

Bladder Carcinogenesis Using Bilharzia-Infested Swiss Albino Mice

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Abstract—Experimental studies were carried out on female Swiss albino mice to evaluate the possible role of: liver injury, urine inborn carcinogens and local irritation of bladder mucosa in the development of bilharzial bladder cancer. In mice, the liver and intestine are only involved by parasite, but the urinary bladder is spared. Mice with induced liver injury due to either: bilharzial infestation alone, hepatocarcinogens alone (2-naphthylamine (2-NA) or 2-acetylaminofluorene (2-AAF)), or both together, did not develop bladder tumors. Also, mice receiving 1% indole (a tryptophan precursor) either alone, or after bilharzial infestation, did not show any change in bladder mucosa. Conversely, the insertion of glass beads in the bladder induced carcinomas in 26.6% of mice after 70 weeks. The induction of bladder tumors by glass beads was enhanced by 2-AAF treatment (40%), but not by bilharzia infestation (25%), 2-NA (27%) or indole (27%). The urinary bladders with glass beads were invariably the seat of bacterial infection (E-coli and gram + ve cocci) and showed epithelial hyperplasia and metaplasia.

INTRODUCTION

THE ETIOLOGIC relationship between chronic bilharzial cystitis and bladder cancer has been established. Since the original report of Ferguson [1], many valuable contributions have been added [2-5], but the exact mechanism of carcinogenesis is still unknown.

Different theories have been postulated including the mechanical effect of ova [1], miracidial toxins [2], associated chronic inflammation [3] and the presence of certain urinary carcinogenic agents such as tryptophan metabolites [4-7].

The aim of the present work is to evaluate the role of liver injury and mechanical irritation of bladder mucosa in the production of bladder cancer in an experimental animal model. Liver injury was induced either by bilharzial infestation and/or chemical carcinogens.

MATERIALS AND METHODS

Animal model and experimental groups

Mice were selected for this study, as in such animals, we get hepatic bilharzial infestation without bladder involvement, since the blood vessels of the mouse bladder are too

small to allow the parasite to reach the bladder. The possible role of mechanical irritation of the bladder by the passage of ova was imitated by surgical insertion of glass beads in the bladder cavity.

Female Swiss albino mice, 6-8 weeks old, about 20 g bred at Cairo Cancer Institute were used. The mice were randomly divided among the different groups (Table 1). Animals were maintained on a standard diet containing 20% protein and were given water *ad libitum*. In groups given oral chemical carcinogens, 1% 2-naphthylamine (2-NA) and 0.06% 2-acetylaminofluorene (2-AAF) were mixed with the diet separately. Indole, a tryptophan precursor, was mixed with the diet in a concentration of 1%.

The majority of mice were sacrificed after 70 weeks. However, in the groups receiving chemical carcinogens (2-NA and 2-AAF), the experiments were terminated after 30 weeks due to the development of severe toxicity. The experiment was started by 480 mice. A total of 130 animals were either sacrificed or found dead during the experiment, thus leaving 350 mice at the termination of study.

Bilharzial infestation

The mice were immersed for 1 hr in water containing cercaria of *S. mansoni*, 20-30 per ml. Six weeks later, the stools of mice were

Table 1. Frequency of bladder carcinomas in Swiss albino mice

Group	Period treatment (WK)	Number of mice	Tumors	
			Number	Percent
i. Normal control	70	45	0	0
ii. Liver injury:				
(a) Bilharzia	70	46	0	0
(b) 2-Naphthylamine (2-NA)	30	20	0	0
(c) 2-Acetylaminofluorene (2-AAF)	30	20	0	0
(d) Bilharzia + 2-NA	30	17	0	0
(e) Bilharzia + 2-AAF	30	22	0	0
III. Local bladder irritation: (glass beads)	70	30	8	26.6
IV. Bladder beads+liver injury:				
(a) Beads + bilharzia	70	20	5	25.0
(b) Beads + 2-NA	30	18	5	27.0
(c) Beads + 2-AAF	30	25	10	40.0
V. Tryptophan precursor:				
(a) Indole	70	25	0	0
(b) Indole + bilharzia	70	20	0	0
(c) Indole + beads	70	22	6	27.7
(d) Indole + beads + bilharzia	70	20	5	25.0

examined for the presence of ova to confirm the infestation. Only mice with positive ova in the stool were selected for the experiments.

Insertion of glass bead in the bladder

Under intraperitoneal nembutal anaesthesia [8], a spherical glass bead with rough surface, about 7–9 mg, was inserted into the lumen of the bladder following the technique of Allen *et al.* [9].

Pathologic techniques

At autopsy, the left lobe of liver and urinary bladder were excised, then fixed overnight in 10% neutral formalin in saline. The fixed bladder was transected horizontally at 3 different planes. Tissues were processed by routine paraffin embedding and hematoxylin and eosin staining.

RESULTS

Pathology of liver

All the mice infested with *Schistosoma* showed bilharzial granulomata in the portal areas, but the normal liver cytology and lobular pattern were preserved. Around the bilharzia ova, histiocytes, fibroblasts lymphocytes and eosinophils were arranged in a concentric pattern. A black granular bilharzial pigment was also observed in the granulomatous areas and bilharzia worms were at times seen in portal veins. Bile ducts showed moderate to marked hyperplasia. The liver of mice treated with 2-naphthylamine (2-NA) or 2-acetyl-

aminofluorene (2-AAF) showed mild fatty degeneration and cellular pleomorphism with hyperchromatic nuclei. However, no hepatomas were observed.

Pathology of urinary bladder

In the normal bladder, the transitional epithelium was 2–4 layers in thickness, with a surface layer of large cells. No epithelial changes were observed in mice infested with bilharzia or fed 2-NA or indole. A mild patchy epithelial hyperplasia was encountered in mice fed 2-AAF. Conversely, the bladders with glass beads showed marked transitional cell hyperplasia, both of surface epithelium and cell nests of Brunns. In addition, squamous metaplasia and columnar metaplasia were commonly observed. An inflammatory reaction was always present in the subepithelial stroma (lamina propria). Bacteriologic studies on urine revealed evidence of *E-coli* and gram positive cocci.

Malignant tumors of the urinary bladder only developed in the groups with glass beads (Table 1). Enhancement of tumor development occurred in mice fed 2-AAF, but not with other treatments (2-NA, indole or bilharzial infestation). All the tumors were deeply infiltrative. Of the 39 induced tumors, 21 were transitional carcinomas, 8 were transitional in type with focal squamous differentiation, and 10 were anaplastic with areas of spindle cells or sarcomatoid pattern. In spite of the advanced stage, and high grade of the majority of tumors, no distant metastases were observed.

DISCUSSION

Impairment of liver function in bilharzial patients, with subsequent disturbance of tryptophan metabolism, was considered a possible factor in bladder carcinogenesis [4]. However, hepatic bilharziasis affects mainly mesenchyme and spares liver cells, hence, very little change in liver function is observed except in very advanced cases [10-12]. In the present study, liver injury due to either: bilharzial infestation, hepatocarcinogens, or both together, failed to induce bladder tumors or atypical urothelial changes. Feeding indole (tryptophan precursor), also failed to produce changes in bladder mucosa.

In the present report, bladder tumors only developed in the groups of mice with intravesical glass beads. The induction of carcinomas was enhanced by treatment with 2-acetylaminofluorene, but not enhanced by bilharzial infestation, 2-naphthylamine or indole. These findings suggest that local factors are more important than systemic factors for bladder carcinogenesis in the present model.

The various hyperplastic, metaplastic and neoplastic changes observed in the bladders with glass beads are similar to those previously reported by Roe [13]. A comparable spectrum of lesions was also reported in the urinary bladder of bilharzial patients [14]. However,

in human, squamous metaplasia and squamous carcinoma that follow bilharzial cystitis tended to show more abundant keratinization than those observed in mice.

Two factors are possibly operable in bladder carcinogenesis following glass bead implantation, namely: (a) chronic mechanical irritation and/or (b) the associated bacterial infection. The bacterial types identified (*E. coli* and *cocci*) were known to be capable of synthesizing nitrosamines from nitrate and secondary amines [15, 16]. These nitrosamines proved to be potent carcinogens in different laboratory animals [17].

Nitrate is a normal dietary component, being present in large amount especially in agricultural areas in Egypt, where nitrate is commonly used as a fertilizer. Secondary amines are also produced by intestinal flora and excreted in urine. Therefore, the environmental conditions of the Egyptian farmers, who are heavily infested with bilharziasis, associated with bacterial cystitis and drinking water with high nitrate content, should raise a great possibility of *in situ* formation of different carcinogenic nitrosamines in the bladder.

At present, further studies are carried out at the Cairo Cancer Institute to evaluate the possible factors involved in the induction of bladder cancer in bilharzial patients.

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