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High Susceptibility of an Analbuminemic Congenic Strain of Rats with an F344 Genetic Background to Induced Bladder Cancer and Its Possible Mechanism

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The susceptibility of an analbuminemic congenic strain of rats (F344-alb) originating from the F344 strain to N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) was examined. F344-alb rats were found to be highly susceptible to induction of urinary bladder cancers. The incidences of bladder cancers in F344-alb and F344 rats were 94% (15/16) and 31% (5/16) in males and 100% (16/16) and 19% (3/16) in females. The bladder weights of these rats, including tumors, were 307±294 mg, 123±26 mg, 183±80 mg and 93±11 mg, respectively. Administration of 0.05%, 0.1% and 0.3% BBN in the drinking water for 2 weeks resulted in greater increases in the bladder content of N-butyl-N-(3-carboxypropyl)nitrosamine in F344-alb rats than in F344 rats. This increase was prevented by the presence of rat albumin.

Key words: N-Butyl-N-(4-hydroxybutyl)nitrosamine — Bladder cancer — Analbuminemic congenic strain — Rat

A mutant strain of analbuminemic rats (Nagase analbuminemic rats, NAR) was established from a stock of Sprague-Dawley (SD) rats.[1] NAR shows very high susceptibility to bladder cancer induced by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN).[2] However, as NAR originated from outbred SD rats that were not uniform genetically, it was uncertain whether their high susceptibility to carcinogens was associated with analbuminemia or with other genetic factors. To elucidate this point, we established three analbuminemic congenic strains of rats from different strains, ACI, F344 and SHR, and named them ACI-alb, F344-alb and SHR-alb, respectively. The coat color and biochemical marker genes of each congenic strain were the same as those of the background inbred strain of rats, except for the *alb* gene locus.[3] Firstly, induction of bladder cancers by BBN was studied in ACI-alb and normal ACI rats.[4] In the present study, which is an extension of our previous work, we examined the carcinogenicity of BBN in F344-alb rats. The possible mechanism of the high susceptibility of the analbuminemic rats to bladder cancer is discussed.

Abbreviations: BBN, N-butyl-N-(4-hydroxybutyl)-nitrosamine; BCPN, N-butyl-N-(3-carboxypropyl)-nitrosamine; F344-alb, analbuminemic congenic strain with an F344 genetic background; ACI-alb, analbuminemic congenic strain with an ACI genetic background; NAR, Nagase analbuminemic rat; SD, Sprague-Dawley; RSA, rat serum albumin.

MATERIALS AND METHODS

Chemicals BBN was obtained from Izumi Chemicals Co. (Yokohama) and N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN) was kindly supplied by Dr. M. Mochizuki, Kyoritsu Pharmaceutical College (Tokyo).

Animals F344 rats obtained from CLEA Japan Inc., Tokyo, and F344-alb rats bred at the Sasaki Institute were used. During the experiment, the rats were kept in cages with wood shavings on Iso-Racks (Sanki Scientific Co., Tokyo) and were maintained in an animal room with air conditioning (23±2° and 55±5% relative humidity) and artificial lighting from 7 a.m. to 7 p.m.

Bladder Tumorigenesis Both sexes of F344 and F344-alb rats (6 weeks old at the start of the experiment) were used. They were given free access to commercial CE-2 animal diet (CLEA Japan Inc., Tokyo) and were weighed once a week. Since the daily water intakes of F344 and F344-alb rats are different, the concentration of BBN was adjusted

so that all animals received the same amounts of BBN per kg body weight: the F344-alb rats were given 0.05% BBN for the first 2 weeks and then 0.04% BBN for 6 weeks, while the F344 rats were given 0.05% BBN throughout the experiment. In week 20, the animals were killed and autopsied and the urinary bladder was fixed for histological observation by injecting phosphate-buffered formalin into it until it was normally distended.

Contents of BCPN in the Urine, Kidneys and Bladder of BBN-treated F344 and F344-alb Rats Female F344 and F344-alb rats were used at 6 weeks old. Groups of 6 animals were given concentrations of 0.05%, 0.1% and 0.3% BBN in their drinking water for 2 weeks. In addition, one group was treated with 0.05% BBN for 2 more weeks. F344 and F344-alb rats received the same amounts of BBN per body weight. After the treatment, each rat was starved overnight and placed in a metabolic cage. Urine was collected for the next 24 hr under protection from light. Then the animals were killed and their bladder and kidneys were removed for examination. BCPN was measured by the method of Wada *et al.*[5]

Effects of Albumin and IgG on the Penetration of BCPN into the Bladder Female F344 and F344-alb rats were used at 7 weeks old. Urine was collected from 9 rats of each strain as described above and filtered. Then 9 vol of urine was mixed with 1 vol of 10-times-concentrated phosphate-buffered saline (PBS). BCPN was added to the solution at a concentration of 0.25 mg/ml. Rat serum albumin (RSA) and IgG were purified from normal rat serum by the methods of Campbell *et al.*[6] and Baumstark *et al.*,[7] respectively. One day after collecting the urine, the rats of each strain were divided into 3 groups of 3 rats each and sacrificed. Their bladders were prompty removed and washed with PBS for one minute by cannulation. Then the bladders of each group were treated as follows. Those of group 1 were filled with 400 μl of PBS containing RSA at a concentration of 10 mg/ml. Those of group 2 were treated with IgG in place of RSA, and those of group 3 were treated with PBS only. After 5 min, they were washed three times with PBS. Then the bladders of the F344 and F344-alb rats were filled with 400 μl of urine from the same strain containing BCPN for 5 min. The bladders were then washed thoroughly with PBS and their BCPN content was measured. The albumin and IgG contents of the bladder were also measured by a single radial immunodiffusion method[8] and protein was determined by the method of Lowry *et al.*[9]

Results

Bladder Tumorigenesis The results of bladder tumorigenesis are summarized in Table I. The body weight of male F344-alb rats was less than that of male F344 rats, but there was no difference in the body weights of females of the two strains. Bladder cancer developed in 94% (15/16) of the male and 100% (16/16) of the female F344-alb rats, but in only 31% (5/16) of the male and 19% (3/16) of the female F344 rats. In both sexes, the average weight of the bladder, including tumors, of F344-alb rats was more than twice that of F344 rats, the difference being significant ($P<0.001$). Histologically, almost all the tumors in both strains were transitional cell carcinomas associated with minor squamous

Table I. Summary of Bladder Tumorigenesis by BBN in F344 and F344-alb Rats

	Male		Female	
	F344	F344-alb	F344	F344-alb
Initial number	16	16	16	16
Effective number	16	16	16	16
Body weight (g)				
Initial	101±4	91±13	89±4	87±11
Final	352±20	303±26	192±7	192±7
Daily intake of BBN (mg/kg body weight)	65±12	64±12	64±12	64±13
Total cumulative dose of BBN (g)	0.72±0.02	0.60±0.03	0.50±0.02	0.47±0.01
Incidence of bladder tumor (%)	31 (5/16)	94 (15/16)	19 (3/16)	100 (16/16)
Transitional cell carcinoma	3	10	2	14
Squamous cell carcinoma	0	1	1	1
Papilloma	2	4	0	1
Weight of bladder with tumors (mg)	123±26	307±294	93±11	183±80

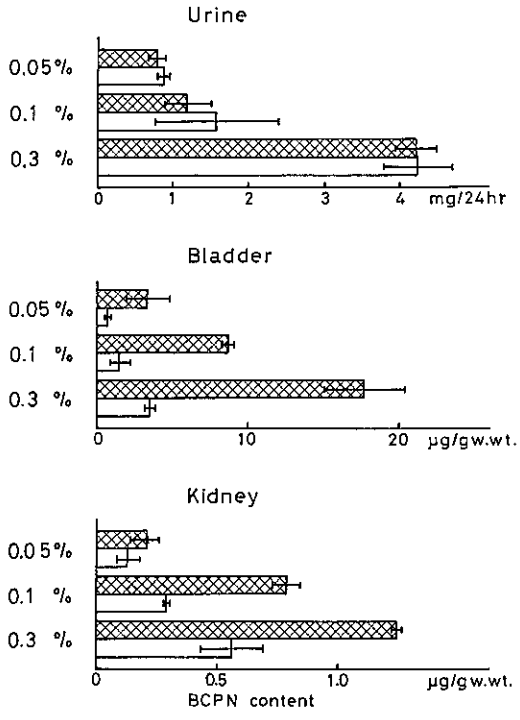

Fig. 1. BCPN contents of the urine, kidneys and bladder of BBN-treated F344 and F344-alb rats. Animals were given 0.05%, 0.1% or 0.3% BBN in their drinking water for 2 weeks. Experimental conditions were as described under "Materials and Methods." ▨, F344-alb; □, F344 rats. Columns and bars represent mean values±SD for 3 animals.

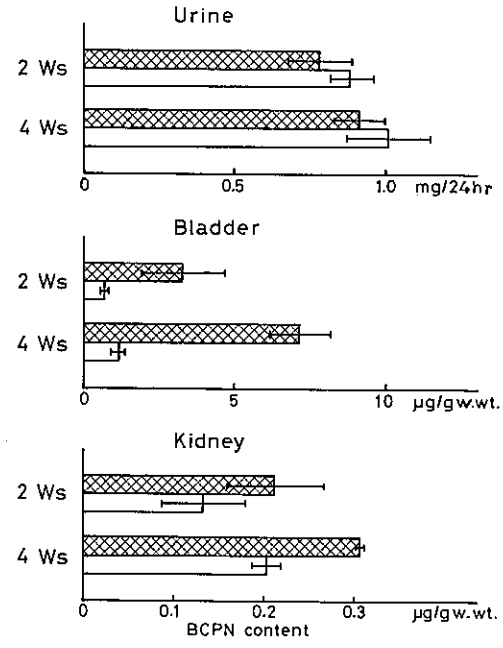

Fig. 2. BCPN contents of the urine, kidneys and bladder of BBN-treated F344 and F344-alb rats. Animals were given drinking water containing 0.05% BBN for 2 or 4 weeks. Experimental conditions were as described under "Materials and Methods." Symbols are as for Fig. 1. Columns and bars represent mean values±SD for 3 animals.

cell carcinoma or papilloma. No strain or sex difference in histological types was seen.

Contents of BCPN in the Urine, Kidneys and Bladder of BBN-treated F344 and F344-alb Rats Figure 1 shows the results for rats given 0.05%, 0.1% and 0.3% BBN in their drinking water for 2 weeks. Clear dose-dependency was seen. The urinary contents of BCPN in the two strains were similar, but those of the bladder and kidney were different. In particular, the bladder content of BCPN was 5 times higher in F344-alb rats than in F344 rats. Figure 2 shows the time dependency of BCPN accumulation in the bladder on continuous administration of BBN. In the group given 0.05% BBN the value in week 4 was higher than that in week 2 and close to the level in rats given 0.1% BBN for 2 weeks.

Table II. Albumin and IgG Contents of Bladders in F344 and F344-alb Rats after Albumin and IgG Treatment

	Protein[a] (mg/ml)	Albumin (μg/mg protein)	IgG
F344-alb			
PBS-treated	2.34±0.15	ND[b]	21.7±5.5
RSA-treated	2.39±0.41	10.4±0.7	23.5±3.6
IgG-treated	2.34±0.12	ND	40.5±2.9
F344			
PBS-treated	2.45±0.32	64.6±4.0	5.2±0.7
RSA-treated	2.45±0.26	70.6±9.3	5.6±0.4
IgG-treated	2.30±0.29	61.2±4.7	21.3±0.4

a) The tissue was homogenized with a Polytron (Kinematika, GmbH, Switzerland) in 9 volumes of PBS, and centrifuged (10,000*g*, 10 min). Experimental conditions were as described under "Materials and Methods." Values are means±SD for 3 animals.
b) Not detected.

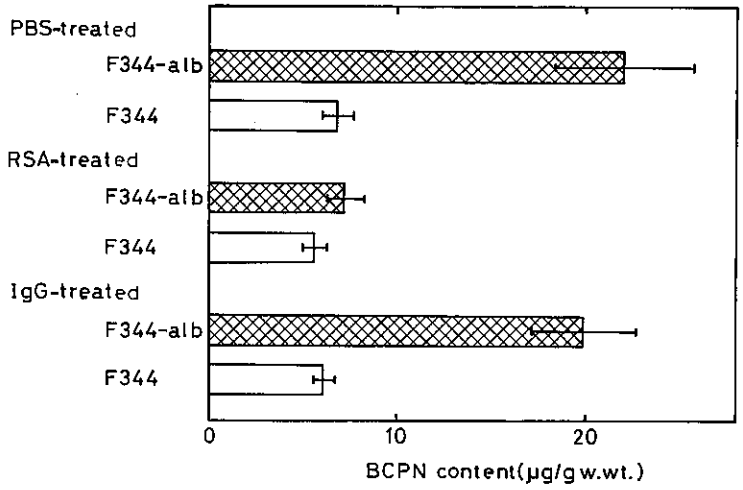

Fig. 3. Effects of albumin and IgG on the penetration of BCPN into the bladder. Experimental conditions were as described under "Materials and Methods." Columns and bars represent mean values±SD for 3 animals.

Effects of RSA and IgG on Penetration of BCPN into the Bladder As shown in Figs. 1 and 2, BCPN excreted in the urine seemed to penetrate into the bladder and the level was higher in F344-alb rats than in F344 rats. Since this difference was mainly due to the presence or absence of albumin, the effect of albumin on the BCPN level in the bladder was examined. The effect of IgG was also studied. A higher content of IgG was seen in F344-alb as compared to F344. This seems to be a reflection of the higher concentration of plasma IgG in the former.[10] The content of albumin in the bladder of albumin-treated F344-alb rats was one-sixth of that in F344 rats. The IgG contents in bladders were increased in IgG-treated rats of both strains (Table II). The penetration of BCPN into the bladder of F344-alb rats was suppressed by albumin, but not affected by IgG (Fig. 3).

Discussion

Like NAR and ACI-alb rats, F344-alb rats showed high susceptibility to induction of urinary bladder cancers by BBN, though their incidences of bladder tumors were different from those of NAR and ACI-alb rats. Thus, the higher tumorigenic response to BBN of analbuminemic rats can be mainly ascribed to lack of albumin. Ito *et al.*[11] examined strain differences in induction of bladder carcinogenesis by BBN in rats, and reported that the incidence of bladder cancer was highest in ACI rats. In our experiments, bladder tumors developed in all ACI and ACI-alb rats, but in the ACI-alb rats the tumors were larger and the average weight of the bladder, including tumors, was more. On the other hand, the incidences of bladder cancers in NAR or F344-alb rats were markedly higher than those in the original strains. The strain differences in incidences of tumors in these analbuminemic strains seem to be related to differences in susceptibility of the original strains.

To determine the mechanism by which BBN caused this high incidence of bladder cancer in analbuminemic rats, we measured the BCPN contents of the urine, kidneys and bladder of normal and analbuminemic F344 rats. No marked difference was found in the urinary excretions of BCPN by the two strains. Although the BCPN concentrations in the kidneys and bladder were low in both strains, they were much higher in analbuminemic rats than normal rats. Thus it seems that the bladder transitional epithelium has a strong barrier effect in normal rats, but the effect is rather weak in analbuminemic rats and so its exposure to a high concentration of BCPN in the urine results in formation

of tumors, or alternatively in analbuminemic rats the epithelium may be gradually altered by BCPN, leading to structural damage and accumulation of BCPN in the bladder.

Albumin is normally the most abundant protein in animals, and is considered to play an important role in general metabolism as a whole. However, despite their lack of albumin, NAR showed only minor clinical and biochemical abnormalities. This fact raises the question of the importance of serum albumin. Findings in NAR have, however, revealed some roles of albumin, such as in renal elimination of mercapturic acid.[12] Formerly, we reported findings related to anemia and potassium permeability of red blood cells in NAR.[13] Namely, we found that serum albumin may play a part in the cation permeability of the membrane of RBC. The present study also suggests effects of albumin on the membrane stability and permeability.

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