ACUTE TOXICITY OF PATULIN IN MICE AND RATS*

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SUMMARY

Patulin had the following 14-day LD50 values when dissolved in saline (pH 7.2) and given as a single i.p. dose: mice, 7.6 mg/kg and weanling rats, 5.9 mg/kg. Administration by stomach tube increased the single dose LD50 value to 17 and 108–118 mg/kg in mice and weanling rats, respectively. Neonatal rats given a single dose of patulin 24 h after birth gained less weight (dose-response relationship apparent than controls during the interval from dosing to 21 days of age. The 14-day oral LD50 in these neonates was 6.8 mg/kg. Patulin potentiated pentobarbital-induced narcosis in adult mice. Because the lethal effect of patulin also was increased by pretreatment with SKF-525A, we suggest that the parent compound, not a metabolite, is the toxic form of this mycotoxin. Death generally occurred within 48–72 h regardless of the route of patulin administration or animal species involved. Focal hepatic necrosis occurred in 10% of the treated mice. Atelectasis was prominent in 64% of the lungs, and alveolar septal congestion and limited intraalveolar hemorrhage were found in an additional 36% of the treated mice and weanling rats. Although stomachs from nursing rats exposed by gavage to patulin were massively distended with milk, microscopic examination showed no changes other than the extremely stretched wall.

INTRODUCTION

Patulin (4-hydroxy-4H-furo (3,2c)pyran-2(6H)-one is a water soluble

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Abbreviation: 3 MC, 3-methylcholanthrene.
β-unsaturated lactone produced by *Byssoschlamys nivea* [1], and by the fungal genera *Penicillium* and *Aspergillus* [2,3]. The first disease linked to patulin occurred when dairy cattle in Japan died after eating feed artificially contaminated with *Penicillium urticae* [4]. Other patulin-related deaths have been reported in cattle in Germany [5] and France [6]. The occurrence of patulin in apples and apple juices has been observed in several countries including the United States [3,7]. Patulin also has been reported in bread [8]. Thus, the presence of patulin in foodstuffs meant for human consumption is probably common and potentially harmful [9,10].

Broom et al. [9] reported LD_{50} values for male mice of 15 and 35 mg/kg after intraperitoneal and oral administration, respectively. The i.p. LD_{50} was confirmed by Hoffman et al. [11]; however, lower values (5.7 and 8.2 mg/kg, respectively) were reported by Ciegler et al. [12] and Moule and Hatey [13]. Some of the variability could be due to differences in technique or methods used to establish the LD_{50}; but none of these reports contained sufficient details to determine the cause of this variability. Furthermore, data on acute toxicity of patulin in neonates have not been reported. Therefore, the purpose of this study was: (1) to determine acute toxicities in adult male and female mice and in weanling and neonatal rats after intraperitoneal, intracranial or oral administration; (2) to determine effects of patulin on morbidity, pentobarbital-induced sleeping time (male mice only) and growth rate (neonatal rats only); and (3) to define tissue alterations as a consequence of acute patulin exposure. These studies should give a better understanding of the degree of toxicity of this naturally occurring food contaminant as well as some insight into its histopathology in experimental animals.

**MATERIALS AND METHODS**

**Chemicals**

The purity of patulin (99%) produced by the method published by Norstadt and McCalla [14], was established by melting point, thin-layer chromatography, infrared and mass spectroscopy. Patulin was stored in the dark at −20°C and fresh solutions were prepared (normal saline vehicle at pH 7.2) before each use. All other chemicals were purchased from a commercial source (Sigma Chemical Co., St. Louis, MO).

**Animals**

Adult ICR mice (20–30 g), and neonatal (6–8 g) and weanling (55–60 g) Sprague–Dawley rats of both sexes were used for these studies (Charles River, Wilmington, MA). Animals were fed appropriate commercial rations (Purina, St. Louis, MO) and were housed in air-conditioned rooms artificially lit for 12 h/day. Feed and water were provided continuously.

We mated adult rats to produce the neonates and weanlings used. 1 female was housed with 2 males overnight and then the female was moved to a separate cage. In the case of neonates used to establish LD_{50} values, pups
were given a single dose of patulin the day after birth and were returned to the mother. Pups were handled with gloves. For the weanling studies, rat pups were allowed to reach 55–60 g before being used. Offspring, regardless of treatment, were observed daily for morbidity and mortality and daily body weights were recorded. Livers, kidneys and hearts were removed from these rats at 21 days and weighed. Liver to body weight ratios were calculated for the neonatal rats at day 21.

Effects of patulin on narcosis and enzyme induction in male mice

The effects of patulin on pentobarbital narcosis were examined in adult male mice. Unique groups of 10 mice were given 3.0 mg/kg patulin in 0.1 ml saline or 0.1 ml saline by i.p. injection at predetermined time intervals. A challenge dose of sodium pentobarbital (60 mg/kg, i.p.) was administered at 30 min, 24, 48, 72 and 96 h post dosing. Immediately before the sodium pentobarbital challenge, body weight was measured. Mice in groups of 10 also were injected i.p. with 0.0 (saline only), 0.5, 1.0, 3.0 and 7.5 mg/kg patulin. After 24 h, the mean sleeping time of each group was determined following sodium pentobarbital challenge. The duration of sleeping time recorded was the interval between loss and reappearance of the righting reflex.

The effects of pretreatment with pentobarbital, 3-methylcholanthrene (3 MC) and SKF-525A on the LD50 of patulin also were evaluated. Mice in groups of 10 were treated, twice with 40 mg/kg, i.p., SKF-525A and 45 min later were given various dose levels of patulin by i.p. injection. Other groups of mice were exposed to pentobarbital either by subcutaneous implantation of a specially formulated pentobarbital pellet for 72 h [15] or by daily injection (i.p.) of 75 mg/kg pentobarbital for 3 days. The LD50 for patulin also was determined on mice preexposed to 4 daily i.p. injections of 20 mg/kg 3 MC. Appropriate placebo-implanted or saline-injected controls were used. All test mice were injected i.p. with 0, 5, 10, 12.5 or 15 mg/kg patulin. Body weight and mortality were monitored for 21 days.

Statistical analysis

All statistical analyses were performed using Student's t-test; statistical significance was accepted for P < 0.05. 14-day LD50 values were estimated by probit analysis [16]. 4 groups of 10–20 animals each were used.

Histopathology

Tissues for histologic examination were removed from animals (adult mice, weanling rats) that died and from those killed by decapitation 14 or 21 days after exposure to a single i.p. dose of patulin. Mice and rats killed at 14 and 21 days were bled out at sacrifice except for those rats and mice used for lung pathology. Lungs from these animals were infused with Lavadowsky's solution immediately after decapitation. Heart, kidneys, liver, lung and in the case of nurseling rats, the alimentary tract (esophagus to colon) were fixed in Lavadowsky's solution (10% formaldehyde, 50 cc, 95%
ethanol, 50 cc and glacial acetic acid, 2 cc). Sections of liver also were fixed
in 10% aqueous formaldehyde to permit frozen sectioning and staining
(Sudan black) for fat. Representative portions from fixed organs were
embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and
eosin and by a method utilizing periodic acid-Schiff's reagent plus hema­
toxylin. Stomachs from selected control and patulin-treated nurseting rats
were stained by Mallory and Masson's method to demonstrate fibrosis and
by a silver technique for demonstrating stromal reticulum [17].

RESULTS

LD₅₀ Determinations

Single doses of patulin killed both rats and mice. The results of LD₅₀
calculations are summarized in Table I. In all cases, patulin was most toxic
when given by the i.p. route. In the adult male mouse, patulin had an LD₅₀
in the range of 6.62-8.58 mg/kg with a mean of 7.60 mg/kg when adminis­
tered i.p. in saline. This value was not significantly different from the LD₅₀
for the female. Also, acute toxicity was not influenced by the vehicle used.
In mice, the LD₅₀ for patulin given orally was twice as large as the LD₅₀ for
patulin given i.p. The intracranial (i.c.) LD₅₀ for male mice was 0.57 mg/kg.
In weanling rats LD₅₀ values were on the order of 5.24-6.46 with a mean
of 5.80 mg/kg body weight when dosed i.p. for females and males,
respectively. Toxicity was reduced significantly in weanling rats given a
single oral dose of patulin (108 and 118 mg/kg in males and females, respect-

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Mean weight (g)</th>
<th>No. of animals</th>
<th>Dosing route</th>
<th>Vehicle</th>
<th>LD₅₀ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>M</td>
<td>60</td>
<td>40</td>
<td>i.p.</td>
<td>PS</td>
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<td>60</td>
<td>48</td>
<td>i.p.</td>
<td>PS</td>
<td>5.80 ± 0.64</td>
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<tr>
<td>Rat</td>
<td>M</td>
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<td>30</td>
<td>p.o.</td>
<td>PS</td>
<td>108 ± 10</td>
</tr>
<tr>
<td>Rat</td>
<td>F</td>
<td>60</td>
<td>30</td>
<td>p.o.</td>
<td>PS</td>
<td>118 ± 15</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td>6.5</td>
<td>90</td>
<td>p.o.</td>
<td>PS</td>
<td>6.80 ± 0.51</td>
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<td>Mouse</td>
<td>M</td>
<td>25</td>
<td>50</td>
<td>i.p.</td>
<td>DMSO</td>
<td>7.60 ± 0.98</td>
</tr>
<tr>
<td>Mouse</td>
<td>F</td>
<td>25</td>
<td>50</td>
<td>i.p.</td>
<td>DMSO</td>
<td>7.80 ± 0.99</td>
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<tr>
<td>Mouse</td>
<td>M</td>
<td>25</td>
<td>50</td>
<td>i.p.</td>
<td>DMSO</td>
<td>6.92 ± 0.87</td>
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<tr>
<td>Mouse</td>
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<td>25</td>
<td>50</td>
<td>i.p.</td>
<td>PS</td>
<td>7.69 ± 0.72</td>
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<tr>
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<td>50</td>
<td>p.o.</td>
<td>PS</td>
<td>17.0 ± 4.0</td>
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<tr>
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<td>50</td>
<td>p.o.</td>
<td>PS</td>
<td>16.1 ± 3.7</td>
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<tr>
<td>Mouse</td>
<td></td>
<td>25</td>
<td>50</td>
<td>i.e.</td>
<td>PS</td>
<td>0.57 ± 0.06</td>
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</table>

a PS = normal saline, pH 7.2; DMSO = dimethylsulfoxide.
b LD₅₀'s ± standard error of the LD₅₀ at 14 days [16].
c Males and females pooled.
d i.e. = intracranial.
ively). In the neonatal rat, the oral LD₅₀ was 6.8 mg/kg, reflecting a difference in susceptibility with age.

Weight gain was decreased (dose-response relationship) in neonatal rats given by gavage a single dose of 2.5, 7.5 or 12.5 mg/kg patulin 24 h after birth (Fig. 1). None of the pups died at 2.5 mg/kg while 60% of the pups died at 7.5 mg/kg. Only 3 of 30 rat pups given 12.5 mg/kg patulin survived 21 days. All pups given 15 mg/kg patulin died within 2 days of treatment. None of the other organs (heart and kidneys) were depressed.

Most rats and mice given patulin, particularly the smaller doses, died 48–72 h after treatment. Regardless of the route of administration, cyanosis of the extremities and convulsions generally preceded death. In surviving animals, these conditions disappeared, and in general survivors appeared normal at the end of the experiment. Furthermore, these responses were more pronounced in mice than rats and were most pronounced after i.c. administration of patulin. No noticeable effects were seen in control animals which received injections of saline by the various routes.

Effects of patulin on enzyme induction and narcosis

Because many animals died several days after treatment, we studied the possibility that there was biotransformation of patulin to a more toxic

![Diagram](image-url)

Fig. 1. Mean body weights of 24-h-old rats given a single oral dose of patulin: 0 (control: ○); 2.5 (●); 7.5 (▲); or 12.5 (★) mg/kg. Values are the means of 15–20 animals.
TABLE II
EFFECT OF SKF-525A, 3-METHYLCHOLANTHRENE (3 MC) AND PENTOBARBITAL ON THE 14-DAY LD_{50} OF PATULIN IN MALE MICE^a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number/group</th>
<th>LD_{50}^b (mg/kg)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>20</td>
<td>7.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>SKF-525A</td>
<td>10</td>
<td>2.3 ± 1.2*</td>
<td>-69.7</td>
</tr>
<tr>
<td>3 MC</td>
<td>10</td>
<td>7.3 ± 1.2</td>
<td>-3.9</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>10</td>
<td>8.5 ± 1.6</td>
<td>+11.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>10</td>
<td>7.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Pentobarbital pellet</td>
<td>10</td>
<td>9.6 ± 1.1</td>
<td>+24.6</td>
</tr>
</tbody>
</table>

^a Animals received a single i.p. dose of patulin in saline, pH 7.2.
^b LD_{50}'s were calculated by the method of Litchfield and Wilcoxon [16], employing 4 groups of 10–20 animals/group.
* Significantly different from untreated control (P < 0.05).

metabolite(s). Experiments were conducted to determine if acute toxicity of patulin was influenced by changes in the hepatic drug metabolizing enzymes (Table II). Mice pretreated i.p. with 75 mg/kg pentobarbital or with a single sodium pentobarbital pellet tended to be less sensitive to patulin. Neither effect, however, was significant. No change in the LD_{50} of patulin was observed in mice pretreated with 3-methylcholanthrene but pretreatment with SKF-525A reduced the LD_{50} significantly.

Data in Fig. 2 show the time course effect of patulin on pentobarbital-induced narcosis in male mice. Values are means ± S.E. of a minimum of 10 animals. * Indicates significantly different (P < 0.05) from solvent-treated animals.
induced narcosis. 24 h after mice were given 3 mg/kg patulin, there was a 1.5-fold increase in the sleeping time induced by pentobarbital. The average sleeping time for mice given the same dose of patulin and then challenged 48 h later with pentobarbital was 125 ± 20 min; although this was less than at 24 h, it was still significantly elevated. The pentobarbital-induced sleeping time of treated animals remained elevated at 72 h but returned to control levels by 96 h post treatment.

The dose-dependent potentiation of patulin on pentobarbital-induced narcosis is presented in Fig. 3. The average sleeping times for mice given 0, 0.5, 1, 3 or 7.5 mg/kg of patulin 24 h prior to pentobarbital challenge were 60, 75, 95, 150 and 240 min, respectively. The average sleeping times of all mice except those given 0.5 mg/kg were increased significantly. The average sleeping times for mice given 3 or 7.5 mg/kg of patulin were significantly longer than the sleeping times for mice given 1 mg patulin/kg body weight.

Histopathology
At autopsy lungs from patulin-treated adult mice and weanling rats were congested. Lungs from controls given only saline appeared normal. There were no abnormal lesions in kidneys and hearts obtained from any of the
CONTROLS

Fig. 1. Stomachs from nursing rats. Numbers 513 and 520 are from control rat pups 9- and 24-days old (last 24 h, separated from dam). Numbers 527, 534 and 471 are stomachs from nursing rats given patulin p.o. at the rate of 12.5 mg/kg/day for 8 days, 7.5 mg/kg/day for 23 days and 12.5 mg/kg/day for 23 days, respectively, and then separated from the dams for the 24-h period prior to being killed.

Patulin-treated animals (adult mice and weanling rats) examined by light microscope. There was, however, focal hepatic necrosis in 10% of the mice treated with 3 mg patulin/kg or higher. Atabectasis was prominent in 64% of lungs (all dose levels) and alveolar septal congestion and limited intraalveolar hemorrhage occurred in 36% of the patulin-treated mice (3 mg/kg and higher) and weanling rats (7.5 mg/kg and higher). Tissue from control mice and rats was normal.

Stomach from neonates exposed p.o. to patulin (7.5 or 12.5 mg/kg) for 8--22 days and then removed from the dam for 24 h remained massively distended with milk (Fig. 4). Distention of the stomach occurred only in nursing rat pups treated with patulin although stomachs from untreated control pups were often engorged with milk but returned to normal after 24 h separation from the dam. When weaned rats (being fed only solid food) were given the same dose regimen of patulin, gastric distension was not observed. The usual anatomical demarcations into cardia, fundus and pyloric portions were obliterated by the distention. The pylorus was not distended, and the pyloric sphincter was constricted. These distended and fragile stomachs (with the associated esophagus and intestinal tract) were fixed enblock in Lavdowsky's solution for a month. The stomachs were normal when examined by light microscope.

DISCUSSION

Neither sex nor the solvent used for administration influenced toxicity in the mouse; sex did not influence toxicity in the rat. The route of administration as well as age affected the lethality of patulin in rats; the oral LD₅₀
(108–118 mg/kg) was higher than the i.p. LD₅₀ (5.8 mg/kg) in weanling rats and the oral LD₅₀ (6.8 mg/kg) for the neonate was lower than for the weanling rat. The reduction in toxicity associated with oral treatment may be attributed to a variety of factors including poor absorption or the chemical instability of patulin in the acidic conditions that exist in the upper gastrointestinal tract. However, patulin is stable for several weeks over the pH range of 3.3–6.3 [18]. Since the new-born rat has a less well developed gut flora than the weanling, the intestinal flora could be involved in degradation of patulin thereby reducing its toxicity in the weanling rat. The poorly developed hepatic mixed function oxidase system in the neonate may explain the increased toxicity of patulin in the neonate, particularly if the parent compound is the toxic form as suggested.

Animals (adult mice and weanling rats) that died after treatment with patulin had massive atelectasis, limited alveolar septal congestion and intra-alveolar hemorrhage in the lungs. Animals that survived, even those given the same or larger doses, had less prominent pathologic alterations. In nursing rats, the results of gross and microscopic examination of stomachs and duodenum support our suggestion that the massive retention of milk resulted from prolonged pylorospasm. Further, it seems likely that the greatly distended stomachs extensively restricted respiration, compounding the incompetence of the already damaged lungs.

Escoula et al. [9] also observed a phase of intense agitation and signs of pain followed by neuromuscular and respiratory responses eventually leading to cyanosis of appendages in mice and rats exposed to a single dose of patulin. Congestion of subcutaneous connective tissue, exudate in the peritoneal cavity and congestion of the gastrointestinal tract, liver, spleen, lung and kidneys were characteristic findings in treated rats and mice. Hemorrhage and congestion were common in all organs. Although there was pulmonary damage and limited focal hepatic necrosis in our study, we did not observe the variety of lesions reported by others.

Although Dailey et al. [20] have suggested that a metabolite(s) of patulin, possibly formed in the gastrointestinal tract, is the toxic agent(s), our results suggest that lethality, at least, is due to the parent compound and not to a toxic metabolite(s). Although pretreatment with pentobarbital or 3 MC did not alter the LD₅₀ of patulin, pretreatment with SKF-525A decreased the LD₅₀ value. These results imply that a decrease in the mixed function oxidase activity may be associated with increased toxicity of patulin. If toxic metabolites were responsible, then pretreatment with pentobarbital or 3 MC would increase the LD₅₀, while pretreatment with SKF-525A would increase the LD₅₀. In fact, the opposite effect was observed, at least in the case of SKF-525A. The possibility exists that there are simultaneous and competing types of metabolism and that microsomal stimulation may decrease formation of a toxic metabolite and increase detoxification. It also is possible that since patulin is water soluble, enzymes other than microsomal enzymes are involved in its metabolism. Nonetheless, these data do suggest that the parent form of patulin is the toxic species.
REFERENCES

8. J. Reiss, Naturwissenschaften, 59 (1972) 37.