Excretion of $^{14}$C-Fumonisin B$_1$, $^{14}$C-Hydrolyzed Fumonisin B$_1$, and $^{14}$C-Fumonisin B$_1$-Fructose in Rats

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$^{14}$C-Fumonisin B$_1$ (FB$_1$) was produced by Fusarium proliferatum M-5991 in modified Myro liquid medium and purified to >95% purity with a specific activity of 1.7 mCi/mmol. Nine male and nine female F344/N rats were each dosed by gavage with 0.69 $\mu$mol of $^{14}$C-FB$_1$, $^{14}$C-hydrolyzed FB$_1$, or $^{14}$C-FB$_1$-fructose/kg body weight. Urinary excretion of $^{14}$C-FB$_1$ and $^{14}$C-FB$_1$-fructose was 0.5% and 4.4% of the total dose, respectively, and was similar between male and female rats. Urinary excretion of $^{14}$C-hydrolyzed HFB$_1$ was significantly greater ($P < 0.05$) in female rats as compared with male rats (17.3% vs 12.8% of the total dose, respectively). There were no significant ($P > 0.05$) differences in biliary excretion of the three fumonisin compounds with a mean of 1.4% of the dose excreted at 4 h after dosing. Lesser amounts continued to be excreted up to 9.25 h after dosing. Although biliary excretion of the $^{14}$C-FB$_1$, $^{14}$C-hydrolyzed FB$_1$, and $^{14}$C-FB$_1$-fructose was similar, increased urinary excretion of the $^{14}$C-hydrolyzed FB$_1$ as compared to $^{14}$C-FB$_1$ and $^{14}$C-FB$_1$-fructose indicated a greater absorption of the hydrolyzed form.

**Keywords:** $^{14}$C-Fumonisin B$_1$; hydrolyzed fumonisin B$_1$; fumonisin B$_1$-fructose excretion

INTRODUCTION

The fumonisins (FBs) are a family of mycotoxins including FB$_1$, FB$_2$, FB$_3$, and FB$_4$ (Gelderblom et al., 1988; Cawood et al., 1991) with FB$_1$ predominant. The FBs are produced by the maize pathogens Fusarium proliferatum and Fusarium moniliforme. Fumonisin consumption causes equine leukoencephalomalacia (Kellerman et al., 1990) and porcine pulmonary edema (Oswiler et al., 1992; Colvin and Harrison, 1992). Fumonisins also cause embryopathogenicity in chickens (Iaved et al., 1993), developmental toxicity in hamsters (Floss et al., 1994), and kidney toxicity and liver cancer in rats (Gelderblom et al., 1991; Voss et al., 1993). Sharma et al. (1997) demonstrated in vivo apoptosis in mouse liver and kidney after doses of 0.35–8.7 $\mu$mol of FB$_1$/kg body weight (bw) were given subcutaneously. The effects of FBs on humans are not known. But epidemiological studies show significant correlations between high levels of FBs in corn consumed by humans and esophageal cancer (Sydenham et al., 1990; Rheeder et al., 1992; Chu and Li, 1994). Fumonisin B$_1$ is listed as a Class 2B carcinogen, a probable human carcinogen (IARC, 1993).

The study of fumonisin metabolism has been facilitated by the production of $^{14}$C-FB$_1$, which permits rapid analysis of the biological disposition of this compound. Fumonisin B$_1$ has been radiolabeled using $^{14}$C-acetate or -methionine in cultures of F. moniliforme or F. proliferatum in liquid medium (Norred et al., 1993; Blackwell et al., 1994; Lebep-Mazur, 1993). In fasted rats, Norred et al. (1993) detected 80% and 2.3% of FB$_1$ administered by gavage (1.4 $\mu$mol of $^{14}$C-FB$_1$/kg bw) in feces and urine, respectively. Liver, kidney, and blood retained a total of 0.6% of the dose 96 h after treatment. In fed rats at 24 h after a dose administered by gavage (10.4 $\mu$mol of $^{14}$C-FB$_1$/kg bw), Shephard et al. (1992) detected 100% and trace levels of FB$_1$ in feces and urine, respectively. Trace levels of $^{14}$C-FB$_1$ were detected in liver, kidney, and blood of these rats. From these two studies, FB$_1$ absorption may be greater in fasted rats than in fed rats. Biliary excretion of FB$_1$ has been suggested by observations of fecal recovery of $^{14}$C-FB$_1$ given by intravenous or intraperitoneal routes (Norred et al., 1993; Shephard et al., 1992). Shephard et al. (1994) recovered 67% of an intraperitoneal dose (10.4 $\mu$mol of $^{14}$C-FB$_1$/kg bw) after 24 h in bile whereas 0.2% of a 0.4-$\mu$mol dose administered by gavage was detected in bile of fed rats. Other forms of fumonisins such as hydrolyzed FB$_1$ have not been evaluated for biliary excretion. Hopmans et al. (1997) analyzed by HPLC the excretion of unlabeled FB$_1$ at three doses. Administration of 0.69, 6.93, and 69.3 $\mu$mol of FB$_1$/kg bw to fed rats resulted in 7.4, 1.2, and 0.5% of the dose excreted in urine, respectively, confirming previous findings with radiolabeled FB$_1$ but also suggesting dose differences in absorption.

FB$_1$ may undergo reactions in food systems that alter its chemical structure and its toxicity. Alkaline hydrolysis of FB-containing corn produced hydrolyzed FB$_1$ (HFB), which was found to promote diethylnitrosamine (DEN)-initiated hepatocarcinogenesis nearly as well as FB$_1$ when fed to rats (Hendrich et al., 1993). Urinary excretion of HFB$_1$ was 2-fold greater than FB$_1$ in rats (Hopmans et al., 1997), suggesting a significant role for increased bioavailability in HFB$_1$ toxicity as compared
with FB1. A Maillard-like reaction between FB1 and a reducing sugar such as fructose can occur during heating (Murphy et al., 1996). Feeding FB1-fructose reaction products caused no development of altered hepatic foci in DEN-initiated rats, whereas an equimolar amount of FB1 promoted hepatocarcinogenesis readily (Lu et al., 1997). The seeming lack of toxicity of FB1-fructose product(s) could not be explained by lesser absorption in Hopmans et al. (1997) because such products were absorbed to a greater extent than FB1 in rats, based on relative urinary excretion. A FB1-glucose reaction product, N-(carboxymethyl)-FB1 has recently been isolated (Howard et al., 1998), but its toxicity is unknown. The mechanism of formation and the nature of the first products in the Maillard reaction are not well-characterized even after over 50 years of study (Ge and Lee, 1997). But fumonisin-sugar products might form during food processing and could diminish or alter fumonisin toxicity.

This study was designed to determine, with 14C-FB1, the extent of urinary excretion of the low doses of 0.69 μmol of FB1, HFB1, and FB1-fructose/kg bw used by Hopmans et al. (1997). We also analyzed biliary circulation of these three forms of fumonisin most likely to be found in foods.

MATERIALS AND METHODS

Reagents were from Fisher Scientific (St. Louis, MO) unless noted otherwise. Milli-Q water (Millipore-Waters, Bedford, MA) was used throughout. All animal procedures and protocols were approved by the Iowa State University Animal Care and Use Committee. FB1 produced from Fusarium cultures is a class 2B carcinogen, and was handled accordingly. We treated HFB1 similarly. We followed Iowa State University Environmental Health and Safety guidelines for the use of 14C.

The 500-ml liquid cultures of F. proliferatum M5991 were prepared as in Dantzer et al. (1996a). These cultures were inoculated with Myro liquid medium (LM), containing MgSO4 at only 0.5 g/l and 1.00% corn hull extract (modified Myro LM). The inoculum culture was incubated for 4 days on a rotary shaker (220 rpm at 23 °C) with 15% (v/v) inoculum were transferred to three replicate rubber-stoppered 125-ml Erlenmeyer flasks containing 50 mL of modified Myro LM. Compressed air was cleaned by passage through five 2-L plastic bottles containing air, 2 N KOH, 2 N KOH, distilled H2O2, and 2 N H2SO4, followed by a moisture trap and a 0.2-μm in-line filter. The purified air was bubbled through the F. proliferatum-inoculated culture at 12-h intervals. At 84 h, the rats were sacrificed by CO2 asphyxiation; blood was drawn by heart puncture, and the hearts, livers, lungs, kidneys, and brains were removed for 14C analysis.

For the biliary excretion study, nine female 15-week-old Sprague–Dawley rats (bw 240–270 g) were used. All rats were housed individually and given AIN-93M diet (Reeves et al., 1993) and water ad libitum for 1 week under 12-h light cycle, groups of three males and three females were administered 0.69 μmol of 14C-FB1, 14C-HFB1, or 14C-FB1-fructose/kg bw by gavage. The rats were housed individually in metabolic cages, and fecal and urine samples were collected at 12-h intervals. At 84 h, the rats were sacrificed by CO2 asphyxiation; blood was drawn by heart puncture, and the hearts, livers, lungs, kidneys, and brains were removed for 14C analysis.
first, second, and third sections (I, II, and III) of small intestines were collected after the rats died or at sacrifice, 9.5 h after dosing. All samples were stored at -20 °C until analysis. 

Fecal samples, oven dried at 60 °C overnight, and intestinal tissues, frozen in liquid N₂, were ground in a porcelain mortar and pestle. Ground feces, intestinal tissues, stomach, stomach wash, kidney, liver, lung, brain, and blood were separately blended in a tissue homogenizer (model TR-10, Tekmar Co., Cincinnati, OH) at 60% power for 0.5–1 min in 5 mL of Milli-Q water, brought to a known volume of 10–35 mL with Milli-Q water, and quantified for ¹⁴C by scintillation analyzer. Quenching of tissues was measured with 0.5–1 mL of tissue extract with and without 18,400 dpm ¹⁴C-FB₁. Data were corrected using percent quenching in the respective sample.

A completely randomized design was used for statistical evaluation of urine and fecal excretion data in the 18 Fisher rats and for the biliary excretion data in the 9 Sprague Dawley rats. Differences among treatments were assessed by a Student’s t statistic (P ≤ 0.05) using SAS (version 6.03, 1995, Cary, NC).

RESULTS AND DISCUSSION

F. proliferatum yielded 1200 μmol of FB₁/L between days 10 and 31 of culture (Figure 1). There was an average production of 0.18 μCi of ¹⁴CO₂/h within the first 24-h period after each addition of 250 μCi of U-¹⁴C-acetate. The production of ¹⁴CO₂ decreased to undetectable levels 3 days after each addition of the ¹⁴C-acetate at an apparent logarithmic rate, suggesting that the production of acetate by the F. proliferatum culture was significantly greater than the addition of the labeled acetate. The culture was harvested at 24 days. The FB₁ was purified to >95% purity with a yield of 24 μmol and a specific activity of 1.7 mCi/mmol. ¹⁴C-Fumonisin B₁ measurement in biological samples allowed for improved monitoring of FB₁ as compared to HPLC fluorescence detection. But scintillation counting of ¹⁴C-FB₁ could not reveal any information on metabolic modification of FB₁ or its related forms.

The doses for the urine and fecal excretion study were 0.14 μmol of ¹⁴C-FB₁, ¹⁴C-HFB₁, or ¹⁴C-FB₁-fructose/mL with specific activities of 1.7, 1.2, and 1.8 mCi/mmol, respectively. The lower specific activity of the HFB₁ suggested that part of the U-¹⁴C-acetate was incorporated into the tricarboxylic side chains of FB₁ that were removed during hydrolysis to produce HFB₁.

Quenching in the blood and liver fractions was determined to be 86 and 28%, respectively, for added ¹⁴C-FB₁. All other tissues had negligible quenching (data not shown). All data were corrected for quenching.

Recently, Howard et al. (1998) identified N-(carboxymethyl)-FB₁ as a reaction product of FB₁ and glucose. Yaylayan and Huyghues-Despointes (1994) reported that nonenzymatic browning reaction products from fructose are much more complicated than those from glucose due to the nature of the products formed from a keto. In addition, our reaction time between FB₁ and fructose was 4-fold longer than that reported by Howard et al. (1998), which probably means that our FB₁-sugar adducts were a much more complex mixture, as is usually seen with the formation of Amadori products (Labuza, 1994). For the sake of simplicity in this paper, we named our fructose-FB₁ reaction product mixture, FB₁-fructose. Our limited attempts to identify the products produced in the FB₁-fructose (and -glucose) model systems by mass spectrometry have been unsuccessful to date. A number of o-phthalaldehyde (OPA) fluorescent peaks were detected in the FB₁-fructose reaction mixture, but we would not expect anything reacting with the FB amine group to be detected by amine derivatization.

Excretion of ¹⁴C in urine over time did not differ significantly between genders, except for HFB₁ during the first excretion interval [female > male (Table 1)], so data were combined (n = 6) (Figure 2). Fifteen percent, 4.4%, and 0.6% of the total ¹⁴C dose was excreted in urine for HFB₁, FB₁-fructose, and FB₁, respectively (Table 1). The average half-life for excretion of FB compounds was 10 h. There were only trace amounts of ¹⁴C-FB₁ excreted in urine with most of the urinary excretion occurring in the first 12 h (Figure 2). The urinary excretion of HFB₁ persisted for up to 60 h after dosing with maximum excretion occurring between 12 and 24 h. The urinary excretion of ¹⁴C-FB₁-fructose persisted for 24 h with the maximum excretion occurring in the first 12 h. The pattern of excretion of HFB₁ and of FB₁-fructose resembled one-compartment models of elimination. Amounts of 2-5-fold greater ¹⁴C-HFB₁ than FB₁ were excreted in urine, suggesting that HFB₁ was better absorbed than FB₁ in these rats. Dietary HFB₁ was nearly as toxic as FB₁ during the promotion phase of a two-stage model of rat hepatocarcinogenesis (Hendrich et al., 1993). Because the similarity between HFB₁ and FB₁ in toxicity may be accounted for by the greater bioavailability of HFB₁, FB₁ would seem to be more toxic at the cellular level than HFB₁. But in primary rat hepatocytes, HFB₁s were more cytotoxic than FB₁s as measured by lactate dehydrogenase leakage (Gelderblom et al., 1993). The toxic dose of FB₁s was 1 mM or more in the hepatocyte cultures. Such high concentrations would be highly unlikely to be achievable through dietary exposures, given the very limited apparent absorption of FB₁. The hepatocyte culture studies are probably not helpful in explaining FB₁ toxicity in vivo.

Total excretion of ¹⁴C-FB₁-fructose was 8-fold greater than FB₁, suggesting that FB₁-fructose may have greater bioavailability than FB₁ in these rats (Table 1). Fumonisin B₁-fructose product(s) seem to be detoxified forms of FB₁ during the promotion phase of a two-stage rat hepatocarcinogenesis model (Lu et al., 1997). The mechanism cannot be due to reduced absorption of FB₁-fructose. Blocking the FB amine group prevented toxicity in primary rat hepatocyte cultures as well as in vivo (Gelderblom et al., 1993). Perhaps the blocked amine group sterically prevents the interaction of FBs with their molecular sites of action.
with 0.69 mol of 14C-FB1 molecule for OPA derivatization. In the current study, hydrolysis of urine produced a free amine group on the total dose of FB 1 in the urine as compared with 4.4\% 14C-HFB1, and 14C-FB1-fructose/kg body weight by rats per 12-h interval.

**Figure 2.** Urine excretion of 14C from 0.69 \( \mu \)mol of 14C-FB1, 14C-HFB1, and 14C-FB1-fructose/kg body weight by rats per 12-h interval. Error bars represent ± 1 standard deviation; \( n = 6 \). Bars within a time interval with different superscripts were different at \( \alpha = 0.05 \). FB1-FRU = FB1-fructose.

The total urinary excretion of 0.69 \( \mu \)mol of HFB1 and FB1-fructose as compared to FB1 over 84 h was comparable to the previous study over 96 h at three dose levels (Hopmans et al., 1997). Both studies reported the same total percent urinary excretion of HFB1 and relative absorptions of HFB1 and FB1-fructose as compared to FB1. In the study by Hopmans et al. (1997), rats dosed with 0.69 \( \mu \)mol of FB1-fructose or FB1/kg bw excreted 4.2\% of the total dose of FB1-fructose and 7.4\% of the total dose of FB1 in the urine as compared with 4.4\% and 0.6\%, respectively, in our current study. Hopmans et al. (1997) correlated FB1 and FB1-fructose to the amount of OPA-HFB1 in the hydrolyzed rat urine. Hydrolysis of urine produced a free amine group on the FB1 molecule for OPA derivatization. In the current study, FB1 and FB1-fructose were measured as the amount of 14C detected in the rat urine. The differences in these two studies may reflect a difficulty in accurate estimation of very small quantities of FBs by sample extraction and HPLC analysis. Norred et al. (1993) found that 2–3\% of a gavaged dose of 1.4 \( \mu \)mol of 14C-FB1/kg bw was excreted in the urine of fasted Sprague–Dawley rats after 96 h, suggesting that their fasted rats had a greater absorption of FB1 than our rats. Our dosing of rats occurred at the end of the light cycle. Rats do not eat much during the light cycle, so our rats should have been fairly comparable to rats fasted for 12 h. The apparent higher absorption by Norred’s rats as compared to our rats could be attributed to differences in strain of rat or, more likely, to differences in absorption of FBs with the extent of food deprivation. In fed rats, food may make FBs less available for GI tract absorption.

The data for fecal excretion of 14C in male and female rats, dosed with the same compound, were combined because they were not significantly different according to gender (Figure 3). The pattern of fecal excretion of 14C-FB1, 14C-HFB1, and 14C-FB1-fructose followed a normal excretion of a compound through the fecal route (Casarett and Doull, 1991) with maximum excretion of 14C from the three FB forms between 12 and 24 h. After 60 h, only trace amounts of 14C were recovered in the feces from these rats. Total fecal excretion was not significantly different between FB compounds or gender and averaged 90\% recovery of total dose after 84 h (Table 1).

The total 14C recovered from the hearts, brains, livers, blood, kidneys, or lungs of rats dosed with 0.69 \( \mu \)mol of 14C-FB1, 14C-HFB1, or 14C-FB1-fructose/kg bw was not significantly different from zero for all rats, indicating that accumulation of these compounds did not occur after 84 h (data not shown).

In the biliary excretion study, Sprague–Dawley rats were used because the Fisher rats were not large enough for successful cannulation. The same FB treatments were used for the biliary excretion study as in the urinary excretion study. However, to obtain proper volumes, the 0.14 \( \mu \)mol of 14C-HFB1 and 14C-FB1-fructose/mL treatments were diluted with unlabeled 0.14 \( \mu \)mol of HFB1 or FB1-fructose/mL, which resulted in specific activities of 0.7 and 1.0 mCi/mmol, respectively. All nine of the cannulated female rats survived for 4 h after dosing. Biliary excretion of 0.69 \( \mu \)mol of 14C-FB1, 14C-HFB1, or 14C-FB1-fructose/kg bw by female rats were not significantly different with an average of 1.35\% and a range of 0.80 (FB1-fructose) to 1.17 (HFB1) of the total 14C dose excreted 4 h after dosing (Table 2). Biliary excretion of the three FB compounds increased

**Table 1. Percent Recovery of 14C from 0.69 \( \mu \)mol of 14C-FB1, 14C-HFB1, and 14C-FB1-fructose/kg Body Weight in Rats\textsuperscript{a}**

<table>
<thead>
<tr>
<th></th>
<th>total dose</th>
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<th>tissues</th>
<th>recovery</th>
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<tr>
<td></td>
<td>urine</td>
<td>feces</td>
<td>tissues</td>
<td></td>
<td></td>
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<tr>
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<td>male</td>
<td>female</td>
<td>male</td>
<td>female</td>
<td>n</td>
</tr>
<tr>
<td>FB1</td>
<td>0.4\textsuperscript{a}</td>
<td>0.7\textsuperscript{a}</td>
<td>85</td>
<td>95</td>
<td>0.28</td>
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<tr>
<td>HFB1</td>
<td>12.8\textsuperscript{d}</td>
<td>17.3\textsuperscript{d}</td>
<td>87</td>
<td>91</td>
<td>0.14</td>
</tr>
<tr>
<td>FB1-fructose</td>
<td>4.2\textsuperscript{b}</td>
<td>4.6\textsuperscript{b}</td>
<td>86</td>
<td>97</td>
<td>0.19</td>
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\textsuperscript{a} Different superscripts indicate that the means were significantly different at \( \alpha = 0.05 \); \( n = 3 \) per gender and treatment.

**Table 2. Biliary Excretion of 14C from 0.69 \( \mu \)mol of 14C-FB1, 14C-HFB1, and 14C-FB1-fructose/kg Body Weight in Rats over 4 h\textsuperscript{a}**

<table>
<thead>
<tr>
<th></th>
<th>dose</th>
<th>percent excretion</th>
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<tr>
<td>FB1</td>
<td>1.55 ± 2.51</td>
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<tr>
<td>HFB1</td>
<td>1.71 ± 1.99</td>
<td></td>
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<tr>
<td>FB1-fructose</td>
<td>0.80 ± 0.82</td>
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\textsuperscript{a} \( n = 3 \).
from 0 to 0.5% of the total dose per 0.5-h interval within 2 h after dosing. The three forms of FB continued to be excreted in the bile by the rats up to 9.5 h after dosing (Figure 4). There seemed to be no cyclical nature to 14C excretion in the bile after administration of the dose by gavage. The large range seen in the biliary excretion data suggest the presence of fluorescent compounds that have been previously observed in rat bile (Hicks et al., 1984). Only two rats from the 14C-FB1 dose and one rat from the 14C-HFB1 and 14C-FB1-fructose doses survived for 9.5 h. Shephard et al. (1994) recovered almost 7-fold less, 0.2% of their total 14C dose in bile duct cannulated rats gavaged with 14C-FB1 as compared to our rats. The rats used by Shephard et al. (1994) were not under anesthesia during gavage or bile collection. Half of the 14C dose of the three forms of FB were recovered in the stomach tissue and contents of our rats (50 ± 23% of the total dose), indicating that only about half of the dosed-FB compounds could have reached the small intestine of these rats (data not shown). Because these rats were under anesthesia throughout the duration of the experiment, there may have been a slower rate of absorption as compared with Shephard et al. (1994). The pooled intestines and bile samples from the rats contained 3 ± 6 and 3 ± 4% of the total dose, respectively. Kidneys and livers contained less than 1% of the 14C dose. Total recovery of the 14C doses from these rats of the bile excretion study averaged 63 ± 17%.

The 25-fold greater absorption of 14C-HFB1 than that of 14C-FB1 in male and female Fisher rats suggested that, once in circulation, HFB1 was less toxic than FB1, on a molar basis, because both have been shown to be equally toxic on a dietary basis to rats (Hendrich et al., 1993). Detoxification of FB1 by the formation of FB1-fructose was not the result of decreased absorption since 14C-FB1-fructose was absorbed 8-fold more than 14C-FB1 by these rats. These data complement and extend the findings of Hopmans et al. (1997), suggesting that HFB1 or FB1-fructose were absorbed more than FB1. In addition, there were no differences in biliary excretion of 14C-FB1, 14C-HFB1, or 14C-FB1-fructose in female Sprague–Dawley rats, lending additional support to the likelihood that the observed decrease in urinary excretion of FB1 as compared with HFB1 or FB1-fructose was due to decreased absorption of FB1 relative to HFB1 or FB1-fructose in rats.

**LITERATURE CITED**


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