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Morphological changes of urothelial and intestinal mucosa after ureterosigmoidostomy during experimental urogenic carcinogenesis*

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Summary. To analyze the effect of chemical carcinogens on urothelial and intestinal mucosa on 214 female Wistar rats an ureterosigmoidostomy was performed. After 10–14 days 123 surviving rats were randomly divided into 4 groups: Group I–III received 0.05 per cent N-Butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) in drinking water over a period of 15 weeks. Group IV received normal drinking water over the same period. The defunctionalized bladders were instilled each second day: Group I: physiologic saline solution, Group II: urine of normal rats, Group III and IV: urine of BBN-treated rats. 30 rats without diversion but BBN treatment served as a control. The evaluation of the histological data gave the following results: In the control group urothelial tumors were found in the bladder exclusively. Dependent on the grade of obstruction in the BBN-treated groups, with diversion urothelial tumors in the renal pelvis and ureter could be documented, whereas in the bladder no tumor growth could be shown. In the intestinal mucosa of BBN-treated animals a high incidence of adenocarcinoma was found. The chemical tumor induction by BBN is related to the urine and takes place by direct contact to the mucosa when the metabolites excreted by the kidneys are activated in the urine. BBN or other urogenic carcinogens seem to have no urothelial specificity. The incidence of bowel carcinoma after diversion must lead to intensive long-term follow up.

Key words: Bladder Carcinogenesis – Ureterosigmoidostomy – Urinary diversion – Intestinal carcinogenesis

Introduction

For a few human cancers the association between an environmental agent and the development of a malignant tumor has been shown. In 1895, Rehn [16], reported the first cases of bladder cancer occurring in a chemical dye factory. The first experimental evidence that an aromatic amine could cause bladder tumors was reported by Hueper et al. [8]. Since then many potential carcinogenic substances have been identified and their pathways and metabolism have been studied [11]. There is much evidence that urothelial carcinogenesis is a multistage process that takes place in man over many years.

The first experiments on staged carcinogenesis were carried out by Berenblum, 1941 [1]. These experiments showed that when the exposure to the initiating carcinogen was short, no tumor development was observed, but if the exposure was prolonged or was followed by another agent, not itself a carcinogen, tumors were detectable. The order in which the carcinogen and the promoter were applied is important, but the time interval between the carcinogen exposure and the promoter did not influence tumor incidence, implying that the changes induced by the carcinogen are irreversible. Multistage carcinogenesis experiments have been performed in the urinary bladder where a subcarcinogenic initiating dose was given and followed by administration of suspected promoters [2, 3, 5, 7].

The role of urine itself in bladder carcinogenesis became of interest when in 1982 Melicow [12] reported 16 cases of spontaneous regression of superficial bladder tumors within 6 months in which cystectomy was preceded by bilateral ureterosigmoidostomy. At first it was thought, that the urine acted only as a carrier for the carcinogens, but the experiments of Rowland et al. [18] and Oyasu et al. [14] have shown that urine may have a more direct role and my act as a promoter. If the urine of rats was diverted from the

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Table 1. Experimental scheme after ureterosigmoidostomy

Group	0.05% BBN 15 weeks perorally	Bladder instillation (each 2nd day)		
		NaCl	Urine	BBN-urine
I	+	+	−	−
II	+	−	+	−
III	+	−	−	+
IV	−	−	−	+

bladder by ureterosigmoidostomy after exposure to FANFT, the incidence of tumors fell from 8/19 in the control group to 1/18 with the diversion [18]. If the bladder was transplanted heterotopically into syngeneic rats after the donor rats had been exposed to BBN the subsequent treatment of the transplanted bladders with rat urine promoted tumor growth, whereas repeated treatment with saline did not [14]. It is possible that the urine itself is one of the promoting agents.

A further question of clinical relevance is whether the initiating carcinogen is transported to the target organ by urine or by blood. In an heterotopic bladder model with a communicating reservoir Oyasu et al. [13], showed that transitional cell carcinomas developed in 25 of 33 heterotopic bladders exposed to MNU, while after exposure to N-butyl-N-(3-carboxypropyl)-nitrosamine (BCPN) they failed to develop tumors. However, 11 of 27 rats with heterotopic bladders that were exposed to BCPN developed tumors in their homotopic or natural bladders. After absorption through the wall of the heterotopic bladder the carcinogen seemed transported to a new target organ to enhance the carcinogenic effect.

Since the introduction of ureterosigmoidostomy 79 cases of adencarcinomas of the sigmoid were described [6]. The incidence of colon carcinoma associated with ureterosigmoidostomy is 500 times greater than in the normal population, indicating about a 5 per cent life time risk [10, 15]. Clinical and experimental data indicate that the excretion of urine and feces together leads to carcinoma [4]. But there are also other factors, like carcinogens excreted by urine or chronic irritation of the bowel mucosa, which could initiate intestine carcinomas. Also tumors in ileum and colon conduit patients and after bladder augmentation were described [6]. The latency period before tumor occurrence was an average of 22 years when the patient was diverted for benign conditions as compared to 7 years when treated for malignant disease [17]. After the introduction of continent urinary diversion systems using small or large bowel segments these observations could become of clinical relevance.

Material and methods

On 214 female Wistar rats with an average weight of 220 g a modified ureterosigmoidostomy was performed. The rats were anesthesized by Fentanyl, Hypnorm® (Janssen GmbH, Neuss, FRG) and after a lower midline incision the ureters were freed from the retroperitoneum. The distal ureters were ligated and transsected. A low colotomy was made and the ureters drawn into the sigmoid. A 9-0 PDS suture was than placed between the adventitia of the ureter and the colonic wall to fix the ureters in the intestine lumen. After closure of the colotomy all animals received 5 ml/100 g body weight of 0.9 per cent saline mixed with 10,000 IU penicillin per kilogram body weight intraperitoneally and the abdomen was closed in layers.

After a postoperative phase of 10–14 days 123 surviving rats were randomly divided into 4 groups (Table 1). Group I–III received 0.05 per cent N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) in drinking water over a period of 15 weeks. Group IV received normal drinking water over the same period. Additionally the defunctioned bladders of all animals were instilled each second day during the 15 weeks. Group I received instillations of physiologic saline solution, Group II was instilled with urine of normal healthy rats and Group III and IV received urine of BBN-treated rats. The animals were anesthesized by Hypnorm® and 0.25 ml of a solution of saline, urine or BBN-urine respectively was instilled transurethrally via an Orion special LX-1 needle in the bladder. Rats voided spontaneously when anesthesia was finished after 2 to 3 hours. Antibacterial prophylaxis was performed by giving 10,000 IU penicillin i.m. per kilogram body weight before each instillation. Sterile urine for instillation was obtained from separate animals housed in metabolic cages where the urine collections were filtered by sterile microfilters, Millex®-HA, 0.45 µm filter unit and Millex®-GS, 0.22 µm filter unit (Millipore GmbH, Eschborn, FRG).

One week after the last instillation 84 surviving rats were killed, the kidneys and ureters, the bladders and sigmoid colon were removed. The organs were fixed in formalin and evaluated by light microscopy after hematoxylin and eosin staining.

30 rats without diversion but BBN treatment over 15 weeks served as a control group.

Results

The results obtained are summarized in four categories: the effect of BBN-treatment in the undiverted animals (control group), morphological changes in the upper urinary tract after diversion, morphological changes in the defunctioned bladder, carcinogenesis of intestinal mucosa after ureterosigmoidostomy.

Undiverted animal group

After 15 weeks of feeding 0.05 per cent BBN, exophytic papillary or invasive urothelial tumors developed in all rat bladders, whereas no tumor growth was observed in the upper urinary tract (Fig. 1).

Morphological changes in the upper urinary tract after diversion

After ureterosigmoidostomy nearly all animals showed mild to severe hydroureteronephrosis. Dependent on

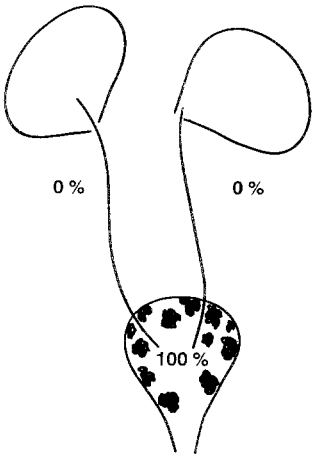

Fig. 1. Distribution of urothelial tumors in the undiverted animal group. $N=30$; 0.05% BBN; 15 weeks perorally

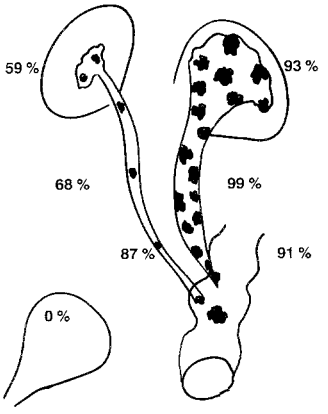

Fig. 3. Frequency of urothelial tumors after ureterosigmoidostomy dependent from the grade of obstruction. $N=63$; 0.05% BBN; 15 weeks perorally

the grade of obstruction in the BBN-treated groups I–III there were found exophytic and invasive urothelial tumors in the renal pelvis and ureters (Fig. 2): In absent or mild hydroureteronephrosis there was an increase in tumor frequency from proximal to distal. In moderate or severe hydroureteronephrosis the frequency of tumor growth was more than 90 per cent independent of the localization (Fig. 3). No tumors could be observed in group IV (normal drinking water over a period of 15 weeks but bladder instillation with urine of BBN-treated rats in two days interval over the same period).

Morphological changes in the defunctionalized bladder

One week after the instillation period, the mucosa of the defunctionalized bladders of group I and II showed only slight focal mucosal necrosis of superficial cell layers and focal mural inflammation or was just returned to normal. In the bladder of the animals group III and IV (instillation with urine or BBN-treated rats) increased hyperplastic proliferations and squamous cell metaplasia of the urothelium could be observed (Fig. 4), but there was no evidence for

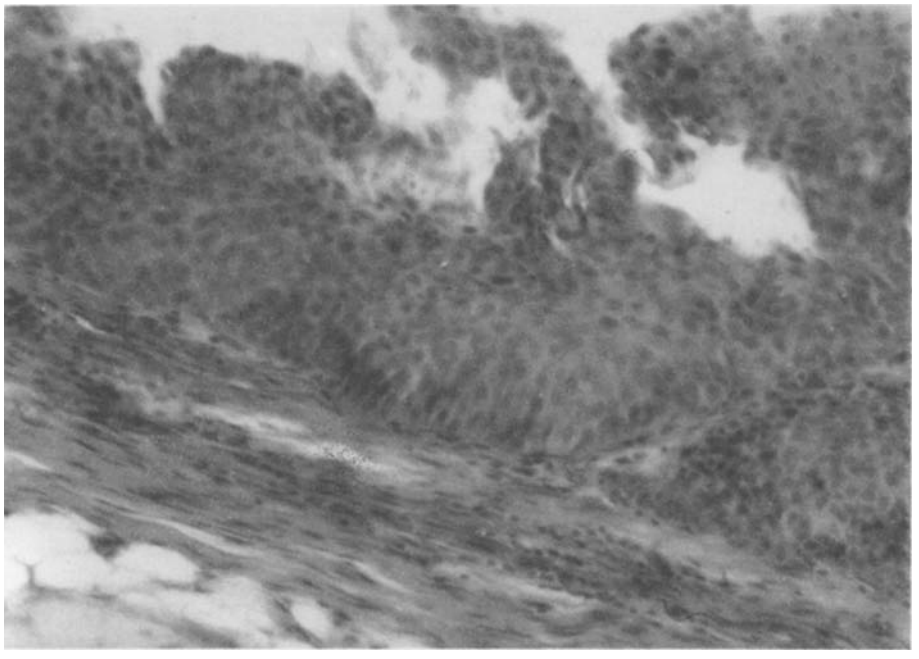

Fig. 2. Urothelial tumor of the ureter in case of moderate hydroureteronephrosis after 15 weeks BBN-treatment perorally (He, ×69)

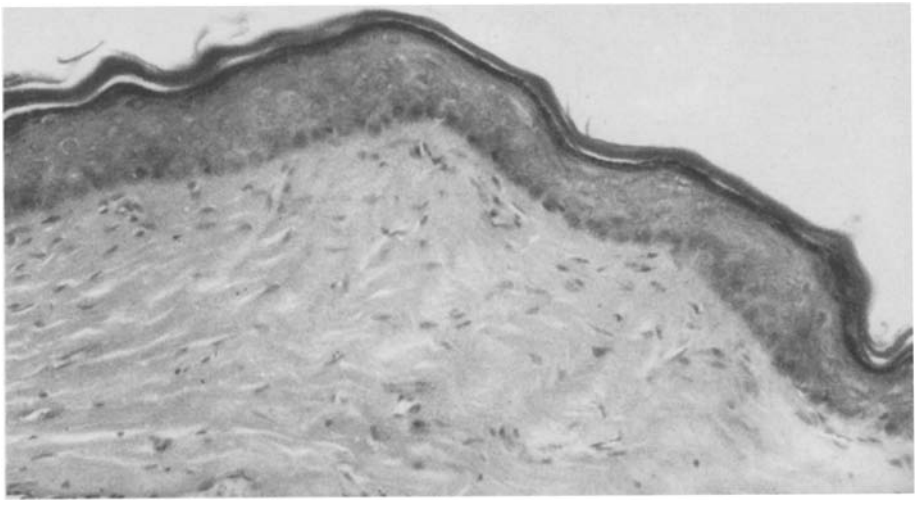

Fig. 4. Squamous cell metaplasia of the defunctionalized bladder mucosa after repeated instillation with BBN-urine (He, ×43)

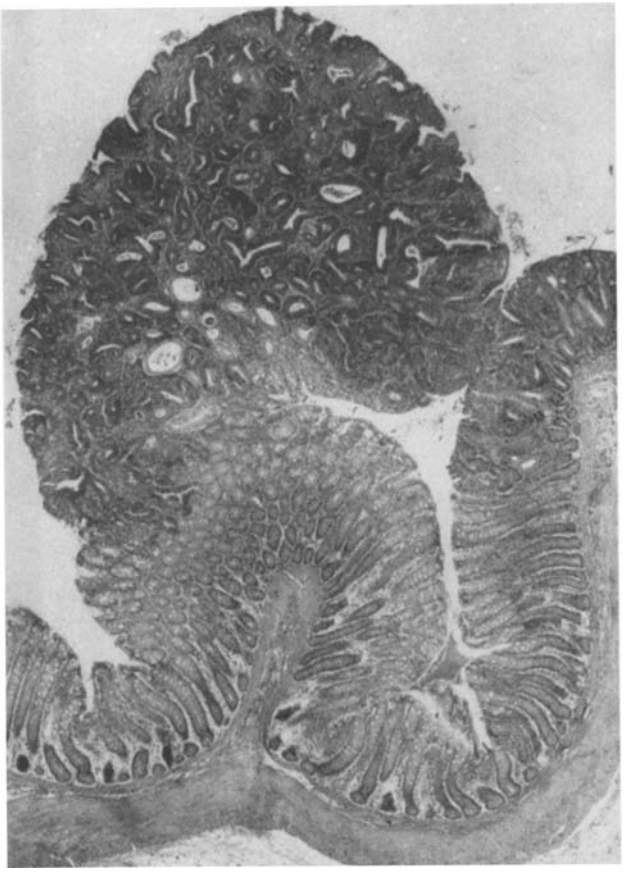

Fig. 5. Adenocarcinoma of the intestinal mucosa at the site of ureteric implantation after 15 weeks BBN-treatment perorally (He, ×26)

papillary or invasive tumors and no severe dysplasia or carcinoma in situ within the observation period (Table 2).

Table 2. Bladder and intestine tumorinduction after ureterosigmoidostomy

Group	No. rats	Bladder (Urothelial Carcinoma)	Intestine (Adeno carcinoma)
I	21	0%	56%
II	23	0%	37%
III	19	0%	43%
IV	21	0%	0%

Carcinogenesis of intestinal mucosa after ureterosigmoidostomy

At the site of the anastomosis in the sigmoid colon but even far from the ureteric implantation site in nearly 45 per cent adenocarcinomas were found in the experimental groups I-III (Fig. 5). There are histopathological evidences that the malignant tumors grow as well from adenomatous polyps as directly as a microcarcinoma. In group IV to tumor of the intestinal mucosa could be demonstrated (Table 2).

Discussion

Many animal models have been developed to study multistage urothelial carcinogenesis. Experiments have been performed using the carcinogens FANFT, BBN, MNU and 2-acetylaminofluorene and demon-

strating that saccharine, cyclamate, tryptophan and phenacetin can act as tumor promoters [3, 5, 7]. Stones and foreign bodies in the bladder also have a tumor-promoting effect [2]. The role of urine itself in bladder carcinogenesis has now come under scrutiny.

BBN is one of several nitrosamines which is known as very potent and specific bladder carcinogen and has a marked dose response [9]. Up to now it is not known that it induces tumors in non-urothelial organs. Most of the experimental data indicate the transport of the carcinogenic agents by the urine to the mucosa. The urine may contain other substances, which act as promoters or cocarcinogens.

The etiology for development of colon carcinoma after urinary diversion has been discussed controversial in the literature. The increased risk has been theorized to relate two different factors: 1) the role of the urine in the colon and 2) the mechanical effect of the fecal stream on the stoma [17].

Another theory of carcinogenesis has been suggested involving bacterial activation of endogenously formed N-nitrosamine [19]. This is in agreement with the experimental data from Crissey et al. [4], who described adenocarcinomas of the colon in rats after ureterosigmoidostomy which could completely prevented by proximal diversion of the feces.

The evaluation of our own data shows that the chemical tumor induction by BBN in Wistar rats is related to the urine. There were not tumors in the defunctioned bladder and the frequency of tumor growth in the upper urinary tract by increased dilatation makes a metabolisation of a primary renal excreted inactive metabolite of the carcinogen possible. The high incidence of adenocarcinomas after ureterosigmoidostomy seems to be related to the excretion of the carcinogen with the urine at which tumor induction takes place by contact of urine and mucosa.

Our results indicate that BBN has no definite urothelial specificity. This may be true for other well known urogenic carcinogens as well. The reported cases in the literature of carcinoma growth in the intestine after urinary diversion and the results from our experiments make an intensive follow up of diverted patients necessary. Because our results show that urinary stasis is an important additional factor in carcinogenesis the different types of diversion have to be considered with respect to "Verweilzeit" of urine. With the increasing number of pouches the oncological aspects particulary in those patients with life expectancy of 20 years or more should be considered more seriously when planing urinary diversion.

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