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The Effect of Aspirin on Preneoplastic Lesions in Rat Bladder Induced by N-Butyl-N-(4-hydroxybutyl) Nitrosamine

Gil Ho Lee, In Ho Sohng, Hyung Jee Kim and Min Chul Lee[1]

Department of Urology, [1]Department of Anatomic Pathology, Dankook University College of Medicine, Cheonan 330-714, Korea

The effects of aspirin on the urinary bladder carcinogenesis in rats treated with N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) were studied. The effects of chemoprevention of aspirin were evaluated with incidence of preneoplastic lesions. Forty-five female Sprague-Dawley rats were divided into three groups. Group A received 0.05% BBN in drinking water for 12 weeks. Group B received 0.05% BBN in drinking water with 0.5% aspirin in the diet for 12 weeks. Group C received control diet without added chemicals. The rats were sacrificed after 12 weeks. There were no typical preneoplastic lesions in the control group. Preneoplastic lesions such as simple hyperplasia, focal hyperplasia and papilloma were noted in Group A and B. The incidence of simple hyperplasia, focal hyperplasia and papilloma in Group A was 100%, 100% and 80%, respectively. The incidence of simple hyperplasia, focal hyperplasia and papilloma in Group B were 100%, 100% and 20%. Counts of focal hyperplasia and papilloma lesion in each rat were significantly reduced in Group B than in Group A ($P < 0.001$). The result suggests that aspirin significantly decreases the incidence of precancerous lesions induced with BBN and it can act as an effective cheomopreventive agent for precancerous lesions in urinary bladder.

Key Words: Carcinogenesis, Bladder tumor, Chemoprevention, Aspirin

INTRODUCTION

Bladder cancer is the most common tumor of urological cancers in Korea.[1)]

Chemoprevention is a rapidly expanding field that promises the hope and potential one in the management of cancer.[2,3)] The concept of field cancerization states that an entire area of epithelium exposed to a carcinogen is at risk of developing cancer. Such changes are associated with subsequent high risk for development of tumor recurrence.[4)] Many clinical trials of chemopreventive agents are now ongoing in various sites of cancer, including the urinary bladder.[6,7)] Chemoprevention agents, including specific vitamins, natural products or toxicologically well-known drugs, are alluring agents in controlling the recurrence of urothelial tumors. Effective chemoprevention by non-steroidal antiinflammatory drugs (NSAIDs) has been demonstrated in experimental models for cancer of colon, urinary bladder and esophagus among other tissues.[2,6,7)] Aspirin is included in this class of compound noted for antitumor activity.

Corresponding author : Gil Ho Lee, Department of Urology, Dankook University College of Medicine, San-29 Anseo-dong, Cheonan 330-714, Korea. Tel: 041-550-6630, Fax: 041-552-2221, E-mail: gilho@anseo.dankook.ac.kr

Aspirin has significant chemoactivity in the colon, and its use is associated with reduced incidence of both colonic adenomas and invasive cancers.[2,3] It is well known according to histogenesis of bladder tumors in rats treated with BBN that hyperplasia, papilloma and cacinoma occur in sequence, suggesting the hyperplasia and papilloma are precancerous lesions to urothelial cancer.[5] A major target of chemoprevention is preneoplastic lesion of urinary bladder which is causal factor for recurrence. The visible cancerous lesion of urinary bladder is well controlled by endoscopic resection. But the result of chemoprevention by aspirin has been controversial in bladder carcinogenesis.[2,6,8~10]

In this study, we investigated the possible inhibitory effect of dietary exposure to aspirin during the initiation and post initiation stages on OH-BBN induced bladder carcinogenesis in female rats.

MATERIALS AND METHODS

1) Animals and animal husbandry

Female SD rats were obtained at 8 weeks of age from colonies at the Ansung Animal Farm.

The rats were housed in groups of three in hardwood bedding in windowless animal rooms.

Animal rooms were illuminated on a 12 hours light/dark cycle and were maintained at a temperature of 22±1°C and a relative humidity of 50 ±20%. After a quarantine period of 1 week, animals were randomized into 3 groups. Animals in Group A with 15 rats were given 0.05% BBN in drinking water for 12 weeks. The animals in Group B with 15 rats were given 0.05% BBN in drinking water and were fed a diet with 0.5% aspirin. The animals in Group C with 15 rats were designated as the control group and were not given BBN or aspirin (Fig. 1).

2) Carcinogensis agent and chemopreventive agent

Aspirin were purchased from Sigma Co. (St. Louis, MO), and BBN was purchased from Tokyo Chemical Industry Co. Ltd, Tokyo, Japan. The purity of each standard used in this experiment was confirmed by high performance liquid chromatography (HPLC). Powdered diet (Sam Yang Co., Korea) was used as a basal diet throughout the experiment.

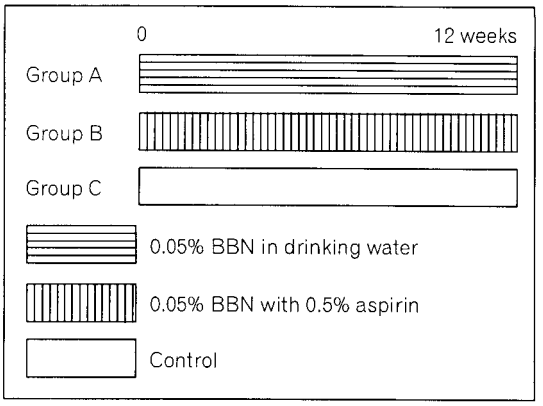

Fig. 1. Experimental protocol.

BBN was given to rats in tap water at a concentration of 0.05%. Drinking water containing carcinogen was prepared every other day. They were allowed as libitum access to food and water, and aspirin was mixed in the basal diet at a concentration of 0.5%. All rats were killed at 12 weeks from the beginning of the experiment under intraperitoneal pentobarbital injection anesthesia. At autopsy, urinary bladders were inflated with 10% buffered formalin, and fixed for 5 hours. The fixed urinary bladder tissues were longitudinally bisected and the specimens were processed for paraffin embedding followed by further transverse cut into 13 slices, and routinely stained with H & E. The classification of different pathologic lesions of the rat bladder, induced by BBN administration, including 3 types of simple hyperplasia, focal hyperplasia and papilloma, was based on previous reports.[11]

3) Statistical analysis

The Students t-test was used to analyze the difference between mean values in data sets.

RESULTS

1) Body weight

Body weights at week 8 showed no significant difference in intergroup, with the average in each group ranging from 140~160 gm. At week 21, body weight of Group A and Group C showed no difference, but at that time the body weight of Group A and Group B was significantly different ($P<0.05$). The difference in body weight seemed to be due to the toxicity of aspirin which interfered with local cytoprotection in the stomach.

2) Incidence of preneoplastic lesions in bladder

There were many cobblestone-like lesions on bladder mucosa of Group A and B under dissecting microscope. Control group did not show any pathologic lesion on the bladder mucosa. There were various pathologic lesions relating to the bladder carcinogenesis on bladder mucosa. Three typical lesions were noted: simple hypreplasia, focal hyperplasia and papilloma (Fig. 2~4). Specific cancer lesions were not noted. Incidence of simple hyperplasia, focal hyperplasia in Group A was 100% and 100%, respectively. The incidence of papilloma in Group A was 80% (12 of 15 rats). The incidence of simple hyperplasia, focal hyperplasia in Group B was 100%, and 100%, respectively. The incidence of papilloma in Group B was 20% (3 of 15 rats)(Table 1).

Mean counts of focal hyperplasia and papilloma lesion in each case of Group A were 28 ± 16 and 5 ± 3, respectively. Mean counts of focal hyperplasia and papilloma lesion in each case of Group

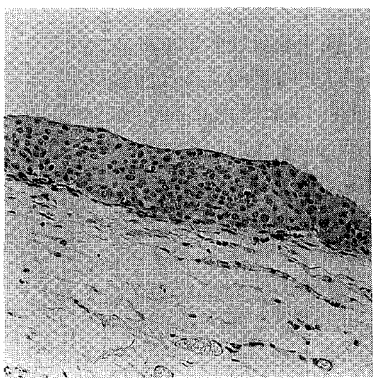

Fig. 2. Hyperplasia of transitional epithelium in BBN-treated rat (H&E, × 200).

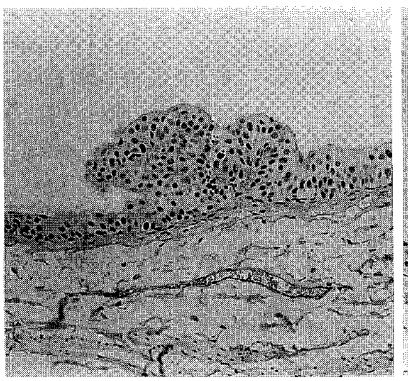

Fig. 3. Focal hyperplasia of transitional epithelium in BBN-treated rat (H&E, ×200).

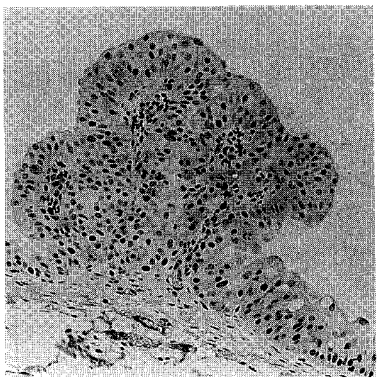

Fig. 4. Papilloma of transitional epithelium in BBN-treated rat (H&E, × 200).

Table 1. Preneoplastic lesions in urinary bladder in rats treated with BBN and aspirin administration

Group	No. of animals	Changes in urinary bladder (%)		
		Simple hyperplasia	Focal hyperplasia	Papilloma
Group A	15	15 (100%)	15 (100%)	12 (80%)
Group B	15	15 (100%)	15 (100%)	3 (20%)
Group C	15	0 (0%)	0 (0%)	0 (0%)

Group A: BBN only, Group B: BBN with aspirin, Group C: control.

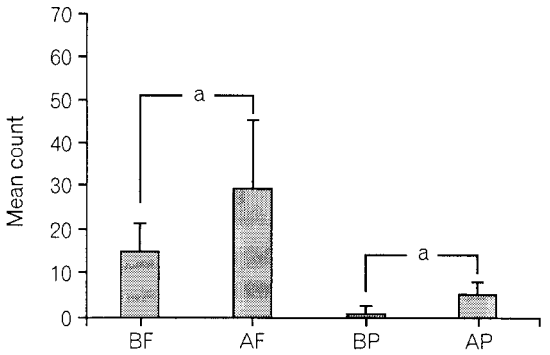

Fig. 5. Mean counts of preneoplastic lesions in each rat given BBN with/without aspirine. [a]Significantly different ($P<0.001$) between two groups. BF: focal htperplasia in group B, AF: focal htperplasia in group A, BP: papilloma in group B, AP: papilloma in group A.

B were 14 ± 6 and 0.6 ± 1.2, respectively (Fig. 5). The counts of focal hyperplasia and papilloma lesion were significantly reduced in Group B than Group A ($p<0.001$ and $p<0.001$, respectively).

DISCUSSION

Bladder tumor is the fifth most common cancer in Korea men. Superficial bladder tumor is treated with transurethral resection of bladder tumor. But with resection only, the bladder tumor will recur in a few years later.[12] Transitional cell carcinoma is usually a field change disease with tumors arising at different times, and with sites in the urothelium and the recurrences often arise many years after the original tumors.[12,13]

The concept of a field change with *in situ* tumor formation in the urothelium surrounding sites of primary or recurrent urothelial cancer has been widely supported. This concept of field cancerization states that an entire area of epithelium exposed to a carcinogen is at risk of developing cancer.[4]

The two-stage process of chemical carcinogenesis, initiation and promotion, is demonstrated in the urinary bladder. The simple hyperplasia is regressed without continuous carcinogenic stimuli, which is a reversible type of the preneoplastic lesions. But the focal hyperplasia and papilloma represent an irreversible change, developing into cancer even in the absence of a carcinogen.[11] Focal hyperplasia and papilloma can be considered as a precancerous lesion of urinary bladder cancer.

The identification of precusor changes offers hope of inducing the regression of lesions which if untreated would lead to invasion and death of the host. Morse et al proposed projected target populations for chemoprevention such as i) individuals who engage in risk-taking behaviors or lifestyles, ii) individuals who have received occupational exposure to known carcinogens, iii) those who are known to be genetically predisposed to the development of cancer, iv) individuals who possess premalignant lesions, and v) survival of primary cancers with a high degree of recurrence or high tendency towards formation of secondary primary tumors.[15] According to this proposal, patients with bladder cancer are generally good target populations for chemoprevention.

Many experimental trials of chemopreventive agents are now ongoing in urinary bladder.[2,3,6~10] A variety of agents inhibiting cyclooxygenase (COX) activity has been known collectively as NSAIDs. The well known agents are aspirin, indomethacin, ibuprofen, piroxicam and sulindac. Among the above suggested agents, aspirin has been well-known drug for several decades. It has a long history of use, thus extensive toxicological profiles have been available for many years.[16]

Eicosanoid biosynthethic pathway has been demonstrated to be appropriate targets for cancer chemoprevention in a wide range of target tissues in experimental animals. The level of prostaglandins (PGs) and activity of PG endoperoxide synthase (COX) are both elevated in tumor cells. Comparison of PGE_2 levels in different bladder lesions has revealed bladder tumors (400~1,000 pg/mg tissue) to have ~200 to 400 fold and ~10 to 25-fold the concentrations of normal epithelium (<3 pg/mg tissue) and papillomatosis (41 pg/mg tissue),[17~19] respectively, directly associating with elevated DNA synthesis. Thus, PGs may participate in the process of bladder carcinogenesis.

The suppression of COX activity by the administration of NSAIDs presents a viable mechanistic approach to cancer prevention.[20] Chemopreventive agents such as aspirin typically have significant chemoactivity in the colon, as its use is associated with reduced incidence of both colonic adenomas and invasive cancers.[2,3,21] The activity of aspirin against N-{4-(5-nitro-s-fury)-2-thiazolyl} formamide (FANFT)-induced tumors in rat urinary bladder has been attributed to inhibition of prostaglandin synthetase-catalyzed activation of FANFT to a carcinogen.[10]

The results of experimental and epidemiologic studies suggesting aspirin as a chemoprevention as above have been confusing. According to Rosenberg et al, an aspirin intake of 4 or more days per week (regular use) was associated with halving colon cancer incidence.[22] In a study of Paganini-Hill et al, daily aspirin intake did not alter the risk of developing colon cancer as in this study.[23] The epidemiology data presented by Thun et al states that the use of aspirin was not associated with reduced risk of human bladder cancer.[2] However, aspirin in high doses has been shown to promote urinary bladder carcinogenicity when administered after FANFT in rats.[8] On the other hand, when aspirin was co-administered with FANFT, inhibition of bladder tumorigenesis was noted.[10] The lack of an inhibitory effect of aspirin in the OH-BBN/BDF mouse model differs from the results of several previous studies in rats, in which aspirin was effective against the induction of urinary bladder cancer by FANFT.[6,10] Interspecies difference and difference in carcinogenic agents, time of administration and dosages of aspirin are possible reasons for different results in different experiments. Rao et al reported that the bladder neoplasm induced by OH-BBN in mice is more aggressive than lesions induced by the same carcinogen in rats. Aspirin may confer protection against the relatively slow growing and primary exophytic lesion induced by OH-BBN in rats. This protection may be overcome by the rapidly growing and highly invasive nature of the lesion induced in mice.[6] In our report, aspirin is effective in preventing precancerous lesions in rat bladder, which is a slow growing and exophytic lesion.

Most of all reports on chemoprevention of aspirin has been focused on the change in incidence of transitional cell carcinoma. The visible tumors of urinary bladder can be well recognized with cystoscopy and they can be resected with endoscopy. The recurrence of tumor is due to preneoplastic lesions that look normal in endoscopy. The focus of our study was preneoplastic lesion, not transitional cell carcinoma lesion. In all specimens, true cancer lesions were not found.

In conclusion, aspirin significantly reduced the incidence of precancerous lesions of urinary bladder that were induced with chemical carcinogen. It may be used as an effective chemopreventive agent for preventing tumor recurrence in urinary bladder.

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