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Mitomycin-C Induced Improvement in Superficial Bladder Tumors: A Morphologic and Immunohistochemical Evaluation*

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Summary. Twenty bladder biopsies from ten patients with superficial transitional cell carcinoma were obtained prior to and following therapy with intravesical mitomycin-C. Each biopsy was evaluated histologically and immunohistochemically for A, B, H blood group antigen (BGA) expression. A, B and H blood group antigens were identified using monoclonal anti-A, B and H antibody and were localized with the avidin-biotin-peroxidase complex (ABC) technique. Particular attention was directed toward flat urothelial lesions and the urothelium adjacent to papillary tumors. Routine histologic evaluation showed improvement in post-therapy biopsies compared to corresponding pre-therapy biopsies in 7/10 cases but was equivocal in 3/10 cases. Immunohistochemical evaluation, however, showed improvement in all 10 cases, as judged by increased urothelial BGA expression. This increase in urothelial BGA expression after intravesical mitomycin-C therapy suggests a therapy induced improvement in dysplastic urothelium which was not uniformly evident on routine histologic examination.

Key words: Blood Group Antigens (BGA), Mitomycin-C, Intravesical chemotherapy.

Introduction

Intravesical chemotherapy is currently being evaluated as a treatment for superficial bladder tumors in many centers. Among agents being tested, mitomycin-C is one which shows considerable promise [7, 8]. Although clinical improvement appears to be present in many of the patients receiving this therapy, histologic evidence of improvement in dysplastic urothelium is often difficult to evaluate in routine hematoxylin and eosin stained specimens. We have, therefore, evaluated pre and post-therapy biopsies for evidence of therapeutic response evidenced by improved urothelial BGA expression. Our results show a uniform improvement in the ability of urothelium to express BGA in mitomycin-C treated patients. This improvement was often not evident in routine histological evaluation. We suggest that evaluation of BGA expression in bladder biopsies following intravesical chemotherapy may be a valuable adjunct to routine histologic examination.

Methods and Materials

Ten patients with recurrent superficial bladder tumors were treated with intravesical installation of mitomycin-C. Multiple pretherapy biopsies were obtained from the bladder trigone, dome, right wall, left wall, anterior wall, posterior wall, and from any visible lesions, and the bladder was mapped. Following this, the patients received intravesical instillation of 40 mg of mitomycin-C once a week for a period of eight weeks, after which they had an eight week "recovery period" before undergoing post-therapy biopsy. Post-therapy biopsies were obtained from the same areas biopsied prior to therapy.

All of the biopsies were fixed in 10% buffered formalin, paraffin embedded and stained with hematoxylin and eosin for routine histological evaluation. Pre-therapy biopsies demonstrating the highest grade papillary tumors and the most dysplastic adjacent flat urothelium were selected for immunohistochemical evaluation in each case. Blocks of corresponding post-therapy bladder biopsies were also studied. Serial sections from these specimens were stained with monoclonal anti-A, anti-B, and anti-H blood group antibodies using the ABC immunoperoxidase technique, slightly modified from that described by Hsu et al. [2]. The procedure used is described briefly as follows: Tissue sections were deparaffinized and trypsinized in 0.4% trypsin in 0.4% $CaCl_2$. Endogenous peroxidase activity was blocked using absolute methanol-H_2O_2 solution. Nonspecific binding to the antigen was blocked using a 1:10 dilution of normal horse serum (GIBCO, Grand Island, NY) in Tris-Hcl saline. Murine monoclonal anti-A, B and H antibodies (Chembiomed, Ltd., Edmonton, Alberta, Canada) were applied to separate serial sections in each case. The sections were rinsed and biotinylated horse antimouse IgG (Vectastain ABC Kit, Vector Labs, Burlingame, CA) was applied. Avidin-biotin-peroxidase complex (Vector Labs) was then applied, rinsed and the site of the peroxidase localization was identified using 3-amino-9 ethyl carbazole (Sigma Chemical Co., St. Louis, MO). The

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Table 1. Correlation of histologic grade and blood group antigen expression[a] in the pre-therapy and post-therapy biopsy of each patient

	Histological grade	Patient #1		Patient #2		Patient #3		Patient #4		Patient #5		Patient #6		Patient #7		Patient #8		Patient #9		Patient #10	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Papillary lesions	Grade I Transitional cell carcinoma		dif	dif[b]			dif							dif[b]		dif	dif				
	Grade II Transitional cell carcinoma	foc				foc						rare[b]						foc[b]		foc[b]	
	Grade III Transitional cell carcinoma							neg[b]		neg[b]											
Flat lesions	Normal				dif										dif						
	Mild/moderate dysplasia	foc	dif	foc		foc	dif		dif			rare	foc	foc			dif		dif		dif
	Severe dysplasia							foc			foc							foc		rare	
	Carcinoma in situ									rare						foc					

[a] As assessed by the immunohistochemical staining pattern: *rare:* 1–10% cells staining positive; *focal (foc):* 10–50% cells staining positive; *diffuse (dif):* >50% cells staining positive; *negative (neg):* No positive cells

[b] No residual papillary tumor present after therapy

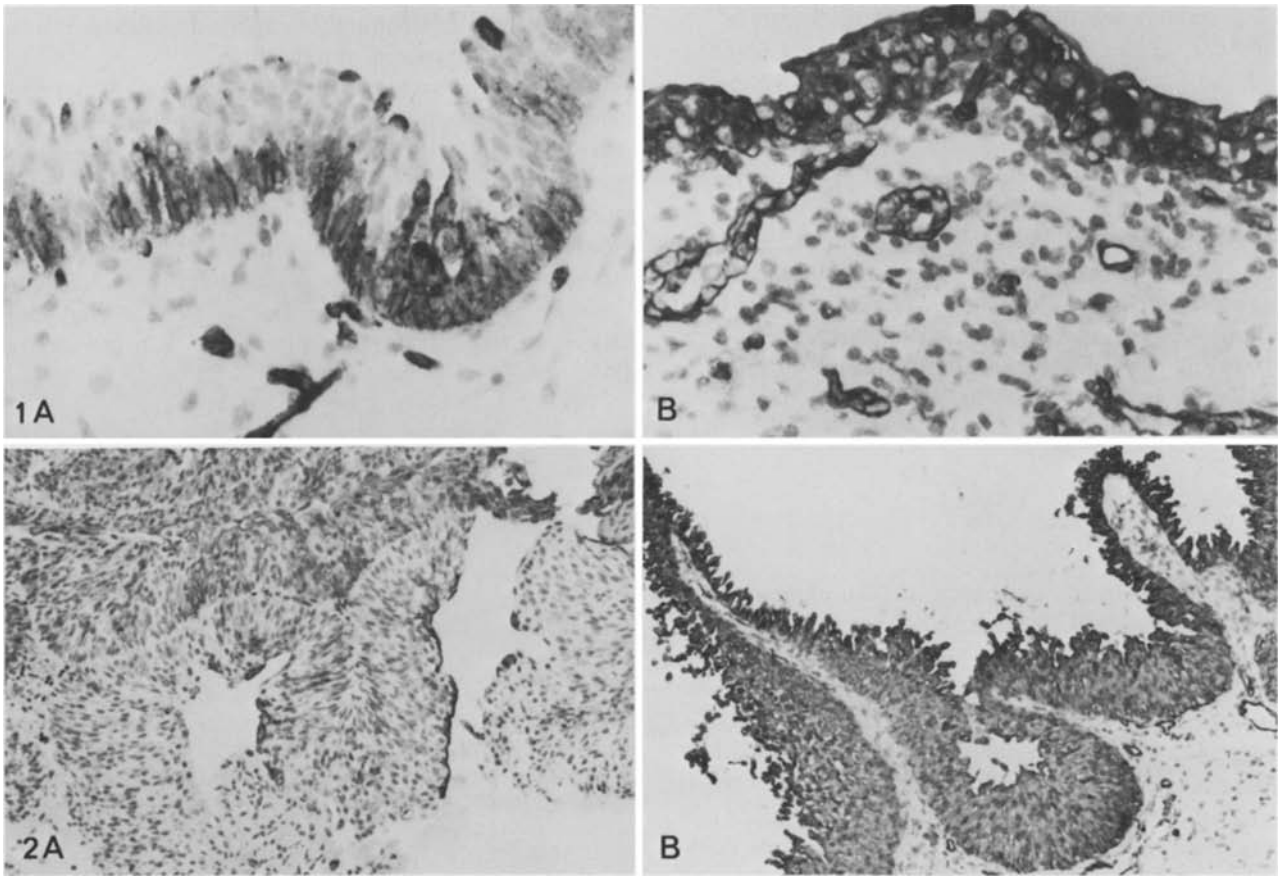

Fig. 1A, B: Focal blood group antigen expression (dark staining cells) present in the flat urothelium prior to intravesical mitomycin-C therapy **(A).** Diffuse blood group antigen expression in the flat urothelium after therapy **(B).** Stromal blood vessels serve as positive controls (52x)

Figure 2A, B: Focal blood group antigen expression in a papillary transitional cell carcinoma prior to intravesical mitomycin-C therapy **(A).** Diffuse blood group antigen expression in a recurrent papillary transitional cell carcinoma after therapy **(B)** (20x)

slides were then washed in tap water, counterstained with Mayer's hematoxylin and coverslipped for reading.

Histological grading of the papillary tumors was performed using criteria proposed by Koss [3], and flat lesions using criteria proposed by Murphy and Soloway [4, 5]. The immunohistochemically stained slides were evaluated at 40X magnification using the following criteria: Negative-no staining, rare positivity-1–10% cells positive, focal positivity-10–50% cells, and diffuse positivity-50% or greater cells positive. Stromal blood vessels served as positive controls, properly identifying the patients blood group in each case. The two nonstaining blood group slides on each biopsy served as negative controls.

All biopsies were evaluated by two independent observers in a blind fashion without knowledge of patient identification or treatment status.

Results

The results of the histological and immunohistochemical evaluation of the biopsies are summarized in Table 1.

Histological Evaluation

Pre-Therapy. Three grade I, five grade II and two grade III papillary transitional cell carcinomas were present in the pretherapy biopsies. Flat urothelium adjacent to these tumors showed mild/moderate dysplasia in five cases, severe dyplasia in three cases, and carcinoma-in-situ in two cases.

Post-Therapy. Three grade I papillary transitional cell carcinomas were present in the post-therapy biopsies. No grade II or grade III papillary carcinomas were present. The flat urothelium in the biopsies was normal in two cases, displayed mild/moderate dysplasia in seven cases, and severe dysplasia in one case.

Immunohistochemical Evaluation

Pre-Therapy. All of the papillary grade I transitional cell carcinomas displayed diffuse staining for BGA expression. In the grade II papillary carcinomas, focal aggregates of cells stained positively in four cases, and rare cells in one

case. No staining positivity was present in the grade III papillary carcinomas. The flat urothelium in these biopsies showed focal staining in seven cases and rare staining in three cases.

Post-Therapy. The three recurrent grade I papillary transitional cell carcinomas stained diffusely. The flat urothelium showed diffuse staining in eight cases, and focal staining in the remaining two. Increased staining was evident in all of the flat urothelium (Fig. 1) and in two of the three papillary carcinomas (Fig. 2) when compared to the corresponding pre-therapy biopsies. The third recurrent papillary carcinoma showed diffuse positive staining before and after therapy.

Discussion

There has been extensive evaluation of BGA expression in neoplastic urothelium as a parameter for predicting aggressive biological behavior [1, 6, 9–11]. In general, it has been shown that BGA expression is inversely related to invasive potential. The evaluation of BGA expression as an indicator or tumor response to chemotherapy is an area which has received relatively little attention. With the availability of highly specific monoclonal antibodies developed to the ABO antigens, highly reproducible results in evaluation of BGA expression are possible [12]. We have compared BGA expression detected with monoclonal antibodies to routine histologic evaluation in bladder biopsies from patients treated with intravesical mitomycin-C.

We found that BGA expression by the papillary transitional cell carcinomas in pretherapy biopsies was inversely related to the grade of the tumor. That is, grade I tumors showed diffuse BGA staining, grade II tumors showed focal staining, and grade III tumors were negative for BGA expression. Therefore, there was good correlation between the histologic grade of the papillary tumors and the degree of BGA expression. Following therapy with intravesical mitomycin-C, only 3/10 cases showed evidence of recurrent papillary transitional cell carcinomas. In one case, both the pre and post therapy tumors were grade I with diffusely positive BGA expression. The remaining two cases demonstrated improvement in tumor grade and BGA expression (see Table 1).

The histological grading of dysplasia in the flat urothelial lesions was sometimes more difficult than the grading of papillary lesions, particularly in post therapy biopsies where artifacts induced by chemotherapy and inflammation occurred. Following intravesical mitomycin-C therapy, the flat urothelium showed histological evidence of improvement in 7/10 cases, but was equivocal in 3/10. However, improved BGA expression was present in all cases (10/10). Evaluation of BGA expression by immunohistochemical methods was less ambiguous and more easily accomplished. We believe this demonstrates that immunohistochemical staining for BGA expression may be a more sensitive indicator of therapeutic response than routine histological evaluation following intravesical mitomycin-C.

In conclusion, we have evaluated papillary and flat urothelial lesions histologically and immunohistochemically in patients treated with intravesical mitomycin-C. Immunohistochemical evaluation of BGA expression showed uniform improvement in post therapy biopsies, while histological evidence of improvement was at times equivocal. This suggests that immunohistochemical evaluation of BGA expression may be a useful additional parameter for evaluating therapeutic response in patients treated with intravesical mitomycin-C.

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