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Influence of intravesical administration of tumor necrosis factor α and systemic γ-interferon on murine bladder cancer: lack of dose response

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Summary. The effect of intravesical administration of high dose recombinant tumor necrosis factor-α (rTNF) and in combination with systemic recombinant γ-interferon (rIFN) on murine bladder cancer was studied. RTNF was given at 12.5 μg/mouse on days 7, 11 and 15 after tumor instillation or at 2.5 μg/mouse on days 7, 9, 11, 13 and 15. Some groups were also injected i.v., 24-h prior to each rTNF treatment with rIFN at a dose of 1.3 μg/mouse. RTNF treatment suppressed tumor growth up to 48% of control, although the difference was not statistically significant. Combined administration of rIFN did not provide additional benefit.

Key words: Human recombinant TNFα – Human recombinant γIFN – Murine bladder cancer – Intravesical therapy

We have demonstrated previously the therapeutic potential of intravesical administration of tumor necrosis factor (TNF) for local bladder cancer control [5]. Administration of low dose TNF (rat serum containing TNF) failed to suppress the growth established (7-d) MBT-2 tumors, whereas, escalation of the dose utilizing recombinant TNF significantly suppressed tumor growth. These observations suggested the response of TNF was dose dependent for suppressing local bladder cancer.

Most recently, a number of studies have shown the beneficial effects of cytokines used in combination [1, 2, 8]. It has been reported that combined use of interferon (IFN) could enhance the cytototoxic effect of TNF and this finding was confirmed by us in vitro using MBT-2 tumor cells [6].

The experiments were conducted to determine whether an escalation in TNF dose or a more frequent schedule would increase the therapeutic potential of intravesical TNF; and whether the combined administration of IFN would provide additional benefit.

Materials and methods

Mice and tumor instillation

18 to 20 g female C3H/HeN mice (endotoxin sensitive strain) were purchased from the Charles River Laboratory (Pittsburgh, PA).

MBT-2 bladder cancer cells [9], syngeneic to C3H mice, were maintained in continuous in vitro culture from cryopreserved tissue, and a single cell suspension was prepared as described previously [5]. Intravesical tumor implantation (5×10^5 MBT-2 cells in 50 μl) was undertaken according to the method described by Soloway [9] and reported by us previously [5]. Following tumor instillation mice were randomly placed into treatment groups consisting of 25 to 30 mice per group. Routine assay of MBT-2 cells for *Mycoplasma* using an agar culture technique were negative.

Cytokines and treatment protocol

Human recombinant TNF-α (rTNF; Lot 3056–63, 2.5×10^7 U/0.5 mg/ml) and murine recombinant γ-IFN (rIFN; Lot 4407–47, 2.3×10^7 U/0.98 mg/ml) were kindly supplied by Genentech Inc. (San Franscisco, CA). Dilution of rTNF or rIFN was made in phosphate buffered saline (PBS).

RTNF was administered intravesically (50 μl/bladder) according to the method reported in detail elsewhere [5]. RIFN was injected i.v. once at 1.3 μg/mouse (30,000 U/mouse) 24-h prior to each intravesical rTNF treatment. Tumor incidence and tumor weight, defined as the weight of tumored bladder, were determined on Day 18 to 21 after tumor instillation. The mice that died before evaluation were excluded from the data analysis.

Statistical analysis

Mean value was expressed as the mean ± SD. Data were compared by using Student's t-test or Chi-square with Yate's modification with significance determined at $P < 0.05$.

Results

Table 1 demonstrates the results of 2 independent experiments; a published observation is also shown in Table 1

Table 1. Effect of intravesical rTNF with or without systemic rIFN on MBT-2 growth

Experiment	Treatment[a]	tumor weight[b]		tumor/total[b] (tumor incidence)
		mg	% of control	
Published observation [5]	PBS×3	430±244	100	7/24 (29)
	rTNF (4.6 μg×3)	237±204*	55	7/20 (35)
Exp. 1	PBS×3	208±210	100	9/23 (39)
	rTNF (12.5 μg×3)	152±123	73	9/23 (39)
	rIFN	184±139	88	12/24 (50)
	rTNF+rIFN	96±101	57	5/21 (24)
Exp. 2	PBS×5	159±140	100	9/29 (39)
	rTNF (2.5 μg×5)	82± 55	52	13/24 (55)
	rTNF+rIFN	115± 81	72	10/24 (42)

[a] RTNF (or PBS) was given intravesically for 3 times (on Days 7, 11 and 15) or for 5 times (on Days 7, 9, 11, 13, and 15). RIFN (or PBS) was given i.v. at 1.3 μg/mouse (30,000 U/mouse) 24-h prior to each intravesical rTNF

[b] Tumor weight and tumor incidence were determined on Days 18 to 21, at which time all mice were sacrificed

* $P<0.05$; treatment vs. PBS control

for comparison. Intravesical administration of high dose or frequent schedule rTNF suppressed tumor growth by up to 48% of control (Exp. 2), though the differences between treatment and control groups were statistically not significant. Intravenous injection of rIFN did not affect the tumor growth nor did it alter the therapeutic outcome of intravesical rTNF. Tumor incidence was not affected by any of the treatments. Histological sections of the tumored bladders (at the time of evaluation) showed no evidence of hemorrhagic necrosis irrespective of treatments (data not shown).

Twelve out of 180 or 6.7% of mice (Exp. 1+2) died prior to evaluation, mostly due to the general anesthesia. Toxic signs associated with rTNF or rIFN (e.g., weight loss and immobility) were not observed within the experiments.

Discussion

Systemic administration of TNF or cachectin causes hemorrhagic necrosis of certain experimental tumors [1, 2, 8]. In vitro TNF is cytolytic or cytostatic for tumor cells with little or no activity on most normal cells. However, subsequent studies revealed that systemic administration of TNF induces generalized toxicities similar to anaphylactic shock, and this lethal toxicity hampers the use of this cytokine for cancer treatment.

Intravesical administration of TNF may have potential advantages. Assuming that TNF is not absorbed through normal bladder mucosa because of its molecular weight (MW 17,000) [7], TNF may not induce systemic toxicity. This allows escalation in TNF doses. Our experimental results support this hypothesis. Mice tolerated at least 37.5 μg total dose (per mouse) of rTNF when it was given intravesically. However, if rTNF was given i.v., mice tolerated at most 2.5 μg per mouse of rTNF in our tumor model [4].

For most cytotoxic agents the relationship between cell survival and dose is close to exponential, so that an escalation in drug dose kills more cell fractions. We have reported previously that low dose TNF utilizing rat serum containing TNF did not affect the intravesical tumor growth, whereas escalation of dose using rTNF mediated tumor suppression (Table 1) [5]. These observations suggested that the dose associated antitumor efficacy existed when rTNF was given intravesically. The present data revealed, however, further escalation in dose and schedule of rTNF did not provide an additional benefit. Lack of the dose response may be due to the poor tumor penetration of rTNF because of its molecular size [3], relative short term exposure (30 to 60 min) of tumors to rTNF and/or an intrinsic tumor resistance to rTNF.

Mice were pretreated with rIFN on the assumption that IFN might sensitize MBT-2 tumors to rTNF based on our own in vitro study [6] and the reports of others [1, 2, 7]. Due to a lack of appropriate pharmacokinetic data of rIFN in rodents, it is unknown if an optimal dose and schedule of rIFN could sensitize in vivo MBT-2 tumors. Our data demonstrated that a single i.v. injection of rIFN at 1.3 g/mouse prior to each intravesical rTNF did not provide additional benefit.

In conclusion, a threshold seems to exist as to the antitumor efficacy of intravesical rTNF. RTNF is able to suppress tumor growth by approximately 50% of control, though escalation in rTNF doses does not provide additional tumor suppression. Pretreatment of mice with IFN does not create therapeutic benefits in combination with rTNF at least at the rIFN doses and schedules we tested.

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