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Chemopreventive Efficacy of Piroxicam Administered Alone or in Combination with Lycopene and β-Carotene on the Development of Rat Urinary Bladder Carcinoma after *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine Treatment

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The effects of the non-steroidal anti-inflammatory drug (NSAID) piroxicam and the carotenoids lycopene and β-carotene, alone or in combination, on the development of rat superficial urinary bladder carcinomas induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) were studied. Male Fischer 344 rats, 6 weeks old, were given 0.05% BBN in the drinking water for 8 weeks followed by administration of piroxicam (0.0075% in the diet), lycopene (0.0025% in the drinking water) and/or β-carotene (0.0025% in the drinking water) for 12 weeks, then killed for histological analysis of urinary bladder lesions. Cell proliferation potential was analyzed by immunohistochemical staining of the proliferative cell nuclear antigen (PCNA). Piroxicam alone, piroxicam + lycopene, and piroxicam + lycopene + β-carotene all significantly decreased the incidences and numbers of transitional cell carcinomas (TCCs), but the combination of piroxicam with carotenoids did not result in a clear improvement in the preventive potential of piroxicam. Piroxicam + β-carotene also caused a significant reduction and lycopene alone a slight but not significant reduction in the number of TCCs. In contrast, β-carotene alone and lycopene + β-carotene were without inhibitory influence on any of the lesion categories examined, and the latter significantly increased the proportion of high-grade TCCs. Nevertheless, all of the chemopreventive agents, either alone or in combination, significantly decreased the TCC PCNA index, the effect extending to the surrounding epithelium in the piroxicam + lycopene and piroxicam + lycopene + β-carotene groups. These results indicate that the NSAID piroxicam may be a more effective chemopreventive agent than lycopene and β-carotene for superficial urinary bladder carcinogenesis.

Key words: NSAID — Carotenoid — BBN — Urinary bladder carcinoma — Rat

Human urinary bladder cancers can be divided into superficial and invasive types, with a significantly higher incidence of the former being normally found. Superficial bladder cancers are usually low-grade transitional cell carcinomas (TCCs) and are easily resected by transurethral intervention. However, careful follow-up is necessary at least for 3 years after surgery since there is a high frequency of recurrence, and some recurrent tumors have the appearance of more aggressive malignancies than the primary ones.[1] Therefore, intravesical instillation of chemotherapeutic agents, such as adriamycin, epiadriamycin[2] and more recently bacillus Calmette-Guerine, has been performed after operations to prevent tumor recurrence.[3] However, this approach is not always successful, and establishment of additional modalities to achieve more satisfactory control of superficial bladder cancers is a high priority.

Evidence of the cancer chemopreventive potential of non-steroidal anti-inflammatory drugs (NSAIDs) in various organs has recently been accumulating from experimental *in vivo* carcinogenesis and epidemiological studies.[4] Furthermore, clinical intervention trials of this type of agent have already been performed in patients predisposed to cancers.[5] Among NSAIDs, 4-hydroxy-2-methyl-*N*-(2-pyridinyl)-2*H*-1, 2-benzothiazine-3-carboximide-1, 1-dioxide (piroxicam), a long half-life NSAID, is known to exhibit relatively strong chemopreventive potential against colon[6] and tongue[7] cancers. NSAIDs are basically inhibitors of cyclooxygenase (COX), and thereby block production of prostaglandins (PGs), thromboxanes and prostacyclins from free arachidonic acid.[8] Recently, important roles of PGs in tumor growth, either by directly stimulating tumor cell proliferation[9] or by inhibiting immunological surveillance,[10] have been postulated. In fact, increased levels of PGs in various tumor cell lines[11] and increased expression of COX-2, an inducible isozyme of COX, and phospholipase A_2 in human

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colon[12] and animal skin[13] tumors have been reported. Urinary bladder epithelium is known to possess relatively high COX (PGH synthase) activity,[14] which can contribute to metabolic activation of carcinogens such as *N*-[4-(5-nitro-2-furyl)-2-thiazoyl]formamide (FANFT).[15] Moreover, elevated levels of PGE_2 in rat bladder following application of tumor promoters[16] and inhibition of their tumor promotion potential by NSAIDs such as indomethacin have been reported.[17] Nevertheless, conflicting data have been obtained when NSAIDs were administered both during and after the carcinogen treatment, with indomethacin enhancing rat bladder carcinogenesis by FANFT[18] and piroxicam inhibiting mouse bladder carcinogenesis by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN).[19] For the present investigation, piroxicam was administered in the post initiation stage to assess its chemopreventive potential, since possible modulating effects of NSAIDs on the metabolic activation of various carcinogens could then be ignored.

Epidemiological,[20–23] experimental *in vivo* carcinogenesis[24] and cultured cancer cell proliferation[25,26] studies have indicated that certain types of carotenoids, including some with and without provitamin A activity, can inhibit tumor development. Among the approximately 600 carotenoids existing in nature, β-carotene, which possesses the highest provitamin A activity and has the greatest abundance in most dark-green, yellow and red vegetables and fruits, and lycopene, which has no provitamin A activity but is abundant in tomatoes, have attracted particular attention. Most carotenoids, including these two, also possess antioxidant activity,[27,28] and it remains controversial whether the provitamin A or the antioxidant properties are responsible for the chemopreventive influence.[29] Recently, enhancing effects of carotenoids including β-carotene and lycopene on gap junctional communication[25] and immunological surveillance[26] have been reported. Astaxanthin, which has no provitamin A activity,[30] but not β-carotene,[31] exerts chemopreventive effects against the development of invasive bladder cancers in mice. But the influence of carotenoids on experimental rat urinary bladder carcinogenesis remains largely unclear.

In the present study, lycopene and β-carotene were chosen, along with piroxicam, for investigation in a BBN model of urinary bladder carcinogenesis in rats, since this model exhibits histological similarities to the human superficial bladder cancers.

MATERIALS AND METHODS

Chemicals BBN was purchased from Tokyo Kasei Kogyo (Tokyo), and piroxicam from Sigma Chemical Co. (St. Louis, MO). Lycopene was kindly supplied by LycoRed Natural Products Industries Ltd. (Beer-Shaver, Israel). β-Carotene and a vehicle emulsion were generously donated by Lion Co. (Tokyo). The emulsion consists of 0.5% sucrose ester P-1570 (Mitsubishi-Kasei Food Co., Tokyo), 1.0% Sansoft 8000 (Taiyo Co., Tokyo), 0.2% L-ascorbyl stearate and 4% peanut oil. *trans*-8′-Apocarotenal was obtained from Sigma, and the other reagents used for the carotenoid estimation were all of special reagent grade.

Animals, animal husbandry, diets and drinking water One hundred and eight male Fischer 344 rats (Japan SLC Inc., Hamamatsu), 6 weeks old at the commencement of the experiments, were used. The animals were housed 4 to a plastic cage, with hardwood chips for bedding, and were given free access to water and diet under conditions of controlled temperature (23±1°C), humidity (60±10%) and lighting (12 h–12 h light-dark cycle). Throughout the experimental period, the animals were observed daily to assess their general health. Body weights and consumption of food and drinking water were measured weekly. The basal diet, CE-2, was purchased from Japan Clea Co., Ltd. (Tokyo). Diet containing piroxicam was prepared once a week by mixing with CE-2. Diets were administered using stainless steel containers and renewed once a week. Drinking water containing lycopene and/or β-carotene was prepared twice a week by diluting the carotenoids, which had already been emulsified in the vehicle emulsion, with distilled water and given in light-opaque bottles. Both lycopene and β-carotene were relatively stable in the distilled water-diluted solution, remaining more than 90% intact after a week.

Experimental protocol The experimental protocol is shown in Fig. 1. Animals were randomly divided into 8 groups of 12 each. Animals in all the groups were given 0.05% BBN in their drinking water for the first 8 weeks. Group 1, serving as the control, was then given the vehicle emulsion alone in drinking water. Group 2 received 0.0075% piroxicam in the diet. Group 3 and 4 received 0.0025% lycopene and β-carotene in the drinking water, respectively. Group 5 received 0.0075% piroxicam in the diet and 0.0025% lycopene in the drinking water. Group 6 received 0.0075% piroxicam in the diet and 0.0025% β-carotene in the drinking water. Group 7 received 0.0025% lycopene and β-carotene in the drinking water. Group 8 received 0.0075% piroxicam in the diet and 0.0025% lycopene and β-carotene in the drinking water after BBN treatment. All the animals were killed after collection of blood samples from the abdominal aorta under ether anesthesia 20 weeks after the beginning of the experiment. Their urinary bladders were ligated at the neck, inflated by intraluminal injection of 10% phosphate-buffered formalin, quickly resected and immersed in fixative. In addition to the urinary bladder, the liver, kidneys, testes, and prostate of all rats were re-

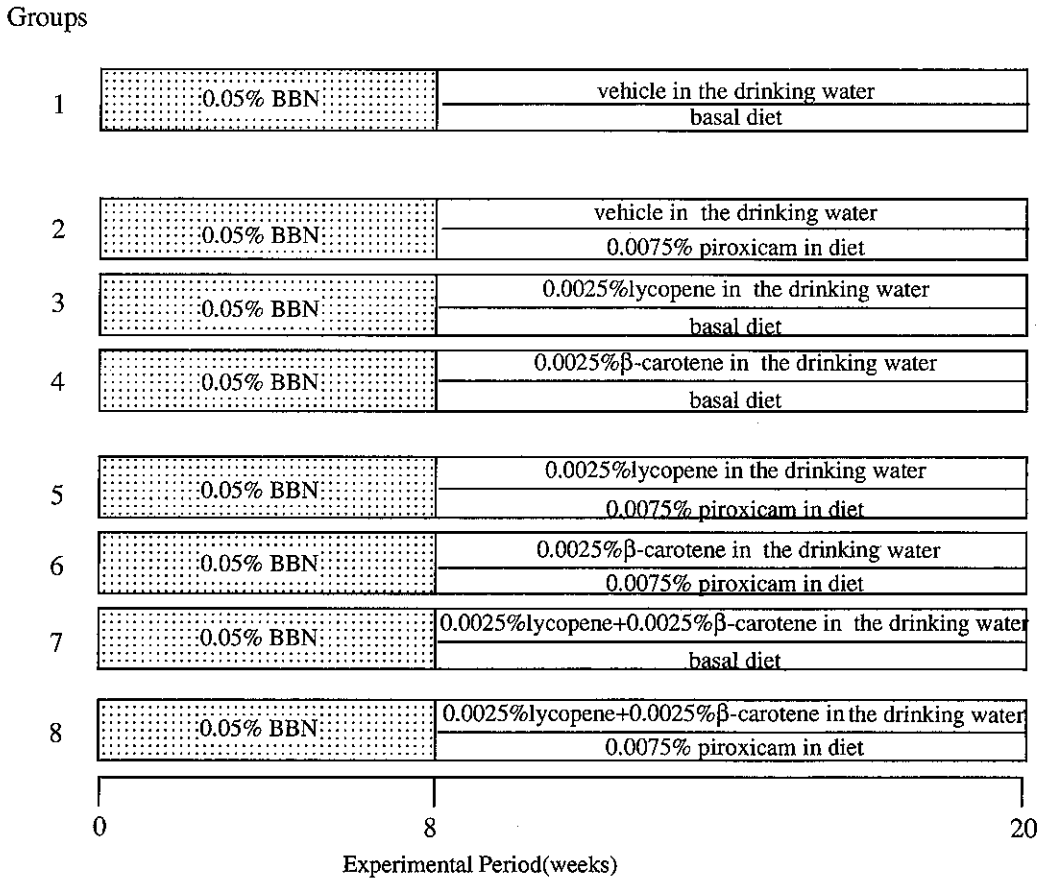

Fig. 1. Experimental protocol.

moved, weighed and subjected to histological examination. The livers and sera from 8 out of 12 rats in each group were frozen for measurement of carotenoid content.

Histopathological examination After overnight fixation, bladders were longitudinally bisected and macroscopically observed, and the lesions were recorded as a guide for the histological examination. Sections through the bladders and the other four organs were routinely prepared and stained with hematoxylin and eosin for histological examination. According to the criteria described previously,[32] the bladder lesions were classified into simple hyperplasia (SH), nodulo-papillary hyperplasia (NPH) and TCC. TCCs were further classified into 3 grades in terms of cell differentiation, and into Ta and T1 depending upon the depth of the invasion.[32]

Proliferative cell nuclear antigen (PCNA) was immunohistochemically stained using an anti-PCNA polyclonal antibody (DAKO Co.,Carpinteria, CA) and an LSAB Kit (DAKO) as described previously.[33] The PCNA indices were determined as the percentages of PCNA-positive nuclei by counting one thousand nuclei in each carcinoma as well as in the non-tumorous areas of bladder epithelium.

Measurement of carotenoids Levels of carotenoids in the sera and liver tissues were estimated individually for 8 rats from each group according to the methods of Oshima *et al.*[27] Briefly, an aliquot of the serum (100–200 μl) was mixed with 1 ml of an ethanol solution of an internal standard, *trans*-β-8′-apocarotenal, and then mixed with 5 ml of *n*-hexane and dichloromethane (4 : 1, v/v) containing 0.02% butylated hydroxytoluene. The mixture was centrifuged at 3,000 rpm for 5 min, and the resultant supernatant (4.0 ml) was evaporated under nitrogen gas. The residue was dissolved in a mixture of methanol, acetonitrile, dichloromethane and water (7 : 7 : 2 : 0.16, v/v/v/v) for high-performance liquid chromatography (HPLC) analysis.

The wet liver tissues (0.1–0.7 g), after adding 1 ml of the internal standard solution, were homogenized with 5 ml of ethanol containing 0.02% butylated hydroxytoluene. One ml of 60% KOH and 1 ml of saline containing 0.5 m*M* diethylenetriamine pentaacetic acid (DTPA)

were added to the homogenate, and the mixture was incubated at 50°C for 30 min. It was made up with saline to 10 ml, then a 1 ml aliquot was mixed with 5 ml of *n*-hexane and dichloromethane (4 : 1, v/v). This mixture was centrifuged at 3,000 rpm for 5 min, and 4 ml of the resultant supernatant was further processed as mentioned above for HPLC analysis.

The levels of carotenoids were measured by HPLC using a column of Lichrospher RP 18-5 (E. Merck, Darmstadt, Germany) with an eluting solvent of methanol, acetonitrile, dichloromethane and water (7 : 7 : 2 : 0.16, v/v/v/v) containing 50 m*M* $NaClO_4$ and 2.0 m*M* DTPA at the constant flow rate of 1.0 ml/min. The effluent was monitored using an ECD-300 electrochem-

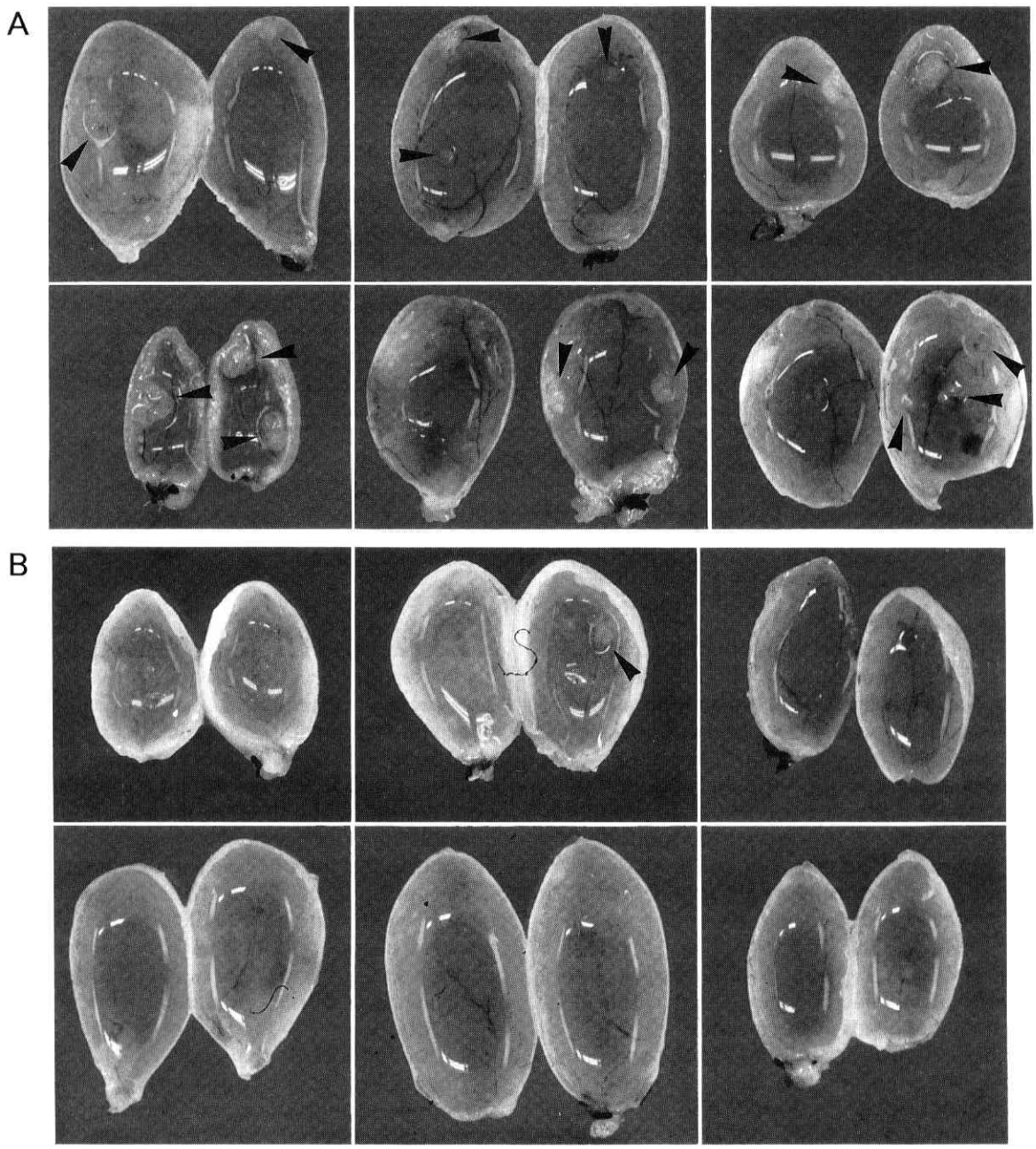

Fig. 2. Macroscopic findings for representative urinary bladders in group 1 and 8. Two or 3 tumorous lesions are evident in each urinary bladder in group 1 (A), while such lesions are rare in group 8 (B).

ical detector (EICOM, Kyoto). The detection limit was 1 ng/ml of the serum for both β-carotene and lycopene.

Statistical analysis Quantitative differences between group values were analyzed for statistical significances using the unpaired Student's t test, Fischer's exact test and the χ^2 test.

RESULTS

Body and organ weights, and intake of diet, water and chemicals No rats died during the experimental period. Body weights at week 8 and the final time point showed no significant intergroup differences, with the average in each group being in the range of 285–294 g at week 8, and 367–376 g at week 20. The weights of liver, kidneys, prostate and testes also showed no significant intergroup differences in terms of either absolute values or ratio to body weight (data not shown). Average diet intake during the experimental period, from week 9 to week 20, in each group was within the range of 47.1–47.6 g/day/kg body weight without significant differences among groups. There was similarly no significant variation in average water intake among groups, the values all being in the range of 68.3–71.6 ml/day/kg body weight. Average intake of piroxicam in groups 2, 5, 6 and 8 amounted to 3.53, 3.57, 3.56 and 3.56 mg/day/kg body weight, that of lycopene in groups 3, 5, 7 and 8 amounted to 1.79, 1.71, 1.78 and 1.72 mg/day/kg body weight, and that of β-carotene in groups 4, 6, 7 and 8 amounted to 1.72, 1.71, 1.78 and 1.72 mg/day/kg body weight, respectively.

Levels of carotenoids in serum and liver The serum levels of lycopene in groups 3, 5, 7 and 8 were less than the limit of detection with the method used in the present study, while those of β-carotene in rats from groups 4, 6, 7 and 8 were 29.27±6.3, 39.49±11.3, 38.80±10.5 and 36.46±10.2 ng/ml, respectively, there being no significant differences among the groups. The levels of lycopene in the livers of rats from groups 3, 5, 7 and 8 were 95.5±22.9, 70.8±10.2, 89.9±11.4 and 127.8±62.5 ng/g tissue, respectively, and those of β-carotene from groups 4, 6, 7 and 8 were 228.9±101.8, 159.9±57.8, 103.8±20.4 and 187.9±77.6 ng/g tissue, respectively, there being no significant differences among the groups. The levels of lycopene and β-carotene in the sera and livers of the rats in the other groups were less than the limits of detection.

Incidences and numbers of urinary bladder lesions As shown in Fig. 2, macroscopically, protuberant, whitish nodular lesions were observed in the bladder mucosa in all the groups. However, the numbers of the lesions were clearly less in groups 2 (piroxicam alone), 5 (piroxicam +lycopene), 6 (piroxicam+β-carotene) and 8 (piroxicam+lycopene+β-carotene) (Fig. 2B) than those in group 1 (vehicle alone control) (Fig. 2A).

Data for incidences of histologically diagnosed urinary bladder lesions are summarized in Table I. There were no significant differences in the values for SH and NPH among the groups. The incidences of TCC were significantly decreased in rats from group 2 (piroxicam alone), 5 (piroxicam+lycopne) and 8 (piroxicam+lycopene+β-carotene) as compared to group 1 (vehicle alone) ($P<$

Table I. Incidences of Urinary Bladder Lesions in Rats Treated with BBN Followed by Piroxicam and/or Carotenoids

Group	Treatment after BBN	Effective number of rats[a]	Incidence (%)						
			SH[b]	NPH[c]	TCC[d]	TCC classified by invasion[e]		TCC classified by differentiation[f]		
						Ta	T1	Grade 1	Grade 2	Grade 3
1	vehicle	12	12 (100)	12 (100)	10 (83)	4 (33)	6 (50)	6 (50)	4 (33)	0 (0)
2	piroxicam	12	11 (92)	9 (75)	4 (33)[g]	2 (17)	2 (17)	2 (17)	2 (17)	0 (0)
3	lycopene	12	12 (100)	12 (100)	10 (83)	2 (17)	8 (67)	3 (25)	6 (50)	1 (8)
4	β-carotene	12	12 (100)	12 (100)	12 (100)	4 (33)	8 (67)	4 (33)	8 (67)	0 (0)
5	piroxicam+lycopene	12	12 (100)	10 (83)	3 (25)[g]	2 (17)	1 (8)	2 (17)	1 (8)	0 (0)
6	piroxicam+β-carotene	12	12 (100)	12 (100)	6 (50)	4 (33)	2 (17)	5 (42)	1 (8)	0 (0)
7	lycopene+β-carotene	12	12 (100)	11 (92)	11 (92)	2 (17)	9 (75)[g]	1 (8)	10 (83)[g]	0 (0)
8	piroxicam+lycopene+β-carotene	12	10 (83)	10 (83)	4 (33)[g]	2 (17)	2 (17)	3 (25)	1 (8)	0 (0)

a) Based on histological examination.
b) Simple hyperplasia.
c) Nodulopapillary hyperplasia.
d) Transitional cell carcinoma.
e) Ta, no invasion. T1, invasion to the lamina propria.
f) Grade 1: either papillary or nodular, showing a minimal cytological atypia and infrequent mitoses. Grade 2: larger and more pleomorphic than grade 1 carcinomas, and nucleoli were prominent. Mitotic divisions were readily detectable. Grade 3: characterized by marked cytological and architectural abonormalities.
g) Significantly different from group 1 ($P<0.05$).

0.05), while the TCC incidence in group 6 (piroxicam+β-carotene) also exhibited a slight decrease but without significance. When the TCCs were classified into different grades for invasion and differentiation, no significant differences were found between groups 2–8 and group 1, except that the incidence of T1 as well as grade 2 TCCs in group 7 (lycopene+β-carotene) was significantly increased. The incidence of grade 2 TCC in groups 3

Table II. Numbers of TCCs and Their Classification in Terms of Invasion and Cell Differentiation

Group	Treatment after BBN	No. of TCC (%)						
		No. per rat[a)]	Total No. counted	classified by invasion		classified by differentiation		
				Ta	T1	Grade 1	Grade 2	Grade 3
1	vehicle	2.25±1.4	27	15 (56)	12 (44)	15 (56)	12 (44)	0 (0)
2	piroxicam	0.42±0.7[b)]	5	3 (60)	2 (40)	3 (60)	2 (40)	0 (0)
3	lycopene	1.67±1.6	20	6 (30)	14 (70)	11 (55)	8 (40)	1 (5)
4	β-carotene	2.58±1.6	31	18 (58)	13 (42)	17 (55)	14 (45)	0 (0)
5	piroxicam+lycopene	0.33±0.7[b)]	4	3 (75)	1 (25)	3 (75)	1 (25)	0 (0)
6	piroxicam+β-carotene	0.58±0.7[b)]	7	5 (71)	2 (29)	6 (86)	1 (14)	0 (0)
7	lycopene+β-carotene	2.00±1.3	24	5 (21)[c)]	19 (79)[c)]	6 (25)[c)]	18 (75)[c)]	0 (0)
8	piroxicam+lycopene+β-carotene	0.33±0.5[b)]	4	2 (50)	2 (50)	3 (75)	1 (25)	0 (0)

a) Values are mean±SD.
b) Significantly different number of carcinomas as compared with group 1 ($P<0.05$ by Student's t test).
c) Significantly different from group 1 ($P<0.05$ by the χ^2 test).

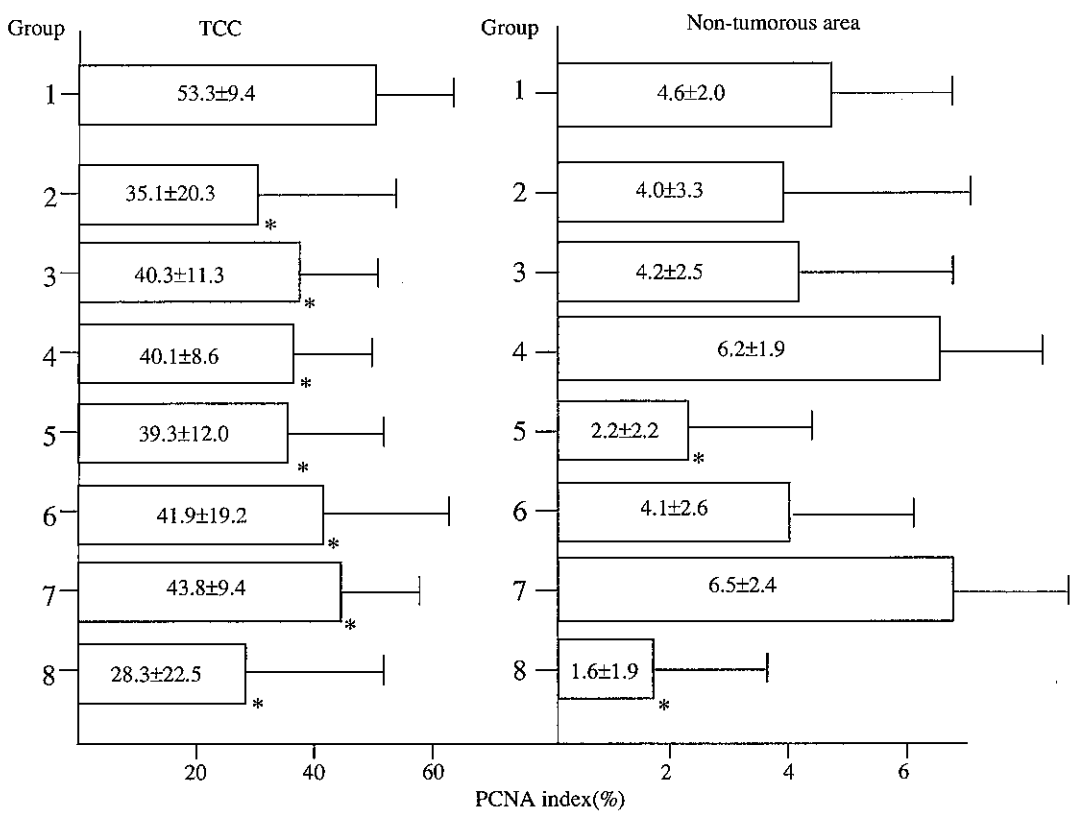

Fig. 3. PCNA indices in TCCs and surrounding non-tumorous epithelia of rats from groups 1 to 8. * Significantly different from group 1 ($P<0.05$).

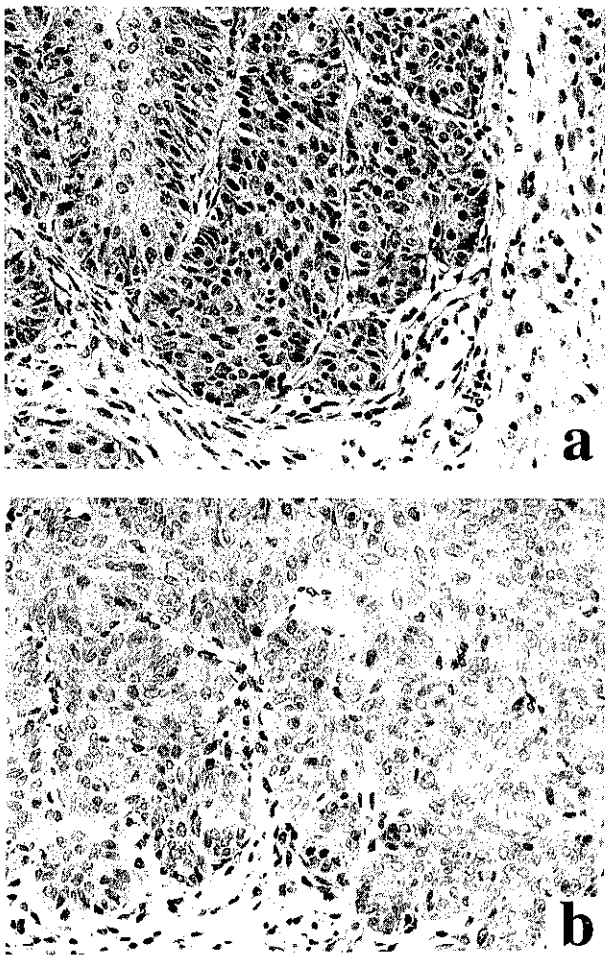

Fig. 4. Immunohistochemical staining of PCNA. A TCC classified as grade 2, T1, in the urinary bladder of a rat from group 1 (a), shows numerous PCNA positive nuclei in the cancer cells, in contrast to a TCC classified as grade 2, T1, in group 8 (b).

(lycopene alone) and 4 (β-carotene alone) was slightly increased, but without significance.

Details of the numbers of TCC and their classification with regard to invasion and differentiation are summarized in Table II. Piroxicam, either alone or in combination with lycopene and/or β-carotene (groups 2, 5, 6 and 8) significantly decreased the numbers of TCCs compared with group 1 ($P<0.05$, respectively), and their combination with piroxicam slightly increased the proportions of TCCs that were classified as Ta and grade 1, and decreased those classified as T1 and grade 2, but without significance, as compared with the piroxicam alone group. Lycopene alone (group 3) also slightly decreased the number of TCC, but without significance. However, lycopene+β-carotene significantly increased the proportions of TCCs that were classified as T1 and grade 2, as compared with group 1 ($P<0.05$).

PCNA index PCNA indices for TCC and surrounding epithelium are summarized in Fig. 3, and representative immunohistochemical findings are shown in Fig. 4. All of the chemopreventive agents, used either singly or in combination (groups 2–8) (Fig. 4b), slightly but significantly decreased the PCNA index in TCC as compared to the group 1 value (Fig. 4a) ($P<0.05$). Further, piroxicam+lycopene and piroxicam+lycopene+β-carotene were also associated with significant reduction in the PCNA index in the surrounding non-tumorous bladder epithelium compared to the group 1 value ($P<0.05$).

DISCUSSION

The present results provide clear evidence that the NSAID, piroxicam, either alone or in combination with lycopene and/or β-carotene, can exert a chemopreventive effect on development of superficial urinary bladder cancers in rats pretreated with BBN. However, the combination of piroxicam with the carotenoids did not result in a clear improvement in the preventive potential of piroxicam, with only the combination with lycopene alone or with lycopene+β-carotene affording a marginal improvement in terms of tumor grade. To the authors' knowledge, this is the first investigation of the chemopreventive potentials of NSAIDs and carotenoids in combination.

The present study, which was designed so that possible modulating effects on metabolic activation of the carcinogen could be avoided, clearly indicated that piroxicam can inhibit post initiation development of superficial urinary bladder cancers. The present dose of piroxicam 0.0075% was the minimum effective and non-toxic dose in a colon carcinogenesis study by Reddy *et al.*[6] The calculated daily intake of piroxicam was approximately 3.5 mg/kg body weight, which is approximately 10 times the maximum tolerated dose in man (20 mg/person/day: according to the Japanese Drug Administration Indication). The PCNA indices in TCCs of rats that received piroxicam, either alone or in combination with lycopene and/or β-carotene, were slightly but significantly decreased as compared to the control group lesions. However, before the biological significance of the decreased PCNA index, if any, can be evaluated, further studies are required, since the indices in TCCs were significantly decreased by all the chemopreventive agents used either singly or in combination in this study. The index may not necessarily correlate well with cancer preventive potential. Inhibition by NSAIDs of COX activity has been postulated to be involved in their cancer chemopreventive effects.[4] However, a single explanation is ruled out by the enhancement of urinary bladder carcinogenesis observed with aspirin when this COX inhibitor was given

after initiation by FANFT in rats.[15] In this context, it is worth noting that one of the metabolites of another NSAID, sulindac, also exerts similar chemopreventive effects against the parent compound in rat colon carcinogenesis, though the metabolite itself does not inhibit COX.[34] Thus, clarification of the differences in the action of piroxicam and aspirin is required before firm conclusions can be drawn regarding mechanisms.

In the present study, β-carotene did not exhibit any clear preventive effect on urinary bladder carcinogenesis, in spite of decreasing the PCNA index of induced cancers. It rather tended to increase the incidence of grade 2 TCC. Epidemiological studies have suggested preventive effects of β-carotene on urinary bladder cancers[20,21] while clinical intervention trials have suggested somewhat enhancing effects on urinary bladder and lung cancers.[35,36] Our results are in line with these two latter reports and the report by Moon.[31] The 0.0025% dose of β-carotene adopted in the present study is known to be non-toxic from a study of its action in the liver, lung and skin.[37] Further dose-response studies, particularly concentrating on whether the decreases observed in the PCNA index might result in long-term prevention of tumor development, are required.

Lycopene did not exhibit a clear preventive effect on urinary bladder carcinogenesis in this study. It exhibited a tendency to decrease the number of TCC, but to increase the incidence of grade 2 and 3 tumors. Thus, the present results are in contrast to the inhibitory effect of asthaxanthin, another non-provitamin A carotenoid, on mouse urinary bladder carcinogenesis by BBN,[30] and to the preventive effect of lycopene on urinary bladder cancers in epidemiological studies.[22] Although the 0.0025% dose of lycopene was selected to be the same as that used for β-carotene, future studies should concentrate on the possible dose-dependence of any lycopene inhibitory potential. Moreover, the present finding that the lycopene + β-carotene treatment appeared to be associated with an increase in the tumor grade, in terms of invasion and differentiation, clearly warrants further study.

The reasons why in the present results, the serum levels of lycopene, but not of β-carotene, were less than the detection limit in the groups given the respective compounds, are unclear. The time courses for the appearance and loss of [^{14}C]lycopene and [^{14}C]β-carotene in rat plasma after their single oral administrations are reportedly similar.[38,39] Since the clearance of lycopene from rat plasma appears to be much faster than that from primates,[39] further pharmacodynamic studies on lycopene in rats, particularly when given continuously at relatively low doses as in the present study, seem necessary.

In conclusion, the present finding of a clear inhibitory potential of the NSAID piroxicam on the post initiation development of superficial urinary bladder cancers appears promising with regard to the human situation, particularly the frequent recurrence of superficial TCC. However, neither β-carotene nor lycopene exhibited clear bladder cancer-preventive potential. Further, the combination of piroxicam with the carotenoids did not result in any clear improvement in the preventive potential of piroxicam.

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