and animals: Some resent research. *Gen. Pharmacol.* **20**: 557–563. [Medline] [CrossRef]
2. Ali, B. H. 1992. Effect of furazolidone on tissue sulfhydryl group, ascorbic acid, and lipid peroxide level in the rat, and the influence of demethylsulfoxide thereon. *Pharmacol. Res.* **25**: 247–254. [Medline] [CrossRef]
3. Ali, B. H. 1999. Phramacological, therapeutic and toxicologic properties of furazolidone: some recent research. *Vet. Res. Commun.* **23**: 343–360. [Medline] [CrossRef]
4. Akin, F. J. and Norred, W. P. 1978. Effects of short-term administration of maleic hydrazine or hydrazine on rat hepatic microsomal enzymes. *Toxicol. Appl. Pharmacol.* **43**: 287–292. [Medline] [CrossRef]
5. Bartlet, A. L., Harvey, S. and Kandorf, H. 1990. Contrasting effects of nitrofurans on plasma corticosterone in chickens following administration as a bolus of diet additive. *J. Vet. Pharmacol. Ther.* **13**: 261–269. [Medline] [CrossRef]
6. Boobis, A. R., Sesardic, D., Murray, B. P., Edwards, R. J., Singleton, A. M., Rich, J., Murray, S., de la Torre, R., Sequra, J. and Pelkonen, O. 1990. Species variation in the response to the cytochrome P450-dependent monooxygenase system to inducers and inhibitors. *Xenobiotica* **20**: 1139–1161. [Medline] [CrossRef]
7. Clark, M. A., Bing, B. A., Gottschall, P. E. and Williams, J. F. 1995. Differential effect of cytokines on the phenobarbital or 3-mthylcholanthrene induction of P450 medicated monooxygenase activity in cultured rat hepatocytes. *Biochem. Pharmacol.* **49**: 97–104. [Medline] [CrossRef]
8. Cooper, J. R. and Brodie, B. B. 1955. The enzymatic metabolism of hexobarbital (evipal). *J. Pharmacol. Exp. Ther.* **114**: 409–417. [Medline]
9. Dupont, H., Davies, D. S. and Strolin-Benedetti, M. 1987. Inhibition of cytochrome P450 dependent oxidation reactions by monoamine oxidase inhibitors in rat liver microsomes. *Biochem. Pharmacol.* **36**: 1651–1657. [Medline] [CrossRef]
10. Farrell, G. C. and Correia, M. A. 1980. Structural and functional reconstitution of hepatic cytochrome P-450 in vivo. Reversal of allylisopropylacetamide-mediated destruction of the hemoprotein by exogenous heme. *J. Biol. Chem.* **255**: 10128–10133. [Medline]
11. Fukuhara, M. and Takabatake, E. 1977. The effects of nitrofuran derivatives on hepatic microsomal mixed-function oxidase activity in rats. *Toxicol. Appl. Pharmacol.* **42**: 571–581. [Medline] [CrossRef]
12. Funae, Y. and Imaoka, S. 1993. Cytochrome P450 in rodents. pp. 221–238. *In*: Cytochrome P450 (Schenkman, J. B. and Greim, H. eds.), Springer-Verlag, Berlin.
13. Handschin, C., Blattler, S., Roth, A., Looser, R., Oscarson, M., Kaufmann, M. R., Podvinec, M., Gnerre, C. and Meyer, U. A. 2004. The evolution of drug-activated nuclear receptors: one ancestral gene diverged into two xenosensor genes in mammals. *Nucl. Recept.* **2**: 1–13. [Medline] [CrossRef]
14. Hu, X. Z., Xu, Y. and Yediler, A. 2007. Determination 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**: 144–149. [CrossRef]
15. Johnston, G., Dowson, A. and Walker, C. H. 1996. Effects of prochloraz and malathion on the red-legged partridge: a semi-natural study. *Environ. Pollut.* **91**: 217–225. [Medline] [CrossRef]
16. Klandorf, H., Rathore, D. S., Iqbal, M., Shi, X. and Van Dyke, K. 2001. Accelerated tissue aging and increased oxidative stress in broiler chickens fed allopurinol. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **129**: 93–104. [Medline] [CrossRef]
17. Koop, D. R. 1986. Hydroxylation of p-nitrophenol by rabbit ethanol-inducible cytochrome P450 isozyme 3a. *Mol. Pharmacol.* **29**: 399–404. [Medline]
18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685. [Medline] [CrossRef]
19. Levin, W., Lu, A. Y., Jacobson, M., Kuntzman, R., Poyer, J. L. and McCay, P. B. 1973. Lipid peroxidation and the degradation of cytochrome P450 heme. *Arch. Biochem. Biophys.* **158**: 842–852. [Medline] [CrossRef]
20. Lennard, P. R. 1990. Image analysis for all. *Nature* **347**: 103–104. [Medline] [CrossRef]
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265–275. [Medline]
22. Nachtomi, E. and Alumot, E. 1972. Comparison of ethylene dibromide and carbon tetrachloride toxicity in rats and chicks: Blood and levels; Lipid peroxidation. *Exp. Mol. Pathol.* **16**: 71–78. [Medline] [CrossRef]
23. Nash, T. 1953. The calorimetric estimation of formaldehyde by means of the Hantzsch reaction. *J. Biol. Chem.* **55**: 416–422.
24. O'Connor, A., Gisbert, J. and O'Morain, C. 2009. Treatment of Helicobacter pylori infection. *Helicobacter Supplement [Remark 2]* **14**: 46–51.
25. Omura, T. and Sato, R. 1964. The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* **239**: 2370–2378. [Medline]

26. Polasek, T. M., Elliot, D. J., Somogyi, A. A., Gillam, E. M., Lewis, B. C. and Miners, J. O. 2006. An evaluation of potential mechanism-based inactivation of human drug-metabolizing cytochromes P450 by monoamine oxidase inhibitors, including isoniazid. *Br. J. Clin. Pharmacol.* **61**: 570–584. [Medline] [CrossRef]
27. Rakitsky, V. N., Koblyakov, V. A. and Turusov, V. S. 2000. Nongenotoxic (epigenetic) carcinogens: pesticides as an example. *A critical review*. *Teratog. Carcinog. Mutagen.* **20**: 229–240. [Medline] [CrossRef]
28. Reimão, J. Q., Taniwaki, N. N. and Tempone, A. G. 2010. Furazolidone is a selective *in vitro* candidate against Leishmania (L.) chagasi: an ultrastructural study. *Parasitol. Res.* **106**: 1465–1469. [Medline] [CrossRef]
29. Sas, B. 1993. Contribution to the pathobiochemistry of furazolidone-induced oxidative toxicity in chickens. *Acta Vet. Hung.* **41**: 103–121. [Medline]
30. Sasaki, N. 1994. Effects of furazolidone on duration of righting reflex loss induced with hexobarbital and zoxazolamine in the rat. *J. Vet. Med. Sci.* **56**: 667–670. [Medline] [CrossRef]
31. Sasaki, N., Matsumoto, T., Ikenaka, Y., Kazusaka, A., Ishizuka, M. and Fujita, S. 2011. Furazolidone induces the activity of microsomal enzymes that metabolize furazolidone in chickens. *Pestic. Biochem. Physiol.* **100**: 135–139. [CrossRef]
32. Sasaki, N., Sakai, N., Ikenaka, Y., Kamiya, T., Heewon, M., Sakamoto, Q. K., Ishizuka, M. and Fujita, S. 2008. Antibiotic furazolidone induces CYP1A but not CYP2E1 subfamily in rat liver. *J. Vet. Med. Sci.* **70**: 223–226. [Medline] [CrossRef]
33. Sinclair, J. F., Wood, S. G., Smith, E. L., Sinclair, P. R. and Koop, D. R. 1989. Comparison of the forms of cytochrome P450 induced by ethanol and glutethimide in cultured chick hepatocytes. *Biochem. Pharmacol.* **38**: 657–664. [Medline] [CrossRef]
34. Stern, I. J., Hollifield, R. D., Wilk, S. and Buzard, J. A. 1967. The anti-monoamine oxidase effects of furazolidone. *J. Pharmacol. Exp. Ther.* **156**: 492–499. [Medline]
35. Timperio, A. M., Juiper, H. A. and Zolla, L. 2003. Identification of a furazolidone metabolite responsible for the inhibition of amino oxidases. *Xenobiotica* **33**: 153–167. [Medline] [CrossRef]
36. Towbin, H., Staehelin, T. and Gorden, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci.* **76**: 4350–4354. [Medline] [CrossRef]
37. Vroomen, L. H., Berahmans, M. C., Hekman, P., Hoogenboom, L. A. and Kuiper, H. A. 1987. The elimination of furazolidone and its open-chain cyano-derivative from adult swine. *Xenobiotica* **17**: 1427–1435. [Medline] [CrossRef]
38. Zolla, L. and Timperio, A. M. 2005. Involvement of active oxygen species in protein and oligonucleotide degradation induced by nitrofurans. *Biochem. Cell Biol.* **83**: 166–175. [Medline] [CrossRef]