Urol Res (1992) 20:383–386

Urological Research

Original articles

Inhibition of two-step urinary bladder carcinogenesis by the somatostatin analogue RC-160

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Accepted: 7 July 1991

Summary. Fisher 344 female rats were exposed for 4 weeks to the initiator carcinogen *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN) 0.05% in the drinking water and thereafter to the promoter carcinogen mitomycin C (0.08 mg per animal per week) intravesically for 12 weeks. High incidence of urinary bladder transitional cell cancers was observed (17 in situ and 17 invasive carcinomas among 40 rats). When the somatostatin analogue RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2) was administered s.c. at the dose of 50 μg per animal per day during 6-week period of promotion with mitomycin C, the incidence of urinary bladder cancer was dramatically reduced. Only 1 in situ carcinoma was observed among 20 rats and only preblastomatous lesions (dysplasias and papillomas) occurred. This effect could indicate that RC-160 interferes with the process of promotion by induction of enhanced apoptosis (programmed cell death) of the dysplastic urothelial cells. RC-160 could be tried therapeutically for the hormonal prevention of malignant transformation of preneoplastic lesions in the urinary bladder.

Key words: Urinary bladder – Carcinogenesis – Somatostatin analogue

The development of urinary bladder cancer is a multistep process [7]. Preblastomatous lesions precede the onset of in situ and invasive carcinoma and persist in several parts of the urothelium for many years, providing the opportunity for a repeated formation of neoplasms[1], which occur frequently in spite of various forms of therapeutic measures [18]. The role of growth factors in urinary bladder carcinogenesis has been discussed recently [1, 12]. Somatostatin and its analogues appear to inhibit the secretion or action of growth factors [6, 10, 15]. In our previous studies, the somatostatin analogue RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2) [4] inhibited the growth of various experimental animal tumors [14, 15, 17]. In the present work, urinary bladder cancer was induced by the slightly modified method of Ohtani et al. [13] in rats, and the effect of treatment with RC-160 on the promotion phase of the multistep carcinogenesis was studied.

[1] Neoplasia is the process of the formation of neoplasms

Materials and methods

Animals

One hundred and twenty Fisher 344 female rats (120 ± 2 g, 10 weeks old) were kept 5 per cage in plastic cages in our Animal Facility. The rats were fed laboratory rodent chow (LATI, Gödöllõ, Hungary) and tap water was given ad libitum. The room temperature was 22 ± 2°C, the relative humidity 55 ± 5%.

Chemicals and route of administration

N-Butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN) was the product of Sigma (St. Louis, Mo., USA). This compound was dissolved in the drinking water (0.05 w/v%) of the groups receiving BBN alone or in combination with other treatment. Mitomycin C (Kyowa, Tokyo, Japan) was instiled via the urethra into the urinary bladder using a specially prepared injection needle. The intravesical treatment was performed under a light Nembutal (Serva, Heidelberg, FRG) anesthesia (70 mg/kg i.p.). The dose was 0.08 mg per animal per week, in aliquots of 0.2 ml physiological saline. Analogue RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2), originally synthesized by solid-phase methods and evaluated in our laboratory [4], was made by classic synthesis by Novabiochem (Laufelfingen, Switzerland). Daily s.c. injections of 50 μg/rat (dissolved in aliquots of 0.2 ml physiological saline) were administered.

Experimental protocol

Group 1 consisted of 10 untreated control animals. Group 2 was composed of 10 animals treated intravesically for 12 weeks with 0.2 ml 0.9% NaCl solution, and sacrificed 1 week after the last treatment. Group 3 was subjected to BBN treatment; 30 animals

Table 1. Pathological changes in the urinary bladder of female F-344 rats after BBN, mitomycin C, BBN + mitomycin C and BBN-mitomycin C + RC-160 treatment

Group	Normal	Mild dysplasia	Moderate dysplasia	Severe dysplasia	Papilloma	Ca in situ	Invasive Ca
1. Untreated							
2. 0.2 ml	10/10						
Saline/week intravesically	10/10						
3. BBN	11/30	4/30			15/30		
4. Mitomycin C	10/10						
5. BBN + mitomycin C	1/40		2/40	6/40	20/40	10/40	12/40
6. BBN + mitomycin C + RC-160	1/20	1/20	1/20	7/20	11/20	1/20*	

* $P < 0.001$ compared with group 5 by X^2 (Chi-square) test

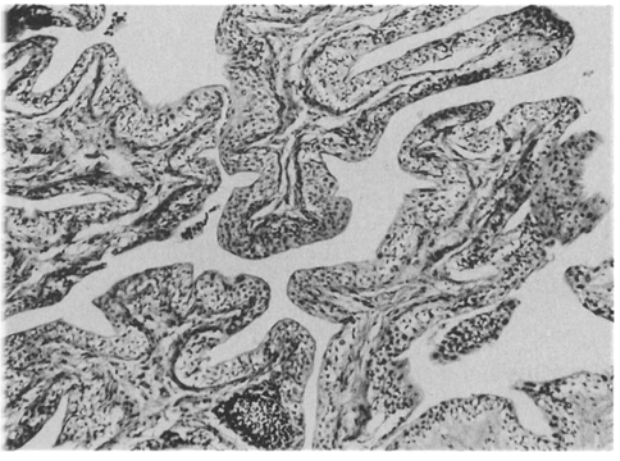

Fig. 1. Papilloma in the urinary bladder of a rat treated with BBN alone. HE, ×130

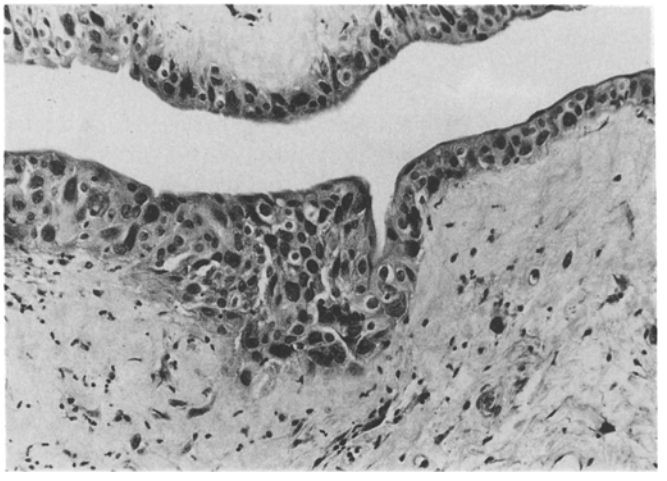

Fig. 2. In situ transitional cell carcinoma in the urinary bladder of a rat treated with BBN and mitomycin C. Note marked atypia of the urothelial cells. HE, ×260

were treated for 4 weeks orally, with 0.05% BBN in the drinking water. After this, a 12-week-long 0.9% NaCl treatment (0.2 ml once a week) was given. The rats were sacrificed 1 week after cessation of the treatment. Group 4 was treated with mitomycin C, 10 animals received mitomycin C treatment, 0.08 mg/animal intravesically, once a week for 12 weeks and were sacrificed 1 week after the last treatment. Group 5 was given BBN + mitomycin C treatment; 40 animals were treated for 4 weeks orally, with 0.05% BBN in the drinking water. Immediately after the cessation of the BBN treatment, intravesical mitomycin C treatment was started (0.08 mg/animal, once a week) and continued for 12 weeks. The animals were sacrificed 1 week after the last treatment. Group 6 was subjected to combined BBN + mitomycin C + RC-160 treatment; 20 animals received the same treatment as group 5 (BBN and mitomycin C), but during the 12-week period when mitomycin C was instilled, daily injections of RC-160 (50 µg/animal) were also administered. The rats were sacrificed 1 week after the last mitomycin C treatment. The animals were sacrificed by exsanguination under Nembutal anesthesia.

Pathological procedure

Autopsy was performed and all organs were examined macroscopically. The urinary bladders were filled via the urethra with 4% buffered formalin and fixed in the same fixative for 1 week. Paraffin embedding was performed and serial 6-µm sections of the whole urinary bladder were cut. Sections from every 1-mm layer were stained with hematoxylin and eosin (H&E) and examined under a light microscope. The following histopathological categories were used in the evaluation of the alterations caused by the various treatments: mild, moderate and severe dysplasia, papilloma, in situ transitional cell carcinoma and invasive transitional cell carcinoma.

Results

The urothelium of the urinary bladder in animals of groups 1, 2 and 4 (untreated control, saline-treated control and mitomycin C-treated rats) showed no histological changes (Table 1). BBN and saline treatment (group 3) resulted in 4 cases of mild dysplasia and 15 papillomas (Fig. 1). Eleven rats had no alteration in their urinary bladder (Table 1). BBN plus mitomycin C treatment (group 5) caused in situ transitional cell carcinoma (Fig. 2) in 10 rats, and an additional 12 rats showed invasive transitional cell carcinoma (Fig. 3, Table 1). In addition, in this group papillomas were observed in 20 cases, moderate dysplasia in 2 cases and severe dysplasia in 6 cases (Table 1). This means that most of the urinary

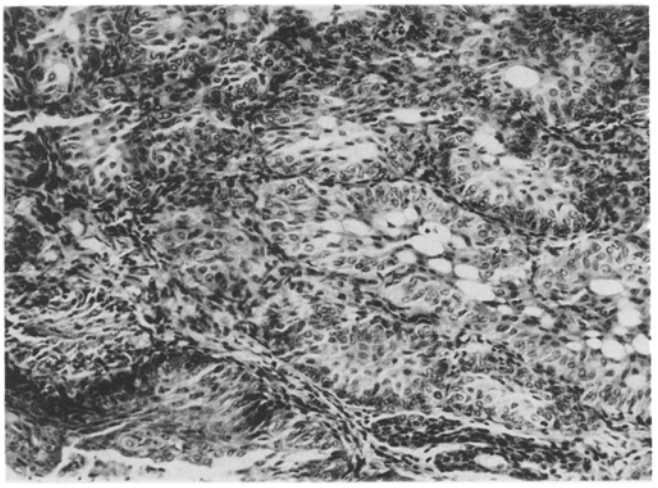

Fig. 3. Invasive transitional cell carcinoma in urinary bladder of a rat treated with BBN and mitomycin C. HE, ×520

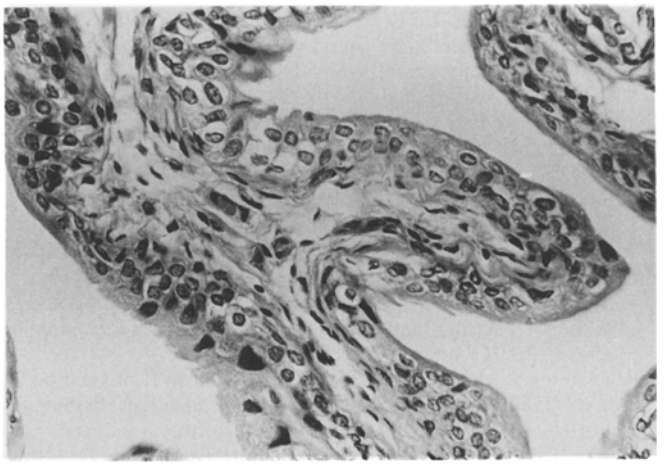

Fig. 4. Moderate dysplasia of the urothelium in the urinary bladder of a rat treated with BBN, mitomycin C and RC-160. Note cellular atypia and desquamating cells with hyperchrome nuclei. HE, ×260

bladders of the animals in this group showed papillomas and dysplasias as well as carcinomas, at the same time. Only one rat proved to be unaffected by the BBN + mitomycin C treatment (Table 1). When the BBN and mitomycin C treatment was combined with analogue RC-160 (group 6), only 1 animal of 20 developed in situ transitional cell urinary bladder cancer. The urinary bladder of 1 rat was intact. Various degrees of dysplasia were observed in 9 cases, and papillomas in 11 instances (Table 1). One rat showed a papilloma and severe dysplasia and another rat sowed papilloma and in situ carcinoma at the same time. A distinctive feature of the RC-160 treated group was that the upper layer of the dysplastic urothelium contained several scattered desquamating cells with large, condensed nuclei (Fig. 4). Such cells were not observed in the urinary bladders of rats in the other groups.

Discussion

Our study demonstrates that RC-160 dramatically reduces the number of urinary bladder cancers in rats, when given together with the tumor promoter mitomycin C, after pretreatment with the nitrosamine derivative BBN. The effect of the induction due to the BBN treatment was not influenced by this treatment and various types of preblastomatous lesions (dysplasia and papillomas) [9] developed in nearly all rats in the group treated with BBN, mitomycin C and RC-160. This suggests that RC-160 may inhibit the process of transition from preneoplastic lesions to neoplasia. It is of interest that similar inhibitory action has been reported for an analogue of LH-RH (luteinizing hormone-releasing hormone) by Matsuki et al. [11]. In our previous studies, we have demonstrated that the somatostatin analogue RC-160 and LH-RH analogues inhibit the growth of some experimental animal tumors by enhancing the occurrence of programmed cell death (apoptosis) in the tumor population [17]. The studies of Bursch et al. [3] as well of Schulte-Hermann et al. [16] showed that the deprivation of promoters from initiated and promoted cells causes a high frequency of apoptosis. The morphological appearance of the desquamating dysplastic urothelial cells, observed by us in the RC-160-treated animals, suggests that these might be apoptotic cells. It appears that RC-160 interferes with the tumor promoter mitomycin C by enhancing apoptosis of the dysplastic urothelial cells, thus preventing them from undergoing malignant transformation. Somatostatin analogue RC-160 can therefore be included among the chemopreventive agents or antipromoters of urinary bladder carcinogenesis, like ascorbic acid or difluoromethylornithine [2, 8], but its action is based on hormonal and not chemical mechanisms. The mechanisms by which RC-160 exerts these effects could include the interference with the action and secretion of growth factors, especially EGF, TGF-α and IGF-I [6, 10, 15].

From the therapeutic point of view, the prolonged administration of RC-160 in the form of sustained delivery systems [5] may provide a promising approach to the prevention of malignant transformation of preneoplastic lesions in the urinary bladder.

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