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Influence of interferon-α2b (IFN-α2b) on the prevention of locally advanced bladder carcinoma in mice

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Abstract In the model of chemically induced bladder carcinoma in mice following application of betahydroxylbutylnitrosamine (BBN) we could not detect a therapeutic influence of systemic treatment with intraperitoneal injection of interferon-α2b ($10 \times 3 \times 10^5$ IU IFN-α2b) on the rate of locally advanced bladder carcinoma (IFN-α2b 37% versus control 41%) nor on the development of tumor precursors (severe dysplasia 100% in both groups).

Key words Bladder carcinoma · Chemical carcinogenesis · Interferon-α2b · Tumor progression · Advanced carcinoma

Introduction

The efficacy of an immunotherapy with intravesical interferon-α in patients with superficial bladder carcinoma is well proven. A complete remission of marker lesions was achieved in 4 of 16 and 4 of 10 patients, respectively [1, 10, 18]. Similar results were reported for the treatment of carcinoma in situ [4]. An influence of IFN-α2b on advanced bladder carcinoma was demonstrated for topical as well as systemic application [6, 12]. We evaluated whether systemic application of INF-α2b influences tumor progression after chemical induction by betahydroxylbutylnitrosamine (BBN).

Materials and methods

Principles of laboratory animal care of the NIH were followed. Female mice of the strain B6D2F1 with a body weight of 25 g and a median age of 11 weeks were selected. The animals were allocated at random with a light–dark cycle of 12 hours. Over a period of 10 weeks 0.05% BBN was added to the drinking water in light-impermeable bottles. Given an average uptake of 6 ml water per day and animal, each mouse ingested approximately 0.2 ml BBN (96%). BBN is a yellow oily liquid, hardly volatile and soluble due to terminal hydroxylation. BBN is synthesized and provided by the German Cancer Research Center, Heidelberg.

From 13 weeks onward therapy with IFN-α2b, a genetically derived, recombinant cytokine, was begun. A solution of 3×10^5 IU IFN-α2b in 0.2 ml of 0.9% saline was injected intraperitoneally without anesthesia or additional medication. The injection was carried out once weekly over a period of 10 weeks between 2 p.m. and 4 p.m. to avoid influence of biorhythm. The mice were allocated to either treatment group ($n = 27$) or control ($n = 27$). Mice in the control group received 0.2 ml of phosphate-buffered saline (PBS) once weekly over a period of 10 weeks. All animals were killed at 23 weeks. To assess the clinical tumor stage the thoracic and abdominal contents were examined macroscopically. Bladderwall penetrating urothelial carcinoma as well as tumor-related obstruction of the upper urinary tract were verified and tumors were classified as locally advanced (≥T3b) in contrast to superficial and early invasive carcinoma (Ta-T2).

After fixation in ethanol the 5 μm frozen sections were stained with hematoxilin-eosin and evaluated at 100 and 400 power fields to assess depth of tumor infiltration according to the UICC. Histopathological examination of the removed organs was performed to disclose therapy-related side effects and the presence of distant metastases (liver, lung, lymphnodes).

Statistical analysis

The results were calculated in P values. Regarding the frequency of tumor development in the animal model either Fisher's exact test, χ^2-test or Mantel-Haenszel test were applied. The primary hypothesis of the animal model was analyzed confirmatively with a P of < 0.05 being significant. All further analysis was descriptive and explorative. Significance levels were not adjusted in view of the problem of multiple testing. All statistical tests were paired.

Results

Initially adult mice had a median body weight of 25 g (21 g to 30 g). At the end of the study at 24 weeks a median body weight of 26.7 g with a range of 21 g to 32 g was disclosed in the IFN-α2b group. The course of body weight in the treatment group is correlated with the control group. Median body weight was lowest in the control group with 25.9 g ± 3.5 g. No side effects occurred in mice treated with either IFN-α2b or PBS

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(WHO grade < II). No influence of immunotherapy on the differential blood count could be detected (Table 1).

Of animals receiving intraperitoneal injection of PBS after exposure to BBN as control, 41% developed locally advanced tumors (11/27, Fig. 1a). No superficial tumors were detected. All tumors were undifferentiated (grade IV, Fig. 1b). Five mice died of tumor-related disease before the end of the 23rd week; four of these five eventually underwent a postmortem examination allowing the assessment of tumor stage in 27 of the 28 animals. In all of the 11 mice with locally advanced tumors the macroscopical findings were consistent with histopathological evidence of urothelial carcinoma ≥pT3b.

After intraperitoneal application of $10 \times 3 \times 10^5$ IU IFN-α2b histopathology revealed urothelial carcinoma in 37% (10/27) of the animals. All tumors were locally advanced and undifferentiated bladder carcinomas. Additionally, obstruction of the upper urinary tract was found in nine mice (Fig. 1a). As compared with the control group no influence of immunotherapy with IFN-α2b on chemically induced bladder carcinoma could be detected ($P = 1.0$) (Table 2). All mice developed severe urothelial dysplasia following BBN.

Table 1 Results of the differential blood count of female B6D2F1-mice aged 36 weeks

		Lymphocytes		Granulocytes	
	n	(%)	$10^3/mm^3$	(%)	$10^3/mm^3$
Control[a]	7	(95)	4.75	(5)	0.25
PBS	25	(96)	4.8	(4)	0.27
IFN-α2b	27	(93)	4.7	(7)	0.26

[a] Non-treated animals

Discussion

To assess the influence of potential drugs on the progression of bladder carcinoma we evaluated the validity of several animal models [16]. The model of chemically induced bladder carcinoma in rats is the most extensively described; application of BBN causes epithelial bladder carcinoma in 98% of the animals. Detailed experimental studies by Linn and Rübben [11] using the model of BBN-induced urothelial carcinoma in Wistar rats provided the basis to determine the effect of the carcinogen on normal urothelium and of cytostatic drugs on urothelium subsequent to BBN application. Based on ultramorphologic, autoradiographic and cytophotometric methods it was concluded that cytostatic drugs can induce tumors and urothelial proliferation in a dose-dependent manner. Autoradiography revealed an increased thymidin-assembly 4 to 6 days following instillation of cytostatic drugs. Chemotherapy led to partial response of BBN-induced tumors. The results were consistent with several other studies [3, 17]. However, the induction of invasive bladder carcinoma by BBN in less than 4%–20% of the rats is apparently low. Additionally Ito [8] demonstrated that nitrosamines apart from causing urothelial hyperplasia can lead to intestinal hyperplasia and adenomas in both thyroid and lungs. Thus the comparability of the results to human superficial bladder carcinoma is limited.

A further disadvantage of this model is the low rate of bladder cancer induction. With intravesical instilla-

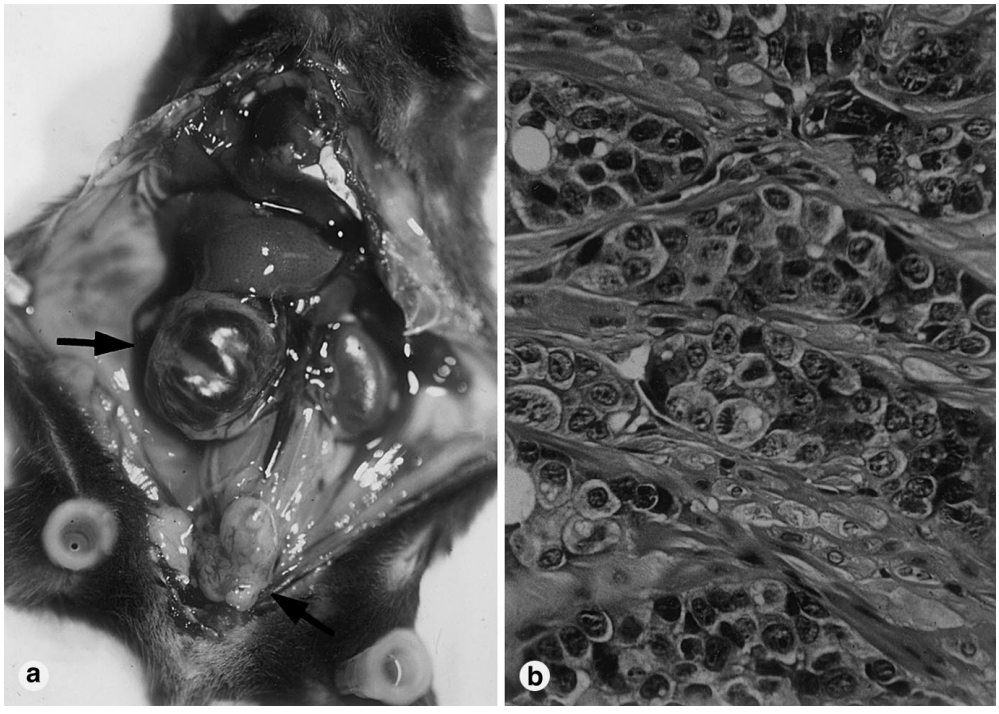

Fig. 1 Macroscopic (a) and microscopic evaluation (b) of a bladder of a mouse treated with PBS. Evidence of locally advanced and poorly differentiated urothelial carcinoma (*arrow*) with additional tumor-related unilateral obstructive uropathy

Table 2 Occurrence of locally advanced urothelial carcinoma (TCC, ≥rpT3b) and severe dysplasia (SD) following application of betahydroxylbutylnitrosamine (BBN) and subsequent treatment with IFN-α2b versus PBS

	n	TCC, ≥pT3b	TCC, ≤T3a	SD
PBS	27	11 (41%)	0	27 (100%)
IFN-α2b	27	10 (37%)	0	27 (100%)
P-value		1.0		1.0

tion of methylnitrosourea (MNU) in F344 rats induction of muscle invasive bladder carcinoma can be achieved in 78%–100% [16]. A drawback of this carcinogen is its high volatility especially when undissolved, limiting its experimental application with regard to safety standards. Another interesting model is the spontaneous tumorigenesis of urothelial carcinoma in rats of the strain BN/BiRij. After 36 months male rats have an incidence of bladder carcinoma of 28%–54% [2]. Concerning the induction of muscle invasive bladder carcinoma, the model of chemically induced bladder carcinoma in mice is closely related to human transitional cell carcinoma. According to those studies advanced transitional cell carcinoma can be induced in 88%–100% of the mice in a time-dependent manner. The strain B6D2F1 especially seems to meet the requirements for a suitable animal model [7]. Contrary to other nitrosamines oral application of BBN causes selective tumor growth in the bladder, ureter and renal pelvis of numerous animal species. The induction of bladder cancer is dose-related and leads to epithelial bladder tumors in mice 13 to 16 weeks after ingestion. The tumors are similar to human epithelial bladder tumors; additionally squamous cell metaplasia and carcinoma occur whereas they are reported in less than 5% of humans [7]. Based on these results we have selected the model of nitrosamine (BBN)-induced bladder carcinoma in female mice (strain B6D2F1) to assess the influence of IFN-α2b on the development of advanced bladder cancer. Though only 41% of the mice in the control arm and 37% in the treatment arm revealed mucle-invasive locally advanced and poorly differentiated transitional cell cancer following BBN, all animals developed severe urothelial dysplasia. We interpreted the severe dysplasia as precursor of further progression to transitional cell cancer and locally advanced tumors if the mice were not killed at 23 weeks.

Intraperitoneal application of IFN-α caused significantly fewer side effects than intravenous drug application [14, 15]. The systemic effect on tumor growth was similar to other application routes, i.e., intracardial or intravenous [14]. Short-term application of IFN-α did prevent metastases [9]. According to other groups (Table 3) we have chosen the intraperitoneal application of IFN-α once weekly for 10 weeks. The dosage was 3×10^5 IU IFN-α × 10 weeks and differed from Jerry et al. [9] in that a higher dose was given once weekly in contrast to 25×10^3 three times daily for 4 weeks.

Table 3 Application of IFN-α in experimental bladder cancer models (*i.p.* intraperitoneal, *i.c.* intracardial, *i.v.* intravenous)

Mice (strain)	IFN-α (dosage)	Application	Schedule	Author
C3H/He	5.000 U	i.p.	1 ×/day × 180	[2]
	50.000 U	i.p.		
NMRI	10^9 U/kg	i.p.	1 ×/day × 5	[14, 15]
	10^9 U/kg	i.c.		
	10^9 U/kg	i.v.		
C3H/He	10^6 U/kg	i.p.	1 ×/day × 5	[13]
NMRI	25×10^3 U	i.p.	3 ×/day × 4	[9]
	3×10^4 U	i.p.	3 ×/week	[5]

We demonstrated that the application of IFN-α2b did not influence the development of locally advanced transitional cell carcinoma. The experimental findings correspond with clinical results regarding topical immunotherapy with cytokines where no influence on the progression of bladder cancer could be detected. Randomized phase II and III trials of topical IFN-α2b versus immunotherapy with bacillus Calmette-Guérin (BCG) [10] or chemotherapy with mitomycin C(1) did not reveal an advantage for therapy with cytokines. Our experimental results do not support topical IFN-α2b in the treatment of potentially progressing superficial or advanced urothelial carcinoma. Whether there is an indication or not for IFN-α2b as intravesical treatment of superficial tumors has to be evaluated by analyzing results of clinical trials.

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ANNOUNCEMENTS

2000

Urolithiasis 2000
The IXth International Symposium on Urolithiasis
13–17 February 2000, Cape Town, South Africa

Information: Prof. Allen Rodgers, Symposium Chairman, Chemistry Department, University of Cape Town, South Africa 7701. Tel.: 27 21 65 02572; Fax: 27 21 68 67647; E-mail: allenr@psipsy.uct.ac.za

2000 Winter Urologic Forum Meeting
23–30 January 2000, Telluride, Colorado

Information: Linda Mace, Meeting Coordinator, Box 3707, Duke Medical Center, Durham NC 27710, USA; Tel.: 919-684-2033, Fax: 919-684-4611

2000 Duke Urologic Assembly
14–19 March 2000, Cancun, Mexico

Subject: Contemporary Issues in Urology
Information: Linda Mace, Assembly Coordinator, Box 3707, Duke Medical Center, Durham, NC 27710, USA; Tel.: 919-684-2033, Fax 919-684-4611

WHO Consensus Conference:
Public Health and Clinical Significance
of Premalignant Alterations in the Genitourinary Tract
June 8–9 2000, Stockholm, Sweden

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