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Effect of Partial Cystectomy on the Induction of Pre-neoplastic Lesions in Rat Bladder Initiated with N-Butyl-N-(4-hydroxybutyl)nitrosamine Followed by Bladder Carcinogens and Promoters

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Summary. The effect of partial cystectomy on the occurrence of pre-neoplastic lesions, papillary or nodular hyperplasia (PN hyperplasia), of the bladder in male F344 rats was studied in an experiment in which bladder carcinogens and promoters were given to the rats after initiation with BBN. The bladder carcinogens tested were N-ethyl-N-(4-hydroxybutyl)nitrosamine (EHBN) and N-4[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) and the bladder promoters were sodium saccharin, sodium cyclamate, and DL-tryptophan. Partial cystectomy significantly decreased the occurrence of PN hyperplasia in rats treated with EHBN and tended to inhibit that in rats given saccharin or tryptophan. Thus partial cystectomy inhibited rather than enhanced the induction of PN hyperplasia.

Key words: Rat bladder carcinogenesis, Initiation and promotion, Partial cystectomy, N-butyl-N-(4-hydroxybutyl)-nitrosamine, N-ethyl-N-(4-hydroxybutyl)nitrosamine, Sodium saccharin.

Introduction

The 2-stage process of chemical carcinogenesis was initially proposed from studies on mouse skin and has subsequently been demonstrated in other organs, such as the liver, colon, and bladder [1, 17, 18]. Sodium saccharin, sodium cyclamate, and DL- or L-tryptophan act as promoters after initiation by carcinogens in the 2-stage process of bladder carcinogenesis [2, 7, 12, 16].

There have been many reports on pre-neoplastic lesions of bladder carcinogenesis, and it is generally agreed that papillary or nodular hyperplasia (PN hyperplasia) is a pre-neoplastic lesion in rat bladders [9, 13]. In general, excessive loss of epithelial cells as a result of mechanical or chemical damage to the bladder is followed by regenerative hyperplasia of the mucosa [5, 6, 19]. Recently we found that partial cystectomy in rats increased DNA synthesis in epithelial cells of all the remaining mucosa and induced regenerative hyperplasia [20]. Therefore, it was thought that partial cystectomy should enhance short-term detection of the pre-neoplastic lesion, PN hyperplasia, in the 2-stage process of bladder carcinogenesis, since partial hepatectomy enhances the induction of pre-neoplastic liver lesions, hyperplastic nodules, in liver carcinogenesis [15, 21].

In the present work, we examined the effect of partial cystectomy on the promoting activities of several chemicals in the promoting stage of N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) bladder carcinogenesis in rats.

Material and Methods

One hundred and seventy 6-week-old male F344 rats (Charles River, Japan Inc., Kanagawa) were used in this experiment.

BBN and N-ethyl-N-(4-hydroxybutyl)nitrosamine (EHBN) were purchased from Izumi Chemical Co., Yokohama, Japan. N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT) was obtained from Saber Labs., Ill., U.S.A., sodium saccharin from Okuno Chemical Co., Osaka, Japan, DL-tryptophan from Sigma Chemical Co., St. Louis, Mo., U.S.A., and sodium cyclamate from Wako Pure Chemical Co., Osaka, Japan.

Rats were divided into five groups. Groups 1 to 4 were given 0.05% BBN in drinking water for 2 weeks and Group 5 was given normal drinking water without BBN. Groups 1, 2 and 5 each consisted of five subgroups treated as shown in Fig. 1. The rats in these five subgroups were given 0.01% EHBN in drinking water and 0.2% FANFT, 5.0% sodium saccharin, 2.5% sodium cyclamate and 2.0% DL-tryptophan in the diet (Oriental M, Oriental Yeast Co., Tokyo, Japan) respectively, for 10 weeks. Groups 3 and 4 were given diet (Oriental MF) without added chemicals. At the end of week 3, the rats in Groups 1, 3 and 5 were subjected to 75% resection of the urinary bladder [20]. Initially there were 10 rats in each subgroup. All rats were killed with ether at the end of week 12. The urinary bladder was inflated with 10% phosphate-buffered formalin and removed. The liver and both kidneys were also removed and weighed. The urinary bladder was cut into left and right halves and four sequential sections were cut from each half. The urinary bladder, liver and kidneys were embedded in paraffin and stained with hematoxylin and eosin for histological examination. For quantitative analysis of bladder lesions, a general purpose colour image processor, model VIP-21C (Olympus-Ikegami Co., Tokyo, Japan) was used.

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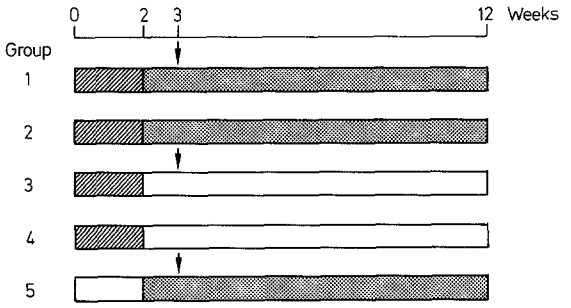

Fig. 1. Experimental design for rapid induction of pre-neoplastic lesions in partially cystectomised rat urinary bladder initiated with BBN

N-butyl-N-(4-hydroxybutyl)nitrosamine, 0.05% in drinking water

75% partial cystectomy

Chemicals: N-ethyl-N-(4-hydroxybutyl)nitrosamine, 0.01% in drinking water; N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide, 0.2% in diet; sodium saccharin, 5% diet; sodium cyclamate, 2.5% in diet; D, L-tryptophan, 2.0% in diet

Basal diet

Results

A few rats in Groups 1 and 5 died during or within 2 weeks after partial cystectomy. After partial cystectomy, the external genital area was wet because of continuous micturition due to mechanical disturbance and test chemicals. This condition continued until 8 or 9 weeks in rats of Group 1, especially in rats treated with EHBN or cyclamate. Adhesion to surrounding fatty tissue was occasionally found at the site of resection. The bladders of one rat each treated with saccharin and tryptophan in Group 5 showed papillomatosis induced by stones, and therefore, these rats were not included in effective numbers. Grossly no tumours were found in the bladder of these rats. There were no differences in total length of basement membrane between cystectomised bladder and non-operated bladder at 12 weeks (Table 1). The criteria used for bladder lesions found in this experiment were simple hyperplasia and PN hyperplasia as described previously [16]. The lesions found at the suture site of the bladder in one to two rats of each subgroup in Groups 1, 3 and 5 are not tabulated in the column of lesions. The incidence and number of lesions of the bladder observed in each group are summarised in Table 1. A high incidence of simple hyperplasia was found in rats that received EHBN, FANFT or saccharin in Groups 1, 2 and 5. The incidence of PN hyperplasia was significantly lower in rats given EHBN in Group 1 than in those given EHBN in Group 2. Moreover, the number of PN hyperplasia per 10 cm of basement membrane was significantly lower in rats given EHBN in Group 1 than in those given EHBN in Group 2. The incidence and number of PN hyperlasia of rats treated with saccharin or DL-tryptophan tended to be lower in Group 1

Table 1. Histopathological changes in the urinary bladder epithelium of rats treated with BBN followed by PC[a] and different chemicals

Group	Treatment			Total length of BM[a] (cm)	Effective No. of rats	No. of rats with simple hyperplaisa (%)	Papillary or nodular hyperplasia	
	BBN	PC	Chemicals				No. of rats affected (%)	No. of lesions[b]/ 10 cm of BM
1	+	+	EHBN	9.66 ± 1.50	10	8 (80)	1 (10)[c]	0.12 ± 0.36[c]
			FANFT	10.45 ± 3.12	10	5 (50)	0 –	0
			Saccharin	8.57 ± 1.92	9	8 (88.9)	4 (44.4)	1.47 ± 1.84
			Cyclamate	8.63 ± 1.13	8	2 (25)	1 (12.5)	0.13 ± 0.35
			Tryptophan	9.79 ± 1.96	9	2 (22.2)	0 –	0
2	+	–	EHBN	9.60 ± 1.10	10	10 (100)	6 (60)[d]	1.31 ± 1.37[d]
			FANFT	9.58 ± 2.16	10	9 (90)	0 –	0
			Saccharin	9.23 ± 1.71	10	10 (100)	7 (70)[e]	2.64 ± 2.80[f]
			Cyclamate	8.88 ± 1.89	10	4 (40	1 (10)	0.12 ± 0.36
			Tryptophan	8.39 ± 1.04	10	2 (20)	2 (20)	0.23 ± 0.47
3	+	+	–	8.24 ± 1.47	10	1 (10)	0 –	0
4	+	–	–	8.41 ± 1.64	10	2 (20)	1 (10)	0.11 ± 0.32
5	–	+	EHBN	9.21 ± 1.84	10	6 (60)	0 –	0
			FANFT	9.52 ± 2.11	10	3 (30)	0 –	0
			Saccharin	9.14 ± 1.94	10	7 (70)	2 (20)	0.78 ± 1.22
			Cyclamate	9.72 ± 1.45	7	1 (14.3)	0 –	0
			Tryptophan	8.03 ± 1.27	7	2 (28.6)	0 –	0

[a] PC = partial cystectomy; BM = basement membrane

[b] Values are means ± S.D.

[c] Significantly different from Group 2 ($P < 0.05$)

[d] Significantly different from Group 4 ($P < 0.02$)

[e] Significantly different from Group 4 ($P < 0.01$)

[f] Significantly different from Group 4 ($P < 0.05$)

than in Group 2. The incidence of PN hyperplasia and their number per 10 cm of basement membrane were significantly higher in Group 2, given EHBN or saccharin, than in Group 4. However, there was no difference in the occurrence of PN hyperplasia in rats given FANFT, cyclamate and DL-tryptophan, respectively, in Group 2 and in rats in Group 4. Moreover, slight PN hyperplasia was observed in rats given saccharin in Group 5. There were no predilection sites of PN hyperplasia in Groups 1, 3 and 5. No bladder tumours were detected in any groups.

Discussion

EHBN and FANFT are carcinogenic to the bladder of experimental animals [4, 10], and there are many reports on the carcinogenic effects of these carcinogens. Saccharin, cyclamate, and tryptophan are promoters of bladder carcinogenesis [2, 7, 12, 16]. This study showed that partial cystectomy significantly inhibited the occurrence of the pre-neoplastic lesion, PN hyperplasia of the bladder, when EHBN was given after initiation with BBN and tended to be inhibitory when saccharin and tryptophan were given in the promotion stage. After partial cystectomy, DNA synthesis is increased in all epithelial cells of the bladder and the bladder is restored almost to normal in 2 weeks [20].

Recently it was reported that ligation of the ureter increased DNA synthesis in the bladder of rats [11]. Partial hepatectomy increases liver cell proliferation, resulting in enhanced induction of hyperplastic nodules by hepatocarcinogens [15, 21]. Therefore, it is speculated that partial cystectomy promotes the induction of PN hyperplasia of the bladder initiated by BBN. In the present study, the bladder in Groups 1, 3 and 5 seemed to be restored to normal size at 12 weeks. However, unexpectedly partial cystectomy did not have a similar effect. On unilateral ligation of the ureter, a high incidence of transitional cell carcinomas of the renal pelvis and ureter was detected in rats that received BBN in drinking water, although BBN does not generally induce tumours in the renal pelvis and ureter [14]. The retention of urine containing carcinogens in the renal pelvis and ureter was the most important factor for enhancing cancer induction. As a result, their mucosa might be exposed to carcinogens for a longer time. In general, bladder carcinogens or promoters of bladder carcinogenesis exert their activities through the urine [14]. Tatematsu et al. [20] described that at 6 h after partial cystectomy the bladder was already filled with urine. However, it is speculated that in rats subjected to partial cystectomy plus test chemicals, the urine probably did not remain in the bladder because of continuous micturition due to irritation of mechanical damage and test chemicals, and so the bladder mucosa was exposed to bladder carcinogens and promoters for a shorter time. Of course, bladder carcinogens and promoters were constantly present in the urine and were constantly in contact with the urothelium. However, they must have been excreted from the bladder before they were metabolised in the bladder. This indicates that carcinogens and promoters of bladder carcinogenesis in the urine need a certain period of contact with the bladder mucosa to exert their activities: urine retention in the bladder is more important than increase of DNA synthesis in the bladder epithelium. This is similar to the case of stomach epithelium in stomach carcinogenesis of rats [8].

In Group 2, EHBN and saccharin increased the incidence of BBN-initiated pre-neoplastic lesions, but FANFT, cyclamate and tryptophan did not. It was reported that 10 weeks feeding of 0.2% FANFT to F344 rats induced marked hyperplasia meaning PN hyperplasia of the bladder [3]. However, in the present work FANFT-treated rats had no PN hyperplasia of the bladder in Group 2. We need further investigation of this difference. The promoting activities of cyclamate and tryptophan are weaker than those of EHBN and saccharin, and they need longer periods to exert these promoting activities.

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