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Mechanisms of action of intravesical treatment

Effect on the ABH surface antigens of urothelial cells

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Summary. *Blood group isoantigens can be demonstrated immune-histologically on normal urothelium of the urinary bladder by means of the specific red cell adherence test. In preneoplastic and reactive changes of the urothelium and in dysplasia and carcinoma in situ, there is a loss of these antigens. Ultrastructural investigations of carcinoma in situ demonstrate a loss of normal membrane structures such as tight junctions, desmosomes, and glycocalyx. Of 18 patients with carcinoma in situ of the urinary bladder, 12 achieved tumor remission after topical chemotherapy with doxorubicin hydrochloride. A recurrence of blood group isoantigens was demonstrated in patients who achieved tumor remission. The antigenicity reappears before complete normalization of the urothelium. Ultrastructural investigations demonstrated normalization of cellular membrane structures in the respective biopsies. According to these findings and the current literature, the mechanisms of action of doxorubicin hydrochloride in carcinoma in situ may be due to retardation of cell cycle, cell loss by desquamation, cell death, and decrease of growth fraction.*

Introduction

Intravesical chemotherapy is attracting increasing interest for the management of superficial, transitional cell carcinoma of the urinary bladder. Several drugs are now available and have proved to be effective. One of these is doxorubicin hydrochloride, which has been used for therapeutic and prophylactic purposes with high efficacy [8, 19]. Although there are several reports dealing with clinical data there are few dealing with the effects of doxorubicin on normal urothelium and on transitional cell carcinoma [31]. The objective of this study is to evaluate the effects of this drug on membrane structures occurring as a consequence of a rigid treatment protocol for carcinoma in situ of the urinary bladder. Therefore we investigated the cell membranes by transmission electron microscopy and immune-histological demonstration of blood group isoantigens.

A, B, and H blood group isoantigens (BGA) are located on the surfaces of many different tissues throughout the body. Since the reports of Davidsohn, Gupta et al., and Bonfiglio and Feinberg it has been known that malignant cells of the cervix, lung, prostate, etc. lose their BGA with increasing cell de-differentiation [6, 10, 14]. Decenzo et al. and other groups have demonstrated that patients with transitional cell carcinoma of the bladder with BGA have a different disease course from those without [4, 11, 17, 22].

In a previous report we were able to demonstrate the loss of BGA in patients with carcinoma in situ (TIS) [17]. Weinstein and co-workers confirmed our findings in cystectomy specimens removed because of extensive TIS [29].

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Clinical data

a Carcinoma in situ. During the period February 1978 to September 1979 every patient with histologically proven TIS without concomitant papillary tumor diagnosed in our Department was treated with topical doxorubicin hydrochloride instillations. Dosages of 40 mg doxorubicin hydrochloride diluted in 20 ml saline (9 patients, group A) or 80 mg doxorubicin hydrochloride in 40 ml saline (9 patients, group B) were instilled into the bladder by way of a 12 F catheter bi-weekly and monthly, respectively. This treatment schedule was planned to extend over 1 year, with prolonged intervals of 1 and 2 months, respectively, thereafter. The treatment was terminated after 24 months. Tumor remission was defined by a negative cytology report and lack of carcinoma in situ or severe dysplasia in any biopsy.

b Random mucosal biopsies. In a prospective study starting in February 1978 and including 143 patients suffering from superficial bladder cancer (pTa, pTl, according to UICC), transurethral resection of the overt tumor was carried out and at the same time two to four biopsies were obtained from bladder areas not involved by the tumor.

Methods

a) Histology. One half of the specimens obtained by cold cup biopsies were fixed in 10% buffered formalin and embedded for routine histology and for SRCA testing. The other half were fixed in 4% glutaraldehyde and embedded for transmission electron microscopy (TEM). Light microscopy grading, including semithin sections, was done according to the following principles. In urothelial dysplasia, the architectural pattern of the urothelium is preserved but the cells are altered from mild over moderate to severe atypia (grade 1–3). In TIS the architectural pattern was destroyed and the cellular atypia was graded analogously to the grading of papillary urothelial tumors using cytological criteria according to Bergkvist et al. [3].

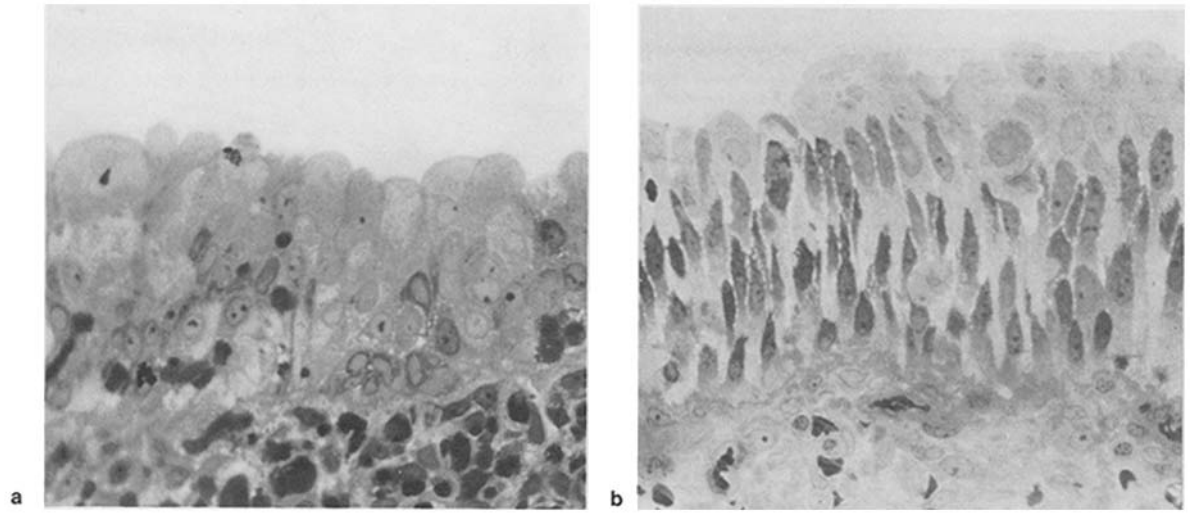

Fig. 1. a Light microscopy shows grade III carcinoma in situ with severe nuclear pleomorphism, decreased cellular adhesiveness, and no discernible histostructural pattern. Semithin section, × 720 **b** Light microscopy after treatment shows grade II (moderate) dysplasia and reappearance of flat superficial umbrella cells. Typical urothelial layering. Semithin section, × 720

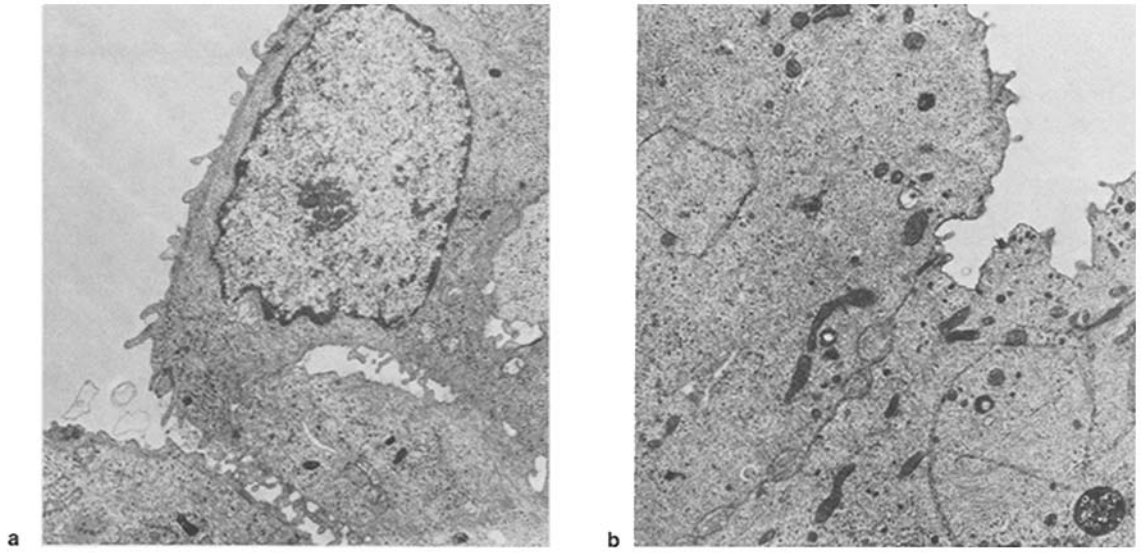

Fig. 2. a Carcinoma in situ with atypical superficial cell. Note numerous microvilli and absence of both tight junction and desmosome-like connections. Ultrathin section, × 20,000 **b** Electron micrograph after treatment shows reappearance of cellular connections, i.e., desmosomes and tight junctions. Ultrathin section, × 20,000

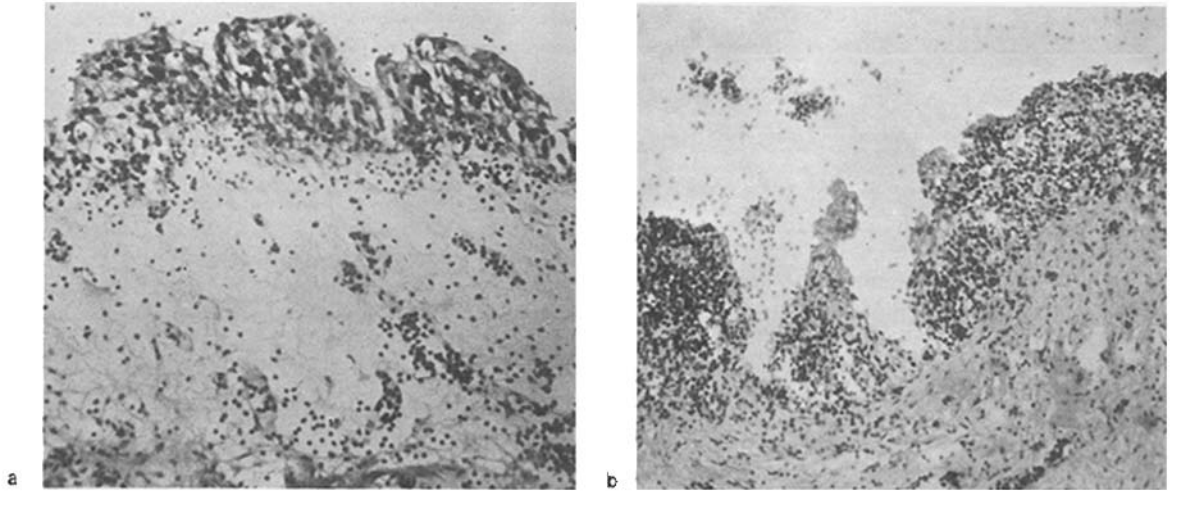

Fig. 3. a Carcinoma in situ. Typical appearance with lost histostructural pattern and severe pleomorphism. No erythrocytes adhering to the urothelial cells, meaning a negative SRCA test result. H.E., × 120. **b** Mild urothelial dysplasia with typical urothelial layering. Note adhering erythrocytes. Positive SRCA test result. H.E., × 120

b) SRCA test. BGA were identified by human anti-blood group antisera and by anti-phytoagglutinin. The reactions were visualized by indicator erythrocytes of the appropriate blood group in a second layer. After removal of paraffin, rehydration, and rinsing in isotonic Tris-HCl buffer the tissue sections were covered with a 2% red blood cell (RBC) suspension in a moist chamber. After a 20-min incubation, the slides were placed on applicator stick supports so that the tissue came in contact with the buffer. The slides were allowed to stand for 10 min to allow the RBC that were not specifically attached to fall to the bottom of the dish. Afterwards the buffer was removed by means of a water jet pump and replaced by 6% phosphate-buffered glutaraldehyde. After 30 min fixation the slides were stained in the same dish with hematoxylin eosin, dehydrated, and mounted with eukitt.

Results

Clinical data

Tumor remission was achieved in 12 of 18 patients. There were six remissions in each group (Table 1). There were no differences between the two therapy regimens with regard to the remissions achieved.

Light microscopy

The light microscopic evaluation before treatment showed the typical signs of TIS in all specimens, with nuclear atypia, polymorphism, destroyed architecture and irregular height of the urothelium, prominent basal capillaries, and varying degrees of edema and inflammation of the stroma (Fig. 1a). Almost uniformly, we observed normal layering of the urothelium, with regular intermediate and superficial cells as the first sign of tumor remission (Fig. 1b). However, nuclear atypia, varying in degree, was present and improved only slowly during subsequent treatment. Squamous metaplasia was seen in the specimens of only two patients. Stromal fibrosis was present in the biopsy specimens of all patients treated for 24 months. The multiple biopsies of seven patients revealed normal urothelium at termination of treatment. The others still had evidence of some dysplastic urothelial alterations. The normalization of the urothelium proceeded in stages; areas of carcinoma in situ were first replaced by moderate to severe dysplasia.

Transmission electron microscopy

Before treatment the lost architectural pattern of the urothelium could be clearly shown. These were an overwhelming number of basal cells and in some cases many irregular cells similar to the intermediate cell type. Well-developed superficial cells were never observed. Cellular cohesion was lost due to missing tight junctions and desmosomes (Fig. 2a). The basal lamina was often thickened and folded. There were proliferating vessels under the basal lamina and between the infoldings of the thickened basal lamina. The stroma was often edematous and infiltrated by lymphocytes and plasma cells. After treatment the architecture of the urothelium was restored, as was seen in the semithin preparations. Tight junctions and desmosomes were present (Fig. 2b). The other signs of TIS remained almost unchanged, as reported above.

Table 1. Duration of tumor remissions achieved in the different treatment groups

Group	Time (months)			Total[a]
	3	6	9	
A	3	2	1	6 (9)
B	4	1	1	6 (9)
Total	7	3	2	12 (18)

[a] Numbers in parentheses indicate the total number of patients treated in each case

Table 2. Comparison of the histologically observed alterations in the bladder mucosal biopsies and the SRCA test result

Biopsy	SRCA			
	Positive		Negative	
	n	%	*n*	%
Histology				
Normal (n = 267)	215	80	52	20
Inflammation (n = 19)	11	58	8	42
Hyperplasia (n = 27)	18	67	9	33
Dysplasia (n = 112)	50	45	62	55
Carcinoma in situ (n = 26)	3	12	23	88

SRCA test

a) Carcinoma in situ. The sequential multiple cold cup biopsies demonstrated no carcinoma in situ 12 of 18 patients after 3–9 months. But there was till evidence of mild to moderate dysplasia. All specimens histologically interpreted as carcinoma in situ were ABH antigen-negative (Fig. 3a). Of the biopsies diagnosed as dysplasia of mild, moderate, or severe type, 92% revealed ABH antigenicity after topical treatment (Fig. 3b).

b) Random mucosal biopsies. There is a decrease of BGA content from normal urothelium to TIS (Table 2). Surprisingly, there was also a loss of BGA in a high percentage of specimens showing only inflammatory alterations of the urothelium. Carcinoma in situ revealed ABH antigenicity only in three biopsies. ABH antigens could not be demonstrated in any of the severe-type dysplasias.

Discussion

Doxorubicin hydrochloride is a potent antineoplastic agent, which has proved to be effective in the treatment of various human tumors [8]. It is assumed that its success in retarding the division of malignant cells is due to its ability (a) to intercalate between parallel stacked bases of DNA, thus resulting in malformation of the double helix being responsible for the subsequent inhibition of DNA and RNA synthesis; (b) to cause DNA strand breakage and therefore considerable chromosomal damage; (c) to alter the integrity and function of cell membranes; (d) to interact with beta-adrenergic compounds; and (e) to produce highly reactive superoxide anions

[5, 12, 20, 21, 23, 24, 27, 28]. Among the above-mentioned mechanisms of action it is now known that cell surface phenomena are at least partially responsible for the growth-inhibitory properties. There are data reported by Kessel and co-workers indicating changes of membrane fluidity resulting in an increase of electronegativity, decrease in hydrophobility, etc. [20, 21]. Murphree et al. demonstrated that Adriamycin induces increased Con A-mediated agglutination of sarcoma ascites cells [24]. Carlson et al. reported incrreased colchicine uptake after exposure of cells to Adriamycin [7].

A single dose of doxorubicin hydrochloride causes nucleolar segregation, vacuolation of the cytoplasm, mitochondrial abnormalities, and the breakdown of secondary lysosomes leading to autolysis of normal urothelial cells as well as transitional cell carcinoma [31]. The cytoplasmic membrane on the surface of these cells may become distended into small blebs, indicating a deficiency in the cytoplasmic membrane system [31].

BGA are constituents of the normal urothelial cell. The biochemical basis of this phenomenon has not been well known until now [30]. In particular, no information is available as to whether these antigens are exactly the same substances as the blood group antigens located on the surface of erythrocytes. Dabelsteen and MacKenzie showed that there are two types of BGA: glycoprotein-associated BGA and alcohol-soluble lipoprotein-bound ones [9]. The depletion of these two subtypes of BGA seems to be different in various epithelial changes: for example, there is a loss of lipid-bound BGA in regenerating oral epithelium, whereas the glycoprotein-associated BGA are preserved.

The loss of BGA at the surfaces of the malignant urothelial cells seems to be a diminution of other cellular functions and membrane antigens in malignant transformation [1, 13]. There are various possible explanations for the deletion of BGA: the true loss of the respective antigens, perhaps caused by blocking of the enzymes engaged in the synthesis of the respective glycolipids and glycoproteins [15]; chemical or immune-chemical antigen modulation; ultrastructural changes of the urothelial surface leading to topical displacement of the antigenic sites; or replacement of the BGA by new (tumor) antigens. BGA loss seems to be a very early event in tumorigenesis; urothelium with a normal appearance in the light microscope shows deletion of BGA in cases where ABH antigen-negative transitional cell carcinomas are present simultaneously [18, 30].

In the study presented here we saw a recurrence of BGA demonstrated by means of the SRCA test in patients with TIS who experienced tumor remission. Surprisingly, the antigenicity reappeared before the urothelium became totally normal. This finding is in contrast to the above-mentioned lack of BGA in dysplasia (55% ABH-negative). A similar observation was made by Alroy and co-workers in specimens from previously irradiated invasive bladder tumors [2]. These authors interpreted their findings by suggesting that an enhancement of the Golgi apparatus (being the site of BGA synthesis) by radiation therapy is responsible for this phenomenon.

The action of adriamycin in vivo is not well understood, since there are data indicating that there is inhibition of RNA and DNA synthesis without inhibition of DNA polymerase. It is also suggested that tumor cell kill may be size-dependent. There is now also evidence from experiments performed by Rowley and associates that Adriamycin may cause an appreciable delay in tumor growth without detectable cell kill [25]. This growth delay may be caused by a cell cycle delay, reduction of growth fraction, increase in cell loss, and/or alteration of the relationship between tumor and host.

Considering the available literature and our own data demonstrating the occurrence of ABH antigens in dysplasia and the normalization of the surface membrane, we can describe the mechanism of action of adriamycin in carcinoma in situ in a very simplified and speculative manner as follows: The intravesical instillation of adriamycin causes a considerable cell loss as a result of desquamation of the superficial urothelial cells due to disintegration following the rupture of the cytoplasmic membrane [31]. Furthermore, the retardation of the cell cycle with the effects on the cell membrane will result in restoration of the cell membrane structures such as tight junctions, desmosomes, and ABH antigens, whereas nuclear atypia even of the severe type is still present. Cell death and decrease of growth fraction may have additional effects in restoration of normal urothelium. The question as to whether the topical chemotherapy with doxorubicin hydrochloride may result in a complete eradication of carcinoma in situ of the urinary bladder is still open to discussion. Long-term follow-up studies may provide us with sufficient data in this respect [19].

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