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Tumorigenicity Test of N-(5-Nitro-2-Furfurylidene)-1-Aminohydantoin by Dietary Administration in BDF_1 Mice*)

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ABSTRACT

The carcinogenicity of N-(5-Nitro-2-furfurylidene)-1-aminohydantoin (nitrofurantoin, NF-153) was examined in (C57BL/6N × DBA/2N)F_1 (BDF_1) mice. Three hundred of mice were divided into three groups, each group consisting about 50 male and 50 female mice. Nitrofurantoin was given in powdered diet at dose levels of 0.3, 0.075, or 0% to the respestive group of mice. The overall incidence of tumors in male and female mice was as follows: 0.3% NF group, 7.7 and 25.9%; 0.075% NF group, 1.9 and 16.7%; in the control group, 22.6 and 29.6%, respectively. In the male, the total incidence of tumors in two experimental groups and that of hepatic adenoma in 0.3% NF group was significantly decreased from that of control by $p<0.05$ and $p<0.05$, respectively. There was no significant difference in the female. The results in the present study conclude that nitrofurantoin is not carcinogenic in BDF_1 mice.

INTRODUCTION

N-5-(Nitro-2-furfurylidene)-1-aminohydantoin (Nitrofurantoin, NF-153) exhibits a wide spectrum of antibacterial activity against both gram-positive and -negative microorganisms. It is both bacteriostatic and bacteriocidal to the majority of strains of Escherichia coli, Streptococcus pyogenes. It is the 5-nitrofuran administered most frequently for systemic human infections, primarily those including the urinary tract.

NF-153 is the only mutagenic[6] and noncarcinogenic nitrofuran in rats[4] that is detoxified relatively slowly compared to the other noncarcinogenic one, such as 5-nitro-2-furoic acid. However, NF-153 has been used clinically for more than 25 years with no reports on the human neoplasias etiologically attributable to this drug[9]. The present study was undertaken to clarify the tumorigenicity of NF-153 in mice.

MATERIALS AND METHODS

Materials. N-(5-Nitro-2-furfurylidene)-1-aminohydantoin (nitrofurantoin, NF-153) was supplied from Yamanouchi-pharmaceutical Industrial. Co. Ltd. Japan.

Animals. Male and female (C57BL/6N × DBA/2N)F_1 (BDF_1) mice 4 weeks old were obtained from Charles River Japan Inc. They were divided into 3 groups of about 50 males and 50 females each and housed 5-6 in plastic polycarbonate cases. They were maintained at 24±2°C and moderate humidity at 55±5%.

Administration of NF. NF-153 was mixed with powdered diet CE-2 (Clea Japan, Inc.) at concentrations of 0.075 and 0.3%. Control mice were fed with the powdered diet CE-2. The powdered feed and water were supplied *ad libitum*. Administration of the test chemical was started when the mice were 9 weeks old.

Pathological Studies. All of the mice were observed once daily and weighed once a month,

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and moribund mice were sacrificed for thorough autopsies. Gross lesions and representative samples of all major organs were fixed in 10% neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

RESULTS

Longevity and General Conditions of Animals. Mice were observed for 24 months and the incidence of survival in groups fed 0, 0.075 or 0.3% NF were 50.5, 42.5 and 46.2% with average of 46.4% at the end of experiment. The average body weight in the control mice was 40 g in males (42.6 g in the maximum at 14 months old) and 30 g in females (33.6 g in the maximum at 15 months old) at 7 months of age followed with plateau in the later life. In mice fed with 0.3% NF, they were about 30 g and 24 g, respectively and always remained significantly lower than that of control ($p<0.05$ in both sexes). In 0.075% group, they remained in between among the two experimental groups.

Tumor Incidence (Table 1). The overall incidence of tumor in male and female mice was as follows; 0.3% NF group, 7.7 and 25.9%; 0.075% NF group, 1.9 and 16.7%; in the control group, 22.6 and 29.6%. The incidence between the experimental groups and the control group in the female did not significantly different, but in the male, the incidences in the experimental groups were significantly decreased from that of control (control vs 0.3%, $p<0.01$; control vs 0.075%, $p<0.05$). Among the tumors examined, uterine tumor in the female and hepatic tumor in the male were highest frequency with numbering 24 and 15, respectively. Uterine tumor was at first detected at

Table 1. Organ Distribution and Incidence of Tumors in BDF_1/CRJ Mice Orally Given with Nitrofurantoin

Concentration of nitrofurantoin	Male			Female		
	0%	0.075%	0.3%	0%	0.075%	0.3%
No. of mice effective	53	52	52	54	54	54
No. of mice with tumors	12	1	4	16	9	14
Genital system						
Uterus: Reticulum-cell sarcoma type A				7(2)[a]	5(3)	12(5)
Endocrine system						
Pituitary gland: adenoma				1		
Ovary : adenocarcinoma				1		
Integument, musculoskeletal system						
Subcutis: fibromyxoma				1		
hemangiosarcoma						1
fibrosarcoma				1		
Hematopoietic system						
Hematopoietic organs: lymphoma	1			3		1
: myelogenic leukemia				1		
Digestive system						
Liver: adenoma	6	1				
hemangioma	3		2	1	2	
Salivary gland: myoepithelioma			1			
Respiratory system						
Lung: adenoma	1		1			
adenocarcinoma					1	
Urinary system						
Kidney: hemangioma	1					
Special senses						
Lacrimal gland: adenoma					1	

a: Numbers in parenthesis indicate metastatic tumor.

44 weeks of age in group of 0.3% nitrofurantoin and its incidence was 22.2% in 0.3% NF group, 9.3% in 0.075% NF group and 13.0% in the control (not significant among each other). It was usually found unilaterally in the uterine horn and showed metastatic foci at the peritoneal cavity, retroperitoneal lymph nodes, liver and lungs. Hepatic tumor was more frequent in the male than in the female ($p<0.05$) and was histologically classified into adenoma and hemangioma. In the male, incidence of hepatic adenoma in the experimental groups was decreased from that of control ($p<0.05$). In the other tumors, no significant difference was noted among each experimental and control group.

DISCUSSION

By the extensive studies of tumorigenicity of nitrofuran derivatives, 40 out of 52 kinds of 5-nitrofurans had tumorigenic effects in experimental animals[4]. Among those representatives were mammary tumor inducing formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide (FNT)[1], urinary bladder tumor inducing N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT)[5], mouse leukemogenic N-[4-(5-nitro-2-furyl)-thiazolyl] acetamide (NFTA)[2] and gastric tumor inducing 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide[11]. NF-153 was, however, initially reported as not having carcinogenic activity in Holtzman female rats[7]. Cohen et al. repeated the experiment using much higher doses in female Sprague-Dawley rats and confirmed the previous results. Tumorigenicity test in mice has not been reported yet[3].

Except tumorigenicity, pulmonary reactions to NF-153 have been reported with increasing frequency. The most common manifestations of 5-nitrofuran toxicity in laboratory animals have been known to be growth retardation as shown in the present study and an increased mortality.

This drug has been demonstrated to undergo nitroreduction *in vitro*[8] and it is mutagenic in bacterial assay systems[10]. In the present study, uterine tumor was most frequently found as much as 14% and half of them had metastatic tumors in the various organs, but the incidence among the experimental groups did not significantly different. It is concluded that dietary doses of 0.075% and 0.3% NF for about 2 years did not show the tumorigenicity in BDF_1 mice.

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