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(参考)

Long-term Carcinogenicity and Toxicity Studies of Patulin in the Rat

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Key words: patulin, mycotoxin, carcinogenicity, rat, reproduction.

Patulin is a mycotoxin produced by a variety of *Penicillium* and *Aspergillus* species which are likely natural contaminants of various foods. The present study was conducted to determine the effects of lifetime administration of patulin in FDRL Wistar rats. Animals received patulin by gastric intubation three times per week at the level of 0.0, 0.1, 0.5 and 1.5 mg per kg body weight. The animals used in this lifetime study were derived from F_0 parents exposed to equivalent levels of patulin for 4 weeks before mating, and throughout mating, gestation and lactation. Patulin treatment at 0.5 and 1.5 mg kg^{-1} to male rats caused a significant decrease in body weight gain in comparison to controls. Body weights of treated female rats were similar to that of control rats. No consistent significant differences among groups were noted in the hematology, clinical chemistry or urine analysis parameters measured during or at the termination of the study. Patulin administered to male and female rats at 1.5 mg kg^{-1} caused a significantly increased mortality rate as compared to respective control animals. The cause of death appeared to be increased pulmonary and laryngotracheal inflammation. No tumorigenic effect of patulin was observed.

INTRODUCTION

Patulin is a water-soluble β-unsaturated lactone, 4-hydroxy-4*H*-furo(3,2c)pyran-2(6*H*)-one. The first disease linked to patulin occurred in Japan when dairy cattle died after eating feed artificially contaminated with *Penicillium urticae*.[1] Patulin has been isolated from several species of *Penicillium* and *Aspergillus*,[2] which are likely natural contaminants of various foodstuffs. The presence of the mycotoxin in different varieties of apples and pears with brown rot was studied; patulin was found in about 50% of the samples investigated[3–5] at levels as high as 1000 ppm. Patulin has also been isolated from flour[6] and malt feed.[1] Furthermore, the rather widespread occurrence of patulin and its stability in apple juice have been established.[7–9]

Dickens and Jones[10] reported that patulin, when administered subcutaneously twice weekly to rats for approx. 15 months, produced sarcoma at the injection sites. However, Osswald *et al.*[11] found that patulin did not display tumorigenic activity when administered orally to rats for 64 weeks (358 mg per kg body weight, total dose). The acute oral LD_{50} in rats has been shown to be 32.5 mg kg^{-1}.[12]

The present study was conducted to evaluate the carcinogenicity and toxicity of lifetime administration of patulin in rats for the purpose of determining the safety of patulin as a potential food contaminant.

EXPERIMENTAL

Animals

Wistar rats (FDRL, Wistar derived) 6–8 weeks of age at the time of the start of the experiment were individually housed in an environment-controlled room, artificially illuminated for 12 h each day, and maintained at 22 ± 2 °C. All animals received Purina Rat Chow (Ralston Purina Company, St Louis, Missouri) and tap water *ad libitum* and were acclimated to their new surroundings for 14 days prior to the start of dosing.

Materials

Patulin was supplied by Makor Chemicals Ltd, Jerusalem, Israel. The patulin used was found to be greater than 95% pure when compared to an ultrapure standard sample and only a single compound was detected when analyzed by UV absorbance, thin layer chromatography and high pressure liquid chromatography. Because the stability of patulin in aqueous solutions is erratic at pH values above 7.0, the crystalline material was dissolved in 1 mM citrate buffer, pH 5.0, immediately prior to each day's dosing.

Design of experiment

The dose levels of patulin used were 0, 0.1, 0.5 and 1.5 mg per kg body weight. These dose levels were selected based on the results of an intermediate duration toxicity study.[12] Test solutions of patulin were administered by gastric intubation 3 times per week (Monday, Wednesday, Friday) except during pregnancy when females were treated 7 days per week. Dosing solutions were adjusted at the end of each week to reflect the changes in body weight. Rats in the control groups were given citrate buffer alone. The temperature of the intubation solution was approx. 22 °C.

Groups of 50 rats per sex per level for patulin and 70 rats per sex for the control group were used for the F_0

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generation. After 4 weeks of patulin treatment, all rats of the F_0 generation were paired one male to one female within groups to breed an F_1 generation. Female F_0 generation rats received patulin throughout the mating, gestation and lactation phases of the study. After weaning, the F_0 generation male and female rats were killed and necropsies were performed. Tissues were fixed using 10% neutral buffered formalin and grossly abnormal areas were subjected to microscopic examination. Microscopic examinations were conducted on paraffin-embedded, hematoxylin and eosin-stained sections.

From the F_1 generation, 70 rats per sex per level for patulin and 110 rats per sex for the control group were selected for the chronic dosing study. No more than 3 rats per sex from each patulin-treated litter and 4 rats per sex from each control litter were randomly assigned to groups. Animals received their first gastric intubation of patulin when they were approx. 28 days of age; this is considered day 1 of the chronic study. Rats were weighed and food consumption was measured weekly. Animals were observed daily for external signs of patulin toxicity. Blood samples for hematology and clinical chemistry determinations were collected from the periorbital plexus from 10 randomly selected rats per sex per group at 6, 12, 18 and 24 months after the initiation of the chronic study. Total and differential leucocyte count, erythrocyte count, hemoglobin, hematocrit, prothrombin time, urea nitrogen, glutamate oxaloacetate transaminase, glucose, sodium and potassium values were determined. Urine was collected at the same time periods and measurement of specific gravity and pH as well as microscopic examinations were made.

Five randomly selected rats per sex per group were killed at 6 and 12 months and 10 randomly selected rats per sex per group were killed 18 and 24 months after the initiation of the chronic studies. All surviving male and female animals were killed after 109 weeks of treatment. Complete gross and microscopic necropsy examinations were conducted on all animals used for the chronic study. Tissues were fixed as above. At necropsy, heart, kidneys, liver, ovaries, spleen and testes were weighed. In addition to the organs weighed, the following organs were examined microscopically from paraffin-embedded, hematoxylin and eosin-stained sections: adrenal glands, aorta, brain, cecum, colon, diaphragm, duodenum, epididymides, esophagus, eyes with optic nerve, femur, forestomach, ileum, jejunum, lungs, lymph nodes, mammary gland, pancreas, parathyroid glands, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, sternum, stomach, thymus, thyroid gland, trachea, urinary bladder, uterus, vagina, and any grossly abnormal tissue.

Statistical analysis

Body weight, food consumption, organ weight and clinical data were evaluated using analysis of variance. Pathology incidence data and indexes of reproduction and lactation were analyzed using a chi-square test with Yate's correction for 2 x 2 contingency tables.

Table 1. Reproduction and lactation data of rats given patulin

	Patulin level (mg per kg body wt)			
	0.0	0.1	0.5	1.5
Number of females mated	70	50	50	50
Number of dams surviving	70	50	49	40
Number of successful matings	68	47	47	42
Total number of pups produced	868	555	553	459
Number of pups per litter:				
Cast alive	12.5	11.6	11.4	11.9
Cast dead	0.3	0.2	0.6	0.2
Indexes:				
Fertility[a]	97.1	94.0	94.0	84.0
Gestation[b]	100.0	100.0	97.9	90.5
Viability[c]	97.0	97.8	96.2	99.1
Lactation[d]	92.6	90.0	91.5	88.6
Mean body weight (g) per pup:				
at birth	6.2	6.4	6.2	6.4
4 days	10.3	10.5	10.6	10.7
21 days	38.6	39.6	38.2	40.0

[a] % matings resulting in pregnancies.
[b] % pregnancies resulting in litters cast alive.
[c] % pups cast alive that survived to 4 days.
[d] % pups alive at 4 days that survived to 21 days.

RESULTS

F_0 generation

A summary of the reproduction and lactation data for the F_0 generation is shown in Table 1. No statistically significant differences were noted among groups in the parameters measured. Body weight gain by F_0 generation male and female rats showed no significant differences among groups. Histopathologic evaluation of grossly abnormal tissues from F_0 generation animals revealed no effect which could be attributed to patulin treatment.

F_1 generation

Body weight data. The group mean body weights of the F_1 generation animals are shown in Table 2. A significant reduction in body weight gain was noted for male rats at the 0.5 and 1.5 mg kg^{-1} dose levels ($p \leqslant 0.05$). No significant differences in body weight gain of female rats was noted among groups.

Food consumption data. Intermittent significant differences in food consumption of male and female rats were noted among groups; however, no consistent or significant pattern was observed.

Table 2. Group mean body weight of rats given patulin

Sex and patulin level (mg per kg body wt per day)	Body weight (g) ± SE at week 1[a]	10	20	30	40	50	60	70	80	90	100
Male:											
0	62±1	354±3	425±4	463±6	499±6	524±6	548±7	549±9	548±10	541±14	543±13
0.1	60±1	352±7	419±5	465±6	493±6	516±7	541±10	549±11	523±17	549±19	530±22
0.5	60±1	349±4	413±5	446±6	470±7[c]	492±8[c]	512±9[c]	520±12	514±16[c]	514±19[c]	499±23[c]
1.5	63±1	349±4	412±6	436±7[c]	455±7[c]	471±9[c]	487±8[c]	485±13[c]	458±11[c]	[b]	[b]
Female:											
0	58±1	217±2	252±2	264±3	280±3	296±4	315±4	330±5	340±7	346±7	348±7
0.1	58±1	220±2	254±3	265±4	279±4	297±5	314±6	331±8	343±10	347±10	355±15
0.5	56±1	214±2	245±3	256±3	269±4	286±4	304±6	322±8	324±10	330±10	344±12
1.5	58±1	221±2	254±2	266±3	274±4	292±5	306±6	322±8	317±10	326±11	340±14

[a] Animals were approx. 28 days of age at the start of the chronic study (week 1).
[b] High dose male group terminated at 83 weeks.
[c] Significantly different from respective control, $p < 0.05$.

Hematology and urine analysis. Hematology data from F_1 generation male animals sampled at 12, 18 and 24 months showed no significant differences among groups, nor were there differences among groups for female animals sampled at 6, 12 and 18 months. At 6 months, a significant decrease in the erythrocyte count was noted in the low and high dose male animals when compared to controls (Table 3). At 24 months, a significant decrease in the leucocyte count was noted on female animals at the middle dose level in comparison to control rats (Table 3). It is important to note that all the mean values in the above groups were within the normal physiological range for rats.

No significant differences were noted in any urine parameters measured throughout the course of the study.

Clinical chemistry. Data from F_1 generation male and female animals sampled at 12, 18 and 24 months showed no toxicologically significant differences among groups. An apparent dose-related decrease in sodium and potassium levels of male animals given patulin was noted at 6 months (Table 4). In female rats sampled at 6 months, an apparent dose-related increase in urea nitrogen levels was noted (Table 4). All sodium, potassium and urea nitrogen values obtained through the course of the study were within the normal range for rats.

Organ weights. Data taken at 6, 12, 18 and 24 months are shown in Tables 5 and 6.

At 6 months, absolute but not relative heart weights of the high dose female rats were significantly increased in comparison to control rats ($p \leqslant 0.05$). Relative and absolute ovary weights were significantly increased in the high dose group in comparison to control rats ($p \leqslant 0.05$) at 12 months but were comparable to controls at other time periods. At 18 months, the absolute but not relative liver and spleen weights of the middle and high dose male

Table 3. Summary of hematology data of rats given patulin[a]

Sex and patulin level (mg per kg body wt)	Erythrocyte count ($\times 10^6$ mm^{-3})	Leucocyte count ($\times 10^3$ mm^{-3})	Hemoglobin (g 100 ml^{-1})	Hematocrit (%)
	6 months			
Male:				
0	7.8±0.1	9.4±0.4	15.4±0.3	38±1
0.1	6.9±0.2[b]	8.0±0.5	14.5±0.5	35±1
0.5	7.4±0.2	8.4±0.4	15.2±0.4	37±1
1.5	6.8±0.2[b]	7.9±0.4	15.1±0.6	34±2
	24 months			
Female:				
0	8.1±0.6	7.0±0.3	14.9±1.0	42±3
0.1	7.7±0.2	7.2±0.5	14.7±0.4	41±1
0.5	7.9±0.2	5.4±0.4[b]	15.0±0.3	42±1
1.5	7.5±0.5	5.8±0.4	14.5±0.6	40±2

[a] Values are means ± SE of 10 rats per group.
[b] Significantly different from respective control, $p < 0.05$.

Table 4. Summary of clinical chemistry data of rats given patulin[a]

Sex and patulin level (mg per kg body wt)	Urea nitrogen (mg 100 ml^{-1})	Glucose (mg 100 ml^{-1})	SGOT (RF units)	Sodium (meq l^{-1})	Potassium (meq l^{-1})
		6 months			
Male:					
0	20±1	84±5	58±4	163±2	6.9±0.2
0.1	18±1	92±3	63±5	147±3[b]	5.6±0.2[b]
0.5	20±1	80±8	57±4	152±3[b]	5.3±0.2[b]
1.5	20±1	91±3	60±4	146±2[b]	4.9±0.2[b]
Female:					
0	20±1	85±5	63±4	155±4	6.0±0.3
0.1	22±1	94±5	67±6	145±2	4.9±0.2
0.5	23±1[b]	89±2	56±2	145±3	5.4±0.2
1.5	24±1[b]	92±3	65±5	146±4	5.5±0.3

[a] Values are means ± SE of 10 rats per group.
[b] Significantly different from respective control, $p < 0.05$.

Table 5. Absolute organ weights of male rats given patulin

Patulin level (mg per kg body wt)	No. of rats	Group mean ± SE organ weights (g) Liver	Spleen	Kidneys	Testes	Heart	Terminal body weight
			6 months				
0	5	17.6±0.3	0.78±0.07	3.1±0.2	3.5±0.1	1.4±0.1	448±13
0.1	5	14.2±0.4	0.73±0.08	2.4±0.4	3.2±0.1	1.2±0.1	427±8
0.5	5	16.5±1.2	0.66±0.04	3.0±0.1	3.5±0.1	1.2±0.1	466±25
1.5	5	16.5±0.8	0.71±0.07	2.2±0.6	3.6±0.1	1.3±0.1	458±10
			12 months				
0	5	16.2±0.4	0.80±0.03	3.1±0.1	3.3±0.1	1.6±0.1	524±18
0.1	5	15.6±1.2	0.75±0.04	3.1±0.2	3.3±0.1	1.5±0.1	470±9
0.5	5	15.6±2.2	0.73±0.06	3.1±0.3	3.4±0.2	1.6±0.1	439±29
1.5	5	14.7±1.7	0.72±0.07	3.2±0.1	2.9±0.4	1.7±0.1	459±34
			18 months				
0	10	19.6±1.9	1.07±0.08	3.8±0.3	3.3±0.2	1.8±0.1	539±29
0.1	10	17.9±0.6	0.92±0.05	3.7±0.1	3.1±0.2	1.8±0.1	547±26
0.5	10	14.9±0.6[a]	0.77±0.03[a]	3.3±0.1	3.3±0.1	1.7±0.1	481±25
1.5	10	14.3±1.2[a]	0.77±0.09[a]	3.3±0.2	3.1±0.2	1.7±0.1	444±32
			24 months[b]				
0	10	16.0±1.1	0.84±0.08	3.7±0.2	2.8±0.3	1.9±0.1	408±33
0.1	10	15.2±1.1	1.20±0.11	4.2±0.5	3.1±0.2	1.8±0.1	540±34
0.5	10	15.9±0.9	1.09±0.07	4.1±0.4	2.9±0.2	1.8±0.1	463±25

[a] Significantly different from respective control, $p < 0.05$.
[b] The high dose group was terminated at 83 weeks.

rats were significantly less than that of control animals ($p \leqslant 0.05$). Spleen weights were significantly increased in the middle dose male rats and the high dose female rats in comparison to controls ($p \leqslant 0.05$) at 24 months.

Mortality and histopathology. Mortality curves of rats are shown in Fig. 1. Data were analyzed using 2 x 2 contingency table with Yate's correction and evaluated either the number of animals that survived through a particular time period or the number of animals that died and were sacrificed moribund through the same time period. Similar results were obtained when the data were analyzed using either parameter. The high dose level of patulin caused a significant increase in the mortality rate in both sexes over the 24-month test period ($p \leqslant 0.05$). The effect was most pronounced during the first 12 months of the study. The cause of death appeared to be increased pulmonary and laryngotracheal inflammation with production of mucofibrinous exudate that apparently obstructed the tracheal lumen.

The same spectrum of tumors observed historically in rats of this strain was noted in the rats of the present

Table 6. Absolute organ weights of female rats given patulin

Patulin level (mg per kg body wt)	No. of rats	Group mean ± SE organ weights (g) Liver	Spleen	Kidneys	Ovaries	Heart	Terminal body weight
			6 months				
0	5	8.4±0.6	0.46±0.03	1.7±0.1	0.09±0.01	0.9±0.02	235±6
0.1	5	8.3±0.4	0.55±0.05	1.7±0.1	0.09±0.01	0.9±0.05	265±14[a]
0.5	5	8.0±0.4	0.46±0.02	1.8±0.1	0.10±0.01	0.9±0.01	242±4
1.5	5	9.2±0.5	0.53±0.02	1.9±0.1	0.10±0.01	1.0±0.04[a]	265±2[a]
			12 months				
0	5	8.1±0.7	0.49±0.02	2.0±0.1	0.09±0.01	1.1±0.07	295±35
0.1	5	8.5±0.5	0.62±0.07	2.4±0.2	0.12±0.02	1.2±0.14	289±15
0.5	5	7.8±0.7	0.50±0.03	2.2±0.1	0.08±0.01	1.4±0.07	274±20
1.5	5	9.4±0.5	0.60±0.05	2.2±0.1	0.16±0.02[a]	1.2±0.02	296±19
			18 months				
0	10	9.5±0.7	0.63±0.07	2.5±0.1	0.13±0.02	1.3±0.05	309±19
0.1	10	9.7±0.3	0.65±0.03	2.4±0.1	0.17±0.06	1.2±0.05	325±23
0.5	10	9.1±0.4	0.60±0.04	2.3±0.1	0.12±0.01	1.3±0.06	336±24
1.5	10	9.6±0.4	0.55±0.03	2.2±0.1	0.12±0.01	1.3±0.05	340±22
			24 months				
0	10	11.4±1.1	0.71±0.09	2.5±0.1	0.14±0.03	1.5±0.10	325±20
0.1	10	11.1±0.8	0.69±0.02	2.5±0.1	0.11±0.01	1.5±0.08	356±10
0.5	10	10.0±0.5	0.66±0.04	2.4±0.1	0.15±0.05	1.4±0.06	299±17
1.5	10	10.1±0.5	1.04±0.13[a]	2.5±0.1	0.11±0.01	1.5±0.07	350±18

[a] Significantly different from respective control, $p < 0.05$.

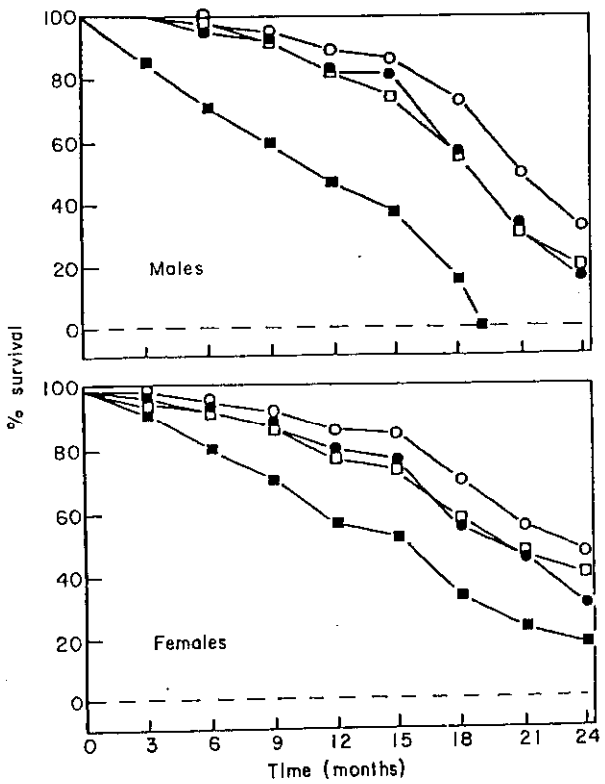

Figure 1. Survival of rats administered patulin by gavage at a level of 0.0 (○), 0.1 (□), 0.5 (●) and 1.5 (■) mg per kg body weight 3 times per week.

study. Statistical analysis of these data showed that the incidence of each type of tumor in any of the patulin-treated groups was comparable to that in the control group. The total numbers of tumors per group, the average numbers per rat and the times of observation of tumors were not affected by any of the levels of treatment. These data in each case were comparable to those of the control group.

DISCUSSION

Patulin, when administered subcutaneously to rats for approx. 15 months, produced sarcomas at the injection sites.[10] However, the validity of the subcutaneous route of administration and the subsequent conclusions drawn relative to carcinogenicity are questionable. Furthermore, patulin did not display tumorigenic activity when administered orally to rats for 64 weeks.[11] Because of the relatively short duration of the oral dosing study[11] and the questionable significance of the subcutaneous study,[10] the present study was conducted to evaluate the carcinogenicity of patulin when administered to rats for approx. 109 weeks. In our study, no tumorigenic effect of patulin was noted. Analysis of the data showed that the incidence of each type of tumor in any of the patulin-treated groups was comparable to the control group.

Patulin at the high dose level caused a significant increase in the mortality rate in both sexes at the 109-week

test period. The effect was most pronounced during the first year of the study. The major cause of mortality in the patulin-treated group appeared to be pulmonary and laryngotracheal inflammation.

It is not clear, however, whether this effect resulted from systemic toxicity or from the mechanics of multiple intubations. Osswald *et al.*[11] have attributed similar findings to dosing methodology. In short-term studies,[17] atelectasis, alveolar septal congestion and intraalveolar hemorrhage were noted in both rats and mice treated with patulin by the intraperitoneal route of administration.

In both rats and mice, patulin has been reported to induce edema of the lungs and brain, visceral organ congestion, and hepatic and renal necrosis.[13,14] In a two-generation toxicity study of patulin in rats, the only lesion observed was gaseous distention of the gastrointestinal tract at levels greater than 1.5 mg kg^{-1}.[12] In chickens, the principal gross lesion related to patulin was extensive intestinal hemorrhage.[15] In the above studies, the gastrointestinal lesions can probably be attributed to patulin's antibiotic activity resulting in changes in the bacterial flora of the intestinal tract; patulin, as an antibiotic, inhibits Gram-positive organisms.[16] In the present study, none of these lesions were noted as test effects.

Although various transient differences were noted in hematology and clinical chemistry values of patulin-treated rats when compared to control rats, none was considered treatment-related or of toxicological significance. Similarly, comparisons of organ weight data did not demonstrate treatment-related effects.

Microscopic examination of tissues and organs other than the respiratory tract did not reveal any treatment-related effects. Lesions observed in patulin-treated animals were comparable to those found in control animals and in historical control animals.

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