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 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-phosphate (G6P) and glucose 6-phosphate dehydrogenase (G6PDH) from Oriental Yeast Co. Limited (Tokyo, Japan); furazolidone (N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone,
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

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

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), HEH (42 mg/kg/day given intraperitoneally for 4 days), AOZ (57 mg/kg/day given intraperitoneally for 4 days), and 2% acacia solution (5 ml/kg/day given orally for 4 days), respectively. 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).
were analyzed using NIH Image v. 1.63 [20].

The membrane was immunos-  
determined spectrophotometrically at 415 nm.

The animals were treated with FZ (62.5 or 125 mg/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).

**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) and the rats and chickens treated with FZ (62.5 mg/kg) or acacia solution. The rats and chickens treated with 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.

**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 measured spectrophotometrically at 415 nm.

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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. 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.

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