Susceptibility of Four F1 Hybrids of Male Rats to the Promoting Effects of Sodium L-ascorbate in Two-Stage Urinary Bladder Carcinogenesis

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Abstract: In the two-stage rat urinary bladder carcinogenesis model with N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) as an initiator and sodium L-ascorbate (Na-AsA) as a promoter, we previously reported that F344/DuCrj (F344) and LEW/Crj (Lewis) rats were sensitive, whereas WS/Shi (WS) and ODS/Shi-od/od (ODS) rats were resistant. In the present study, for the development of a model useful to the QTL analysis of host genes, we examined the susceptibility to Na-AsA promotion in (F344 × WS)F1, (F344 × ODS)F1, (Lewis × WS)F1, and (Lewis × ODS)F1 hybrids of male rats. Rats were given 0.05% BBN in their drinking water for 4 weeks and then a basal diet with or without a 5% Na-AsA supplement for 32 weeks. In urine of all F1 hybrids, Na-AsA elevated pH and concentrations of sodium ion and total ascorbic acid. There were not significant differences for susceptibilities to BBN alone among the four F1 hybrids. In all F1 hybrids, administration of Na-AsA increased urinary bladder carcinogenesis when compared to the matched control rats given BBN alone. Susceptibilities to Na-AsA promotion were high in (F344 × WS)F1 and (F344 × ODS)F1 hybrids, whereas they were mild in (Lewis × WS)F1 and (Lewis × ODS)F1 hybrids. The present results therefore indicate that F1 hybrids among the F344, ODS, Lewis and WS strains may be a useful model for analyzing host genes susceptible to Na-AsA promotion in rat bladder carcinogenesis. (J Toxicol Pathol 2006; 19: 87–91)

Key words: sodium L-ascorbate, genetic factors, F1 hybrids rat, transitional cell carcinoma

Introduction

Sodium L-ascorbate (Na-AsA) and sodium saccharin have promoting effects in two-stage urinary bladder carcinogenesis of male rats. The factors, which influence the promoting activity, are urinary qualities which indicate (1) the elevation of pH, and high concentrations of sodium ion and total ascorbic acid1,2,9, (2) diet differences2,9, and (3) the presence of high concentrations of alpha 2u-globulin, which is one of the essential factors for calcium phosphate-containing precipitate formation in the urine of rats exposed to high doses of sodium saccharin3,4. Na-AsA-promoting effects on rat bladder carcinogenesis were decreased by co-treatment of amiloride, a Na’/H’ exchanger blocker, but not by that of ouabain5. The genes encoding Na’/H’ exchanger isozymes are in rat chromosome 56 and the genes may be relevant to Na-AsA-promoting activity. Moreover, host genes such as p53 mutations7, overexpression of metallothionein and cyclin D18, and strain differences2,9 influenced the risk of bladder cancer development. The susceptibility to the tumor-promoting action of Na-AsA in rat urinary bladder carcinogenesis is dominant to an autosomal trait between the sensitive F344/DuCrj (F344) and the resistant WS/Shi (WS) strains10. Quantitative trait analysis using F344 and WS strains showed that the “Bladder Tumor Susceptible-1” (BTS-1) locus on rat chromosome 17 and possibly another locus on chromosome 5 regulate rat strain differences in Na-AsA-promoting effects of bladder carcinogenesis10. However, these candidate genes have not been analyzed in new, more suitable F1 hybrid rats.

We therefore investigated whether the F1 hybrids of Na-AsA-sensitive F344 and LEW/Crj (Lewis) and resistant WS and ODS/Shi-od/od (ODS) parents are sensitive or resistant to Na-AsA promotion.
**Materials and Methods**

*Animals*

ODS and WS strains of male rats were obtained from Aburabi Laboratories of Shionogi Co., Ltd. (Koka, Shiga, Japan). The WS is an inbred strain of rats from a stock of Wistar rats which has been maintained for over 100 generations by brother-sister matings\(^1\). WS rats have susceptibility to melamine but not NaHCO\(_3\) or butylated hydroxyanisole promotion of urinary bladder carcinogenesis\(^2,9\), and they are also susceptible to N-nitrosomorpholine-induced hepatocellular carcinoma with high metastatic potential\(^11\). The ODS strain lacks L-ascorbic acid-synthesizing ability\(^11\), and is a mutant substrain of the above-mentioned Wistar rats. WS and ODS strains are resistant to the Na-AsA promotion of rat bladder carcinogenesis\(^9,10,14\). F344 and Lewis strains of female rats were purchased from Charles River Japan, Inc. (Hino, Shiga, Japan). F344 and Lewis strains are sensitive to the Na-AsA promotion of bladder carcinogenesis\(^2,9\). All rats were obtained at 4 weeks of age and were acclimatized until use. They were housed 2–3 rats per plastic cage (RT type: Charles River Japan, Inc., Japan), in a room maintained on a 12-h (7:00 a.m. – 7:00 p.m.) light-dark cycle, at constant temperature (25 ± 1°C) and relative humidity (55 ± 5%). ODS rats were given Clea CA-1 diet (containing 250 ppm T-AsA, Clea Japan Inc., Osaka, Japan). The other rats were fed Oriental MF diet (Oriental Yeast Co., Tokyo, Japan). All rats were given drinking water *ad libitum*.

*Chemicals*

BBN was obtained from Tokyo Kasei Kougyo Co. (Tokyo, Japan) and Na-AsA was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) at a food additive grade (99.8% pure).

*Experimental protocols*

Rats that were the offspring of a F344 mother and WS father, were given the F\(_1\) designation of (F344 × WS)F\(_1\) hybrid. Forty (F344 × WS)F\(_1\), 36 (F344 × ODS)F\(_1\), 40 (Lewis × WS)F\(_1\), and 40 (Lewis × ODS)F\(_1\) hybrids, all male rats, 6 weeks of age, were randomly divided into two groups each, groups 1–2, 3–4, 5–6, and 7–8, respectively. In the first 4 weeks of the experiment, rats in groups 1, 3, 5 and 7 were given drinking water containing 0.05% BBN by volume, and then fed basal MF diet with 5% Na-AsA for 32 weeks. Rats in the matched control groups 2, 4, 6 and 8 were treated with 0.05% BBN for the first 4 weeks and then received basal MF diet without Na-AsA for the same period. Body weights, food consumption, and water intake were measured weekly up to week 4 and every other week from week 5 to the end of experiment. The amounts of food and water consumed during two consecutive days of a week were assessed. At the end of the experiment, all survivors were sacrificed for pathological examination.

*Urinalysis*

For measurement of pH, total ascorbic acid (T-AsA) concentration and sodium ion concentration in the morning (09:00 – 11:00 a.m.) at weeks 12, 24 and 35, fresh urine samples from rats of each group were obtained by forced urination. Assessments were made with pH test paper (ranges 6.6–8.3 and 7.9–10.0; E. Merck Inc, Darmstadt, Germany), the ascorbic acid test (range 50–2000 mg/dl, E. Merck Inc, Germany), and atomic absorbance chemical analysis (atomic absorption spectrophotometer AA-175, Varian Techtron Co., Canberra, Australia).

*Pathological examination*

At the end of week 36, all rats were sacrificed under ether anesthesia. The urinary bladders were inflated through the urethra with 10% phosphate-buffered formalin solution (pH 7.4) and sliced into strips (12 per bladder) for routine processing and histological examination of sections stained with hematoxylin and eosin.

Urinary bladder tumors were classified into papillomas and carcinomas as described previously\(^15\). For quantitative analysis, carcinomas in urinary bladders were counted under a microscope, and the total length of the basement membrane was measured with the aid of an image processor (COSMOZONE-S, Nippon Kogaku KK, Tokyo, Japan) under a stereoscopic microscope\(^6\).

*Data evaluation*

Statistical analyses of incidences of histopathological lesions were performed with the Fischer’s exact probability test. After testing for homogeneity by Bartlett’s test, the other data were evaluated by either (1) the F-test for analysis of variance, and then the Scheffe’s test, or (2) the Kruskal-Wallis test using the rank sum and chi-square analysis, and then Dunn’s test.

*Results*

Scorbutic symptoms or signs of toxicity, such as diarrhea, due to the chemical treatments were not demonstrated by any rat. During weeks 24–36, all rats given BBN plus Na-AsA (groups 1, 3, 5 and 7) showed higher incidences of hematuria than the matched control rats given BBN alone (groups 2, 4, 6 and 8). All rats given BBN (all groups) consumed similar amounts of BBN. The final average body weights of (Lewis × WS)F\(_1\) and (Lewis × ODS)F\(_1\) hybrids given BBN plus Na-AsA (groups 5 and 7) were significantly lower, by approximately 10%, than those of the matched control groups (groups 6 and 8, respectively) and a similar trend was seen in the other F\(_1\) hybrids (Table 1).

The Na-AsA treatment elevated the urinary pH and the T-AsA and sodium ion concentrations in all groups given BBN plus Na-AsA (groups 1, 3, 5, and 7) during experimental weeks 12, 24 and 35 (Table 2, the results of the urinalyses at experimental week 24).

Macroscopically, (F344 × WS)F\(_1\) and (F344 × ODS)F\(_1\) hybrids given BBN plus Na-AsA (groups 1 and 3) had large,
multiple tumors of the urinary bladder, and mild order of tumor induction was seen in (Lewis × WS)F₁ and (Lewis × ODS)F₁ hybrids (groups 5 and 7). In contrast, rats in all groups given BBN alone (groups 2, 4, 6, and 8) had very small numbers of tumors. Urolithiasis was not evident in any of the rats. The data for the histopathologically diagnosed lesions of the urinary bladder epithelium are summarized in Table 3. All carcinomas of the urinary bladder induced by treatments of BBN plus Na-AsA (groups 1, 3, 5, and 7) were transitional cell carcinomas. All F₁ hybrids given BBN alone (groups 2, 4, 6, and 8) had no carcinoma, and also there were no significant differences of incidences (20, 28, 5 and 10%) and numbers of papillomas among groups 2, 4, 6, and 8. F₁ hybrids administered Na-AsA had significantly increased induction of neoplastic lesions in groups 1, 3, 5, and 7, when compared with their respective matched control groups 2, 4, 6, and 8. In F₁ hybrids with different maternal
strains (F344 vs. Lewis) and the same paternal WS strain, the incidences of carcinoma and papilloma were higher in group 1, (F344 × WS)F₁ hybrid given BBN plus Na-AsA, than in group 5, (Lewis × WS)F₁ hybrid given the same treatment. Also, a similar trend was significantly observed between groups 3 and 7. Moreover, a similar trend in numbers of lesions per 10 cm of basement membrane for papillomas and carcinomas was also presented. The order of Na-AsA-promoting effects was group 1 = group 3 > group 5 = group 7.

Discussion

We investigated susceptibilities to Na-AsA in four F₁ hybrid rats of strains sensitive and resistant to the Na-AsA promotion of bladder carcinogenesis. All F₁ hybrids given BBN alone had no carcinomas. Also, the induction of papillomas by BBN alone was not significantly different among the four F₁ hybrids (groups 2, 4, 6 and 8). The susceptibilities to the BBN initiation in (F344 × WS)F₁ hybrid group 2 resemble our previous data².⁹.

The urine in the all F₁ hybrids of rats given Na-AsA showed increased pH and increased concentrations of sodium ion and T-AsA. The increasing magnitudes of these urinary parameter changes were the same among the four F₁ hybrids. These results are consistent with previous observations of the effects of Na-AsA treatment on changes in the urinary components in F344, Lewis, ODS, WS, and (F344 × WS)F₁ hybrid rats².⁹.¹⁴. In the present study, urinalyses also indicated that the epithelial cells of the urinary bladder were chronically exposed to high concentrations of sodium ion and T-AsA at a high pH. However, the urinary changes of pH, concentrations of sodium ion and T-AsA were reported to have less influence on the promotion of urinary bladder carcinogenesis by Na-AsA than strain difference².⁹.

The administration of Na-AsA clearly promoted BBN-initiated urinary bladder carcinogenesis in all the four F₁ hybrids. The present results for group 1, (F344 × WS)F₁ hybrids given BBN plus Na-AsA, were consistent with our previous report¹⁰. Results similar to those of the (F344 × WS)F₁ hybrids were seen in (F344 × ODS)F₁ hybrids, whereas F₁ hybrids of the Lewis maternal strain, (Lewis × WS)F₁ and (Lewis × ODS)F₁, had approximately half the magnitude of urinary bladder carcinogenesis induced by BBN plus Na-AsA in comparison with the F₁ hybrids of the F344 maternal strain, (F344 × WS)F₁ and (F344 × ODS)F₁ hybrids. Genetic factors such as strain have been reported to have important roles on the promotion of urinary bladder carcinogenesis by Na-AsA².⁴.⁹.¹⁰.¹⁴.¹⁶. and sodium saccharin¹⁷. It has been reported that the male F344 strain is nearly twice as sensitive to urinary bladder carcinogenesis induced by BBN plus Na-AsA as the male Lewis strain²,⁹, and a similar phenomenon was observed in the results of the present study with F₁ hybrid male rats; results which were both novel and statistically significant. Genetic factors from maternal strains such as F344 and Lewis rats may be related to the susceptibility to Na-AsA-promoting effects on urinary bladder carcinogenesis, whereas those from paternal strains such as between WS and ODS, did not apparently influence the Na-AsA-promoting effects. The genetic factors may be related to p53 mutations², cyclin D₁³, or Na⁺/H⁺ exchanger isoforms⁹.

The present results therefore indicate that F₁ hybrids among the F344, ODS, Lewis and WS strains may be a useful model for analyzing the host genes of Na-AsA promotion of rat bladder carcinogenesis.

References