Urol. Res. 9, 153-156 (1981)

Urological Research

ABH Antigenicity of in situ Carcinoma of the Urinary Bladder During Intracavity Treatment with Doxorubicin Hydrochloride

G. Jakse and F. Hofstädter

Department of Urology and Institute of Pathology, University of Innsbruck, Innsbruck, Austria

Accepted: November 26, 1979

Summary. Ten patients with carcinoma in situ of the urinary bladder were treated topically with doxorubicin hydrochloride. Blood group antigens were evaluated by the specific red cell adherence test. Carcinoma in situ was associated with the loss of antigenicity, the reappearance of which could be demonstrated in patients with tumour remission. This occurred in spite of a persistent nuclear atypia.

Key words: Carcinoma in situ, Doxorubicin hydrochloride, SRCA test, ABH antigenicity.

INTRODUCTION

A, B and H blood group antigens (BGA) are located on the surface of many different tissues throughout the body. Since the reports of Davidsohn, Gupta et al. and Bonfiglio and Feinberg it is known that malignant cells of the cervix, lung, prostate etc. lose their BGA with increasing cell de-differentiation (4, 6, 8). Decenzo et al. and others demonstrated that patients with transitional cell carcinoma of the bladder with BGA have a different disease course compared to those without (3, 7, 9, 12).

In a previous report we were able to demonstrate the loss of BGA in patients with carcinoma in situ (Tis) (10). Weinstein and co-workers affirmed our findings on cystectomy specimens, which were removed because of extensive Tis (14).

Abbreviations: Carcinoma in situ = Tis (WHO, 1978), Blood group antigens = BGA, Doxorubicin hydrochloride = DOX, Transmission electron-microscopy = TEM, Transitional cell carcinoma = TCC

In a preliminary report we showed that topical treatment of Tis with doxorubicin hydrochloride (DOX) could be an alternative to cystectomy in some patients (11). In bladder specimens obtained at other times we observed a stepwise re-differentiation from Tis to moderate dysplasia. Because of this observed tumour remission we were interested in finding out whether besides the morphological cellular re-differentiation an immunological change also occurred.

MATERIAL AND METHODS

a. Clinical Data

Ten patients (6 men, 4 women) with Tis of the bladder were studied; their average age was 64.6 years. The symptoms were mainly dysuria. Four patients had been treated at least 6 months previously by transurethral resection for a superficial TCC of the bladder. The diagnosis of carcinoma in situ was made only on the cystoscopic appearance of the bladder mucosa - red patches, granular or oedematous areas without concomitant papillary tumour. If there was a single area and biopsies of the other sites were negative, we called it unifocal Tis. A multifocal lesion was diagnosed on cystoscopic appearance and/or histologically demonstrated Tis change. The follow-up was 3 to 18 months, mean 9.6 months (Table 1). In 7 patients 40 mg of DOX diluted in 20 ml saline were instilled into the bladder through a 12 Charrier catheter every 14 days. In 3 others the dosage was 80 mg/40 ml saline once a month. Cold cup biopsies were taken every 3 months on selected sites and/or cystoscopically suspicious areas with Storz forceps. The selected sites were located on each side of the ureteric orifices, the trigone and the dorsal and ventral walls.

0300-5623/81/0009/0153/$ 01.00

Table 1. Clinical presentation of 10 patients with carcinoma in situ of the urinary bladder

Name	Sex	Age	Symptoms	Histology	Growth	Cytology	Previous tumour
B.A.	m	68	dysuria haematuria	Tis III	multi-loc.	IV	T_1/IV
G.J.	m	71	haematuria	Tis III	uni-loc.	IV	T_1/III
J.A.	m	28	dysuria haematuria	Tis III	multi-loc.	V	-
K.Z.	f	54	microhaem. cytology	Tis II	multi-loc.	IV	T_1/III
M.I.	f	65	microhaem. cytology	Tis III	multi-loc.	IV	-
P.F.	m	52	dysuria	Tis II	uni-loc.	V	-
R.A.	m	39	haematuria	Tis III	uni-loc.	IV	-
R.I.	f	71	dysuria	Tis IV	multi-loc.	V	-
R.M.	f	76	haematuria	Tis III	uni-loc.	V	-
U.J.	m	69	microhaem. cytology	Tis III	multi-loc.	IV	T_1/II

b. Histology

One half of the specimens obtained by cold cup biopsies were fixed in 10% buffered formalin and embedded for routine histology. The other half were fixed in 4% glutaraldehyde and embedded for transmission electron microscopy (TEM).

c. SRCA-Test

BGA were identified by human anti-blood group antisera and by anti-phytoagglutinin. The reactions were visualised by indicator erythrocytes of the respective 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 the tissue entered into contact with the buffer. The slides were allowed to stand for 10 min so that the RBC which were not specifically attached could 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 haematoxylin eosin, dehydrated and mounted with eukitt.

d. Evaluation

The paraffin embedded, haematoxylin-eosin stained slides were evaluated according to the criteria of Bergkvist (2). Dysplasia was defined as an increased but intact layering of the urothelium with nuclear pleomorphism recorded as grade 1 to 3, corresponding to transitional cell carcinoma grades 2 to 4 according to Bergkvist et al. (2). The same grading was applied to Tis, where the pattern of the cellular arrangement was destroyed. The intensity of the SRCA-test reaction was graded on a 0 to +++ scale with 0 indicating no reactions, + strongly reduced reaction, ++ moderate and +++ marked. Reactions +++ and ++ were considered positive. The SRCA test was performed without knowledge of the blood group and the clinical course of the patient. Controls were a. normal tissue as a positive control, b. the positive reaction of the blood vessel endothelium in the case of an antigen negative tumour specimen and c. the failure of

Table 2. SRCA test results correlated with histological findings of the last specimens obtained from each patient

Name	Histology	SRCAtest
B.A.	Dys. II	+++
G.J.	Tis IV	-
J.A.	Dys. III	+++
K.Z.	normal	+++
M.I.	Dys. I	++
P.F.	Dys. II	++
R.A.	normal	+++
R.I.	T2/IV	-
R.M.	Dys. III	+++
U.J.	Dys. II	++

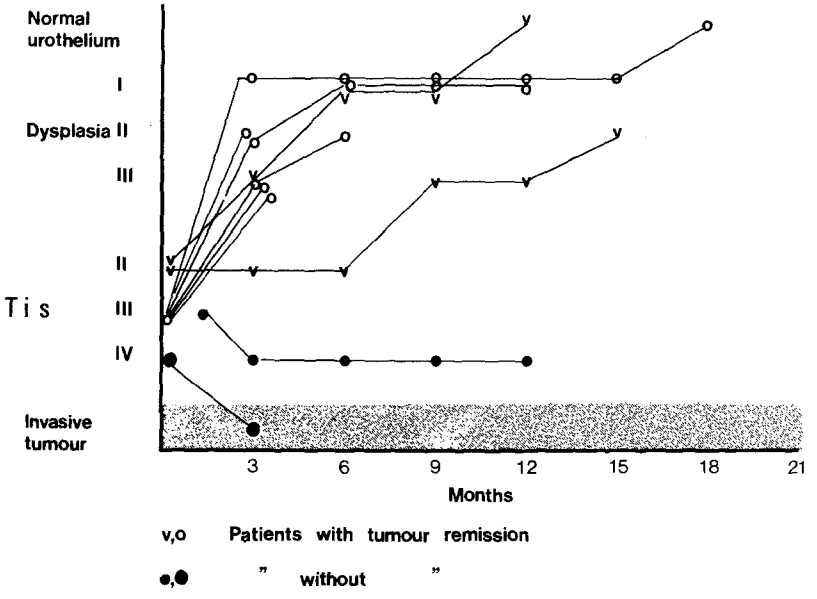

Fig. 1. Histological results of specimens obtained by cold cup forceps biopsies before and after varying times of doxorubicin hydrochloride treatment. In this table only the results of the worst biopsy are recorded

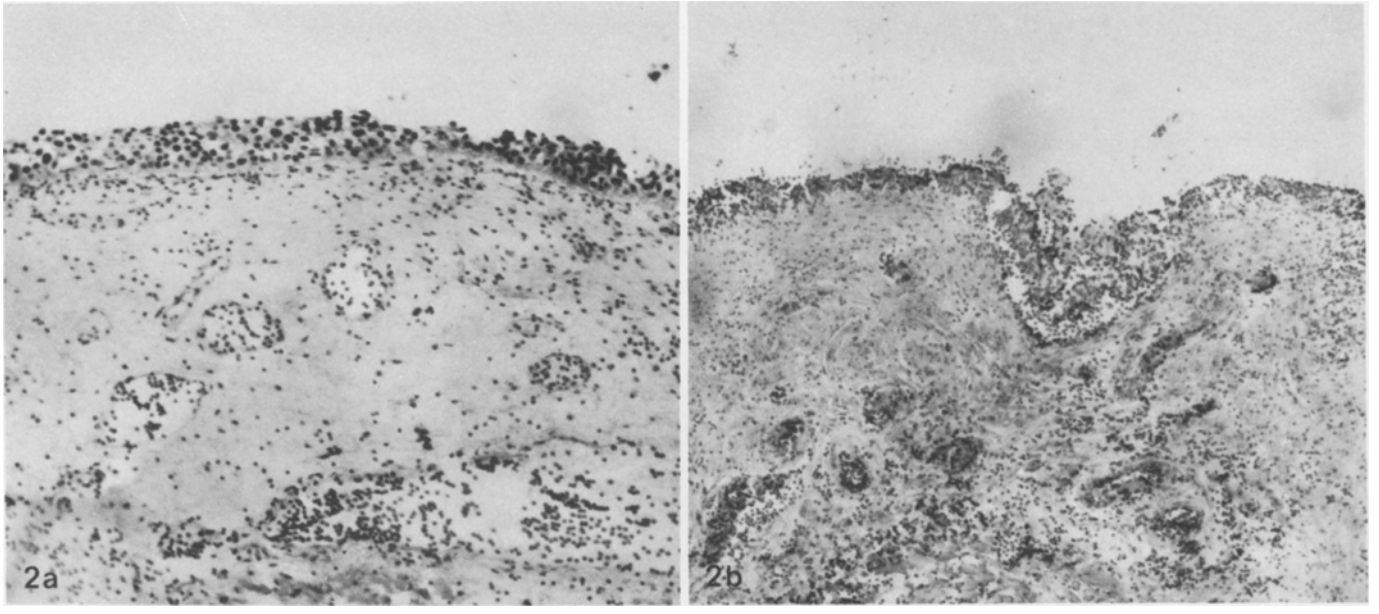

Fig. 2 a, b. a Carcinoma in situ, grade 3. SRCA Test. No BGA are demonstrable on the urothelium. Blood vessel endothelium shows reaction (HE x120); b Dysplasia, grade 1. SRCA Test. Marked reaction of the urothelium and of the blood vessel endothelium (HE x100)

tissue to react with non-related antiserum of indicator erythrocytes.

RESULTS

In 8 out of 10 patients biopsies revealed tumour remission, on the finding that no Tis was detected in any of the multiple biopsy specimens after treatment (Fig. 1). However, in 2 patients a marked dysplasia was observed. In spite of this, the SRCA-test demonstrated a recurrence of BGA in all patients with tumour remission (Table 2, Fig. 2). The electron microscopical findings after DOX treatment, which will be the subject of a separate report, were briefly: the reappearance of superficial cells exhibiting typical asymmetric unit membrane and tight junctions. These features were not detectable in Tis.

DISCUSSION

DOX is a potent antineoplastic agent which has proved to be effective in the treatment of various human tumours (5). It is assumed that its success in retarding the division of malignant cells is due to its ability to intercalate between the parallel stacked bases of DNA (13), which results in malformation of the double helix which is responsible for the subsequent inhibition of DNA and RNA synthesis.

In our study we saw recurrence of BGA on the cell surface demonstrated by means of the

SRCA test in patients who showed tumour remission. Our TEM specimens revealed luminal surfaces which were nearly intact after DOX treatment. Therefore, we assume that the retardation of the cell cycle caused by DOX enables the basal cells to mature into regular superficial cells. Surprisingly, antigenicity reappears before the urothelium becomes totally normal. That means that BGA can be demonstrated in dysplastic urothelium showing marked nuclear atypia. This is in contrast to our previous findings, that untreated dysplasias reveal the loss of BGA (10). A similar observation was made by Alroy et al. in specimens from previously irradiated invasive bladder tumours (1). These authors interpreted their findings by suggesting that there was an enhancement of the Golgi apparatus, which is also the site of BGA synthesis. They also supposed that the phenotype of the malignant cell was masked by radiation therapy, and therefore that the SRCA test result had no prognostic value in this group of patients.

Although the mode of action of DOX on urothelial cells in Tis is speculative, we do not think that the reappearance of BGA is evidence of masking of the tumour phenotype. However, it seems clear that a recurrence of ABH-antigenicity heralds a re-differentiation of malignant urothelial cells. This is confirmed by our electron microscopical findings, which revealed a reconstruction of the luminal surface after DOX treatment.

Whether or not the immunological re-differentiation demonstrated by the SRCA test can be used as a tumour marker in patients who are treated topically with DOX is unclear from our investigation. Only a longer period of observation and a larger group of patients could answer this question.

Acknowledgement. This investigation was supported by Fonds zur Förderung der wissenschaftlichen Forschung No. 3761.

REFERENCES

1. Alroy, J., Teramura, K., Miller, A.W., Pauli, B.U., Gottesman, J.E., Flanagan, M., Davidsohn, I., Weinstein, R.S.: Isoantigens A, B and H in urinary bladder carcinomas following radiotherapy. Cancer 41, 1739 (1978)
2. Bergkvist, A., Ljungquist, A., Moberger, G.: Classification of bladder tumours based on the cellular pattern. Preliminary report of a clinical pathological study of 300 cases with a minimum follow-up of eight years. Acta Chirurgica Scandinavica 130, 371 (1965)
3. Bergman, S., Javadpour, E.: Cell surface antigens A, B or O as indicator of malignant potential in stage A bladder carcinoma: A preliminary report. Journal of Urology 119, 49 (1978)
4. Bonfiglio, T.A., Feinberg, M.R.: Isoantigen loss in cervical neoplasia. Archives of Pathology 100, 307 (1976)
5. Carter, S.K.: Adriamycin. A review. Journal of the National Cancer Institute 55, 1265 (1975)
6. Davidsohn, I.: Early immunological diagnosis and prognosis of carcinoma . American Journal of Clinical Pathology 57, 715 (1972)
7. Decenzo, J.M., Howard, P., Irish, C.E.: Antigenic deletion and prognosis of patients with stage A transitional cell bladder carcinoma. Journal of Urology 114, 874 (1975)
8. Gupta, R.K., Schuster, R., Christian, W.D.: Loss of isoantigens A, B and H in prostate. American Journal of Pathology 70, 439 (1972)
9. Jakse, G., Hofstädter, F., Friessnig,F.: ABH-Antigene beim Blasentumor Stadium O und A. Aktuelle Urologie 9, 15(1978)
10. Jakse, G., Hofstädter, F.: Further experiences with the specific red cell adherence test in bladder cancer. European Urology 4, 356 (1978)
11. Jakse, G., Hofstädter, F.: Intravesical doxorubicin hydrochloride in the management of carcinoma in situ of the bladder. A preliminary report. European Urology (in press, 1980)
12. Lange, P.H., Limas, C., Fraley, E.E.: Tissue blood group antigens and prognostis in low stage transitional cell carcinoma of the bladder. Journal of Urology 119, 52 (1978)
13. Marco, A. di, Arcamone, F.: DNA complexing antibiotics: daunomycin, adriamycin and their derivatives. Arzneimittel-Forschung 25, 368 (1975)
14. Weinstein, R.S., Alroy, J., Farrow, G.M., Miller, A.W., Davidsohn, I.: Blood group isoantigen deletion in carcinoma in situ of the urinary bladder. Cancer 43, 661 (1979)

Dr. G. Jakse
Department of Urology
University of Innsbruck
Anichstraße 35
A-6020 Innsbruck
Austria

Responsible for the text: Professor G. D. Chisholm, Western General Hospital, Edinburgh
Responsible for advertisements: L. Siegel, Kurfürstendamm 237, D-1000 Berlin 15 Springer-Verlag Berlin Heidelberg New York
Printed in Germany by Julius Beltz oHG, D-6944 Hemsbach.