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Strain Differences in N-Butyl-N-(4-hydroxybutyl)nitrosamine Bladder Carcinogenesis in Rats

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Differences in susceptibility of the urinary bladder epithelium to N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) in various strains were examined. In experiment 1, 5 strains of male rats were given 0.025% BBN in the drinking water for 8 weeks followed by drinking water without BBN for 32 weeks. Analbuminemic rats (NAR) and ACI rats had high incidences of urinary bladder lesions (papillary or nodular hyperplasia, papilloma and carcinoma), F344 and Wistar rats had low incidences, and Sprague-Dawley (SD) rats showed an intermediate incidence. Carcinoma area was largest in NAR rats followed in decreasing order by SD, ACI and F344 rats. The extent of tumor invasion was higher in NAR and ACI rats than in SD rats. In experiment 2, the 5 strains of male rats were administered 0.025% BBN in the drinking water. Some rats from each group were killed after each of weeks 4 and 8. The urinary bladder of ACI and NAR rats given BBN had the most marked lesions observed by scanning electron microscopy, with less marked changes in SD rats. F344 and Wistar rats showed the weakest response. Cytochrome P-450 content of the liver in ACI rats treated with BBN for 4 weeks was significantly higher than those of the controls. Cytochrome P-450 and cytochrome b_5 contents of the control and BBN-treated rats were significantly higher in ACI and SD rats than in Wistar, F344 or NAR rats. These results indicate that there are strain differences in the urinary bladder response to BBN.

Key words: Strain difference — Urinary bladder — N-Butyl-N-(4-hydroxybutyl)nitrosamine

Comparing the carcinogenicity of chemicals in different strains and species of animals is important for an understanding of their carcinogenicity to man. Genetic factors present in different strains and species have considerable influence on the development of cancers. There are many reports of strain differences in chemical carcinogenesis.[1–7] For example, in a previous report on urinary bladder carcinogenesis, a strong carcinogen, N - butyl - N - (4 - hydroxybutyl)nitrosamine (BBN)[8] had greatest activity in the ACI strain of rats followed in decreasing order by Wistar, Sprague-Dawley (SD) and Lewis strains. Also, rats showed a higher susceptibility to the urinary bladder carcinogenicity of BBN than mice, hamsters and guinea pigs.[9, 10] Strains and species differences are thus modifying factors in chemical carcinogenesis. Understanding the mechanisms of these differences provides clues to understanding of carcinogenesis in general. It is likely that there are differences in expression of initiation and promotion in two-stage carcinogenesis between strains and species of animals. For example, the promoting activity of sodium saccharin, was most potent in ACI rats followed by Wistar, F344 and SD rats.[1] The purpose of this study was to confirm and further evaluate the differences in susceptibility to BBN carcinogenesis of the rat urinary bladder epithelium.

Materials and Methods

Animals and Carcinogen The following strains of male rats were used in Experiments 1 and 2: ACI/A (Fuji Animal Farm Co., Tokyo), Wistar (Shizuoka Laboratory Animal Co., Shizuoka), F344 (Charles River Japan, Inc., Kanagawa), SD (Shizuoka Laboratory Animal Co.) and analbuminemic rats (NAR) (Sasaki Institute, Tokyo). All rats were 6 weeks of age at the start of the experiment. The rats were housed five per cage in

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stainless steel cages with mesh bottoms. The animal room was maintained at 23±2° and 55±5% with a 12-hr light/dark cycle.

BBN was obtained from Izumi Chemical Co., Yokohama.

Experiment 1 Twenty-six SD, 26 F344, 26 Wistar, 26 ACI and 22 NAR rats were given drinking water containing 0.025% BBN for 8 weeks followed by drinking water without BBN for 32 weeks. The control groups, 10 rats in each strain, were given drinking water without BBN for 40 weeks. All rats were administered Oriental MF pelleted diet (Oriental Yeast Co., Tokyo) during the experiment. Body weight, and food and water consumption were measured weekly for 14 weeks and then every 2 weeks until the end of the experiment. After 40 weeks, surviving animals were killed by exsanguination from the aorta. The liver, kidney and urinary bladder were removed, weighed and fixed in 10% phosphate-buffered formalin solution. The urinary bladder was fixed by filling it, and then immersing it in 10% phosphate-buffered formalin. The bladder was cut in half sagitally and each half was cut into four strips. A portion of liver and all bladder strips were embedded in paraffin and sections were stained with hematoxylin and eosin for histological examination. For quantitative analysis, the carcinoma area of the urinary bladder was measured with a color video image processor (VIP - 21CH; Olympus - Ikegami Tsushin Co., Tokyo).

Experiment 2 The experimental groups, 15 rats for each strain, were given drinking water containing 0.025% BBN for 8 weeks. The control groups, 15 rats for each strain, were given drinking water without BBN for 8 weeks. All rats were fed Oriental MF. Diet and water were available *ad libitum* during the experiment. Body weight, and food and water consumption were measured twice a week. Seven rats from each group were sacrificed at each of weeks 4 and 8 after the beginning of the experiment for scanning electron microscopic examination. The urinary bladder was inflated with 2% glutaraldehyde in 0.2*M* phosphate buffer and was processed for scanning electron microscopic examination as described previously.[1] In week 4, drug-metabolizing enzyme activities (cytochrome P-450 and cytochrome b_5) of the liver were measured in 3 rats from each group, but in 6 rats from the NAR strain. Animals were killed by decapitation, and the liver was removed and perfused with ice-cold 0.9% saline followed by homogenization in 0.02*M* Tris-1.15% KCl (pH 7.4) in a motor-driven Potter-Elvehjem Teflon and glass homogenizer with 6 complete strokes at 500–2000 rpm. Microsomes were isolated by centrifugation of the homogenate at 12,000*g* for 10 min. The supernatant fraction was filtered through glass wool and centrifuged at 105,000*g* for 60 min. Protein content in microsomes, and microsomal cytochrome P-450 and cytochrome b_5 contents were determined by the methods of Lowry *et al.*[11] and Omura and Sato,[12] respectively.

Results

Experiment 1 The average body weights and urinary bladder weights are shown in Table I. Body weight increases were less for all strains of rats treated with BBN than the controls. SD, Wistar and NAR rats treated with BBN showed significantly less average final body

Table I. Average Body Weight, Urinary Bladder Weight and BBN Intake in 5 Strains of Rats Treated with BBN

Strain	Treatment with BBN	Effective No. of rats	Body weight[a] (g)		Average urinary bladder weight[a] (g)	Average BBN intake (mg/kg/day)
			initial	final		
SD	+	25	184±18	568±52*	0.67±0.20	24.4
	−	10	182±16	612±46	0.32±0.02	—
F344	+	26	126±15	443±27	0.25±0.01	25.8
	−	9	127±16	456±43	0.26±0.02	—
Wistar	+	25	130±12	422±27*	0.25±0.01	25.4
	−	10	132±14	455±25	0.28±0.03	—
ACI	+	26	104±10	330±31	1.65±0.56*	26.0
	−	7	105±10	340±25	0.27±0.02	—
NAR	+	17	159±15	498±19*	5.15±1.51**	25.5
	−	9	159±15	535±17	0.22±0.02	—

a) Mean±SD.

* Significantly different from the corresponding control at $P<0.05$.

** Significantly different from the corresponding control at $P<0.01$.

Table II. Histological Lesions of the Urinary Bladder and Area of Carcinoma in 5 Strains of Rats Treated with BBN

Strain	Treatment with BBN	Effective No. of rats	Urinary bladder lesions (%)			Carcinoma area[a] (mm^2)
			PN hyperplasia	Papilloma	Carcinoma	
SD	+	25	20 (80.0)	16 (64.0)*	5 (20.0)***	66±48
	−	10	0	0	0	−
F344	+	26	19 (73.0)*	15 (57.7)***	1 (3.8)***	10
	−	9	0	0	0	−
Wistar	+	25	18 (72.0)**	18 (72.0)**	0***	−
	−	10	0	0	0	−
ACI	+	26	25 (96.2)	21 (80.8)	18 (69.2)	35±34
	−	7	0	0	0	−
NAR	+	17	17 (100.0)	17 (100.0)	15 (88.2)	138±84
	−	9	0	0	0	−

a) Mean±SD.
Significantly different from the incidence in NAR rats at * $P<0.05$, ** $P<0.01$, and *** $P<0.001$.

weight than the respective controls. The average weights of the urinary bladders in ACI and NAR rats treated with BBN were significantly increased compared to the respective controls. In SD rats, the average weight of the urinary bladders tended to be increased in the BBN-treated group compared to the control group. There were no differences in the average BBN consumption by any of the 5 strains of rats.

Macroscopically, both the number and size of tumors of the urinary bladder in SD, ACI and NAR rats were greater than in F344 and Wistar rats. Histopathological lesions of the urinary bladder are summarized in Table II. The lesions were classified using the criteria described previously.[13, 14] The incidences of papillary or nodular hyperplasia (PN hyperplasia), papilloma and carcinoma were highest in NAR rats. The incidence of PN hyperplasia in NAR rats was significantly higher than in F344 and Wistar rats. Urinary bladder papillomas in NAR rats were also significantly increased compared to those in SD, F344 and Wistar rats. Moreover, the incidence of carcinoma in NAR rats was significantly higher than in SD, F344 or Wistar rats. ACI rats also had high incidences of the urinary bladder lesions. Schistosoma was found in the bladder of 2 of 7 control ACI rats and in 3 of 26 BBN-treated ACI rats, but was not observed in rats of any of the other strains. SD rats showed an intermediate incidence of urinary bladder carcinoma, whereas F344 rats showed low incidences. Carcinoma was not observed in Wistar rats. Carcinoma area was greatest in NAR rats followed in decreasing order by SD, ACI and F344 rats. The histological type of urinary bladder carcinoma in the 4 strains in which it occurred was usually transitional cell carcinoma, and squamous cell carcinoma was observed occasionally in NAR, ACI and SD rats. In F344 rats, squamous cell carcinoma was not observed. Staging of the urinary bladder carcinomas is presented in Fig. 1. The extent of tumor invasion was classified as described previously[15] and was higher in ACI and NAR rats than in SD rats.

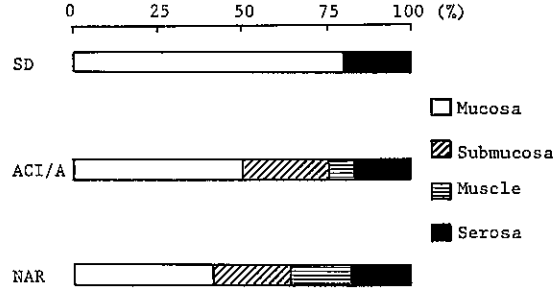

Fig. 1. Staging of urinary bladder carcinoma in SD, ACI and NAR rats treated with BBN.

Table III. Scanning Electron Microscopic Findings of the Urothelial Surface in 5 Strains of Rats Treated with BBN

Strain	Treatment with BBN	No. of rats[a]	Weeks:	Pleomorphic microvilli		Short, uniform microvilli		Ropy or leafy microridges	
				4	8	4	8	4	8
SD	+	7, 8		+[b]	+	+	++	+	++
	−	7, 8		−	−	−	−	−	−
F344	+	7, 8		+	+	+	+	+	+
	−	7, 8		−	−	−	−	−	−
Wistar	+	7, 8		+	+	+	+	+	+
	−	7, 8		−	−	−	−	−	−
ACI	+	7, 8		+	++	++	+++	++	+++
	−	7, 8		−	−	−	−	−	−
NAR	+	7, 8		+	++	++	+++	++	+++
	−	7, 8		−	−	−	−	−	−

a) Number sacrificed at each time period: 7 rats at 4 weeks and 8 rats at 8 weeks.
b) −, no change; +, slight; ++, Moderate; +++, marked.

Table IV. Cytochrome P-450 and Cytochrome b_5 Contents in 5 Strains of Rats Treated with BBN

Group	Strain	Treatment with BBN	Cytochrome P-450[a] (nmol/mg protein)	Cytochrome b_5[a] (nmol/mg protein)
1	SD	+	$1.006\pm0.068^{b,c,d}$	$0.515\pm0.036^{b,c,d}$
2		−	$0.914\pm0.047^{e,f,g}$	$0.452\pm0.037^{e,f,h}$
3	F344	+	0.756 ± 0.037	0.402 ± 0.012
4		−	0.727 ± 0.023	0.410 ± 0.005
5	Wistar	+	0.794 ± 0.034	0.386 ± 0.009
6		−	0.734 ± 0.032	0.426 ± 0.015
7	ACI	+	$1.086\pm0.024^{i,j,l,m}$	$0.543\pm0.015^{i,k,m}$
8		−	$0.968\pm0.014^{n,p,q}$	$0.487\pm0.018^{o,p,r}$
9	NAR	+	0.554 ± 0.054	0.327 ± 0.017
10		−	0.632 ± 0.049	0.318 ± 0.015

a) Mean±SE.
Values for the following groups were significantly different: *b*) group 1 and group 3, $P<0.05$; *c*) group 1 and group 5, $P<0.05$, *d*) group 1 and group 9, $P<0.01$; *e*) group 2 and group 4, $P<0.05$; *f*) group 2 and group 6, $P<0.05$; *g*) group 2 and group 10, $P<0.01$, *h*) $P<0.02$; *i*) group 7 and group 3, $P<0.01$; *j*) group 7 and group 5, $P<0.01$, *k*) $P<0.001$; *l*) group 7 and group 8, $P<0.02$; *m*) group 7 and group 9, $P<0.001$; *n*) group 8 and group 4, $P<0.001$, *o*) $P<0.02$; *p*) group 8 and group 6, $P<0.01$; *q*) group 8 and group 10, $P<0.01$, *r*) $P<0.001$.

Experiment 2 Scanning electron microscopic findings of the surface of the urinary bladder of rats administered BBN are summarized in Table III. The luminal surface of superficial epithelial cells of the urinary bladder mucosa of control rats in all 5 strains had peaked microridges. Foci of epithelial cells having pleomorphic microvilli, short, uniform microvilli and ropy or leafy microridges as reported previously[10, 16–20] were observed on the luminal surface of the urinary bladder in rats treated with BBN. At week 4, pleomorphic microvilli were observed only occasionally in all 5 strains of rats. Short, uniform microvilli and ropy or leafy microridges were prominent in ACI and NAR rats. At week 8, the urinary bladder of ACI and NAR rats had more extensive pleomorphic microvilli, short, uni-

form microvilli, and ropy or leafy microridges on their luminal surfaces. The extent of these changes was slightly greater in SD rats than in F344 and Wistar rats.

Drug-metabolizing enzyme activities are shown in Table IV. Cytochrome P-450 content in ACI rats treated with BBN was significantly higher than those of the controls. However, in the other strains no differences were observed between BBN-treated and control rats. With the exception of NAR rats, the content of cytochrome P-450 in microsomes tended to be higher in the BBN-treated group than in the control group. Cytochrome P-450 and cytochrome b_5 content of the control and BBN-treated rats were significantly higher in ACI and SD rats compared to Wistar, F344 or NAR rats. There were no differences in cytochrome b_5 content between the 5 strains of rats compared with the controls.

Discussion

Our data indicate that there are differences in the susceptibility to BBN of the urinary bladder among various strains of rats. Induction of urinary bladder tumors at high incidences in ACI and NAR rats by BBN is consistent with previous experiments.[8, 21] Thus, the urinary bladder of ACI and NAR rats is extremely susceptible to BBN. NAR rats also showed a high susceptibility to the induction of renal tumors by N,N-dimethylnitrosamine[22] and of gastric tumors by N-methyl-N′-nitro-N-nitrosoguanidine.[23] However, NAR rats have a low susceptibility to mammary tumor induction by 7,12-dimethylbenz[*a*]anthracene[24] and to bladder cancer induction by N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide.[25] NAR are a mutant strain established from a stock of SD by Nagase *et al.*[26] in 1979 and characterized by serum albumin deficiency and hyperlipidemia. Serum albumin is known to be a carrier protein of many compounds including bilirubin, bile acids, hormones and probably some carcinogens in the plasma, but its role in carcinogenesis is unknown.

In the present study, urinary bladder cancer was not observed in Wistar rats treated with BBN, but previous studies[8] showed that administration of BBN resulted in relatively high incidences of urinary bladddder cancer in this strain. The cause of these conflicting results is unknown. SD rats showed an intermediate incidence of urinary bladder cancer, at similar incidences to those in previous experiments.[8] The extent of tumor invasion in NAR and ACI rats was higher than in SD rats. Also, ACI and NAR rats had clearer and more extensive urinary bladder lesions by scanning electron microscopy during the first 4 weeks of BBN administration than the other strains. In general, pleomorphic microvilli, short, uniform microvilli and ropy or leafy microridges appear with mucosal hyperplasia, whether reversible or irreversible.[16-20, 27] Many chemicals which induce these scanning electron microscopic changes show a carcinogenic effect on the urinary bladder.[16, 19, 28] In the present study, ACI and NAR rats showed clear and extensive scanning electron microscopic changes. The present experiment supports the hypothesis that scanning electron microscopic changes of the urinary bladder induced by chemicals are well correlated with the urinary bladder carcinogenesis.

Biochemical studies on susceptibility factors have focused on genetic differences in carcinogen metabolism. Differences in metabolic pathways for activation of carcinogens may account for the differences in response among strains and species. If microsomal cytochrome P-450 plays a central role in the initiation of chemical carcinogenesis, its inducibility by administration of a carcinogen should be an important factor. In our experiment, there were no marked differences of cytochrome P-450 or cytochrome b_5 content in any strain treated with BBN compared to the respective controls, except in ACI rats. However, with the exception of NAR rats, hepatic cytochrome P-450 activity tended to be higher in BBN-treated groups than in the corresponding control groups.

Okada *et al.* have demonstrated that the induction of bladder tumors in rats by BBN is ascribable to their major urinary metabolite, N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN).[29-31] BCPN is formed by ω-oxidation in the liver by drug-metabolizing enzymes. In the present experiment, cytochrome P-450 and cytochrome b_5 activities in the liver were related to the incidence of urinary bladder lesions: high frequencies of urinary bladder lesions appeared in ACI and SD rats (with high cytochrome P-450 and cyto-

chrome b_5 activities), while a low frequency of urinary bladder lesions was seen in F344 and Wistar rats (with low cytochrome P-450 and cytochrome b_5 activities). Thus, increased urinary excretion of BCPN secondary to an increased content of hepatic drug-metabolizing enzymes may result in higher incidences of urinary bladder lesions. However, no marked differences in the urinary excretion of BCPN among the 4 rat strains (Wistar, ACI/N, SD and NAR) were found.[32] Therefore, the observed strain differences in the susceptibility of rats cannot be explained simply on the basis of the extent of urinary excretion of BCPN. Thus, other factors (DNA adduct formation, DNA repair, cell cycle within the urinary epithelium and immunological response) than BBN metabolism must play an important role in host susceptibility.

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