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A novel method for monitoring patients with superficial bladder tumors which are under intravesical IFN α -2b installation

ABSTRACT

Background: Transitional cell bladder tumors are common and they have rising incidence. Most of them appear, at the beginning, as superficial ones. Superficial bladder tumors, despite the favorable prognosis will recur in 70% of the cases within 36 months after the transurethral resection (TUR) if no additional treatment with intravesical instillation is administered. Recurrences increase the risk of disease progression and the appearance of infiltratory tumors. Among the various instillation protocols active worldwide, interferon- α -2b (INTRON) has been used and many studies report encouraging results, thus establishing it as a potent agent for intravesical instillation therapy.

Materials and methods: The measurement of 2'5'oligo(A)synthetase was estimated by following the conversion of ³H-labelled ATP to adenosine oligomers as previously described by the authors. Frozen tissues from bladder biopsies were used as source obtained from more than thirty patients who were enrolled in the protocol.

Results: The most important finding of our observations is the excellent correlation between the induction of 2'5'oligo(A)synthetase and the response to the therapy. Ninety percent of the responders have increased level of the enzyme. No difference was observed in comparison to patients that received combined therapy with IFN α -2b, plus epirubicin. The control values of the enzyme are increased in comparison with the levels of the enzyme in other tissues.

Conclusion: The results of the induction of 2'5'oligo(A)synthetase could be used as a novel reliable marker for monitoring patients with superficial bladder tumors which are under a protocol with IFN α -2b instillation.

Key words: 2'5'oligo(A)synthetase; Bladder cancer; IFN α

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INTRODUCTION

Bladder cancer is the fifth most frequent neoplasm in developed countries with an unusual incidence of 18 new cases per 100000 total population and a mortality ratio of 4 cases per 100000 total population a year (1). Eighty percent of the new cases present with tumors that do not reach the muscular layer of the bladder wall (superficial), featuring an excellent prognosis (2). Transurethral resection (TUR) would be the standard first line treatment for this kind of bladder tumors, otherwise, they will progress to muscle - invasive neoplasm, espe-

cially those that reach the lamina propria. At the same time superficial bladder tumors feature a 50% mortality ratio in spite of combined treatment with radical surgery and cytotoxic chemotherapy (2).

Intravesical therapy has been successfully used for several decades in the management of superficial transitional cell carcinoma for the treatment of residual tumors, the prevention of tumor recurrence and the prevention of tumor progression (3).

For more than 20 years, superficial bladder tumors have been demonstrated to be sensitive to several biological response modifiers and especially immunomodulators. Among the new approaches with immunomodulators for superficial bladder cancer therapy, IFNs have been the object of numerous studies. IFNs are known to have antiviral and direct antiproliferative activity, they inhibit angiogenesis, regulate cell differentiation, activate immune effector cells, induce cytokine production and enhance tumor-associated antigen expression (4). Both IFN α and IFN β 1, which bind to the same receptor,

exhibit direct and indirect antitumor effects *in vitro* (5). Interestingly, high grade bladder cancer cells express increased IFN α receptors (6). Recombinant IFN α has been demonstrated to have an activity against bladder cancer cell lines through the increase of lymphokine-activated killer cell activity (7).

Clinical studies have suggested that intravesical therapy with recombinant IFN α -2b increased the cytotoxic activity of T-cells and natural killer cells by increasing the infiltration of these cells into the bladder wall (8).

Intravesical therapy with recombinant IFN α -2b was demonstrated to have both a systematic and a local activity on cells of the immune system and the bladder respectively (9,10).

The induction of 2'5'oligo(A)synthetase is one of the biochemical pathways that is induced in cells after treatment with interferon. It has been suggested, therefore, that measuring the 2'5'(A)synthetase on peripheral mononuclear lymphocytes can be helpful as an indicator for determining the biological effects of IFN *in vivo*.

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The presence of ds-RNA, 2'5'(A)synthetase leads to the formation of oligoadenylates that activate an endoribonuclease (RNA-se L) resulting in the inhibition of protein synthesis. This so-called 2'5'(A)system is important for the antiviral and probably cell growth inhibitory effect of IFN (11). Few reports show that the same enzymatic system might operate in experimental mice treated with interferon or interferon inducers - since one of the enzymes involved in the system, i.e. the 2'5'oligo(A) synthetase is induced in different organs of these animals (12,13).

It has been suggested, therefore, that measuring the level of 2'5'oligo(A)synthetase in peripheral mononuclear lymphocytes can be helpful to determine the biological effects of IFN *in vivo* and to monitor IFN production and/or responsiveness in individuals with infections or individuals receiving IFN therapy (14). The usefulness of the 2'5'oligo(A)system as tumor marker for monitoring the patients' response to IFN α -2b effect was confirmed in renal cancer (11), thyroid cancer (15), multiple myeloma (16), hepatitis (17). The results obtained suggested an excellent correlation between the induction of 2'5'oligo(A)synthetase and the clinical response of patients to IFN α -2b.

From this point of view we decided to study the induction of 2'5'oligo(A)synthetase in biopsies from patients with bladder cancer who, after TUR, have IFN α -2b instillation as monotherapy or in combination with epirubicin.

MATERIALS AND METHODS

Tissue extracts

Tissues, obtained from bladder biopsies, frozen in liquid nitrogen, were homogenized mechanically (Dounce homogenizer) in buffer containing 10mM KCl, 1.5mM MgCl₂, 0.5mM DDT and 20mM Hepes pH 7.4. the cell homogenate was then centrifuged at 10.000 x g for 10 min and supernatant representing the S10 cell fraction was stored in liquid nitrogen.

Assay of 2'5'oligo(A)synthetase

The activity of 2'5'oligo(A)synthetase is estimated by following the conversion of ³H-labelled ATP (Amersham) to adenosine oligomers. Incubations (S10+³H ATP) were for 24h at 30°C followed by precipitation of the mixture with 6 volumes of acetone. The proportion of ATP which is polymerized is calculated by separating the synthesized oligomers from monomers with small DEAE-cellulose columns. The activity of the 2'5'oligo(A)synthetase is expressed as the percentage of the substrate (³H-labelled ATP) which is converted to a mixture of oligonucleotides.

Patients

Thirty eight patients with superficial bladder cancer were enrolled in the protocols with IFN α after the TUR resection of the tumor. The 2'5'oligo(A)synthetase was measured before and during the treatment with IFN α -2b.

Twenty five patients were in a protocol with IFN- α for a period of one year. They had an instillation of 50x10⁶ IU IFN α weekly for eight weeks, and later, eight times, the same amount, every two weeks. Finally, they had instillation every month for six months to complete a one year therapy. The other thirteen patients had, for the first three months, four weekly instillations of epirubicin every fifteen days. The next nine months, the patients were under IFN α instillation of 50x10⁶ IU. The first four instillations were every week, the other four every two weeks, and the last six every month.

RESULTS

Induction of 2'5'oligo(A)synthetase in different tissues

Among the various instillation protocols active worldwide, interferon α -2b (INTRON-A) has been used and many studies report encouraging results. It was unknown if the human tissues have measurable levels of 2'5'oligo(A)synthetase even though the authors (12,13) had published some years ago, the results which showed the induction of the enzyme in liver and spleen of CH3 and BALB/C mice with corresponding cancer. It is obvious that the levels of enzyme are more than twice higher in patients with bladder cancer in comparison with the patients with renal cell cancer and prostate cancer as well (Table 1).

Table 1. Induction of 2'5'oligo(A)synthetase in different tissues

Origin of the tissue	Induction of the enzyme (% converted ATP)
CH3 mice with *EAT	
Liver	0.58
Spleen	1.43
BALB/C mice with EAT	
Liver	1.6
Spleen	1.3
Human neoplasm	
Renal cell cancer	1.2
Prostate cancer	1.4
Bladder cancer	3.4

* Ehrlich ascitic tumor.

Induction of 2'5'oligo(A)synthetase in bladder biopsies

Thirty eight patients were enrolled in protocols with IFN α -2b instillation after TUR. Twenty five patients with bladder cancer had instillation of INTRON-A whereas thirteen patients had a combined therapy with IFN α -2b instillation and epirubicin as described in

materials and methods. There is no significant difference in response between the two protocols ($p=0.843$ by chi square test, $p>0.05$) (Table 2).

Table 2. Induction of 2'5'oligo(A)synthetase in bladder biopsies

No of patients	Protocol	Response		Increased level of induced enzyme (% converted ATP)	
		Yes	No	Yes	No
23	IFN α -2b	20/25 (80%)	5/25 (20%)	18/20 (90%)	1/5 (20%)
13	IFN α -2b + Epirubicin	10/13 (77%)	3/13 (23%)	8/10 (80%)	0/3 (0%)

The duration of the response was at least eighteen months. It was obvious that increased levels of 2'5'oligo(A)synthetase were observed in more than 80% of the responders while only 20% were the false positive.

The Induction of 2'5'oligo(A)synthetase was measured four times in each patient. Before the treatment, after the first instillation, three months later and finally one year after their first visit to the hospital. There is a reliable increase of the enzyme levels which approaches to three times of the cut-off prices after one year (Table 3).

Table 3. Induction of 2'5'oligo(A)synthetase in bladder biopsies

Protocol	*Induction of 2'5'oligo (A) synthetase (% converted ATP) Before treatment	After 1 st instillation	After 3 months	After 1 year
IFN α -2b	3.2	7.1	7.4	11.2
IFN α -2b + Epirubicin	3.1	7.5	7.3	11.0

*The values indicated here represent the median value of all the patients that responded to IFN- α .

It is obvious that there is no difference between the two protocols.

DISCUSSION

One of the unsolved problems of oncology is the reliability of the tumor markers. With the exception of very few cases, like PSA-CEA-Hydroxyproline, there are no parameters with the required sensitivity and specificity. The simultaneous measurements of more than one markers is usually profitable strategy but very often the results obtained are very confusing.

The induction of 2'5'oligo(A)synthetase is the main biochemical pathway that is induced in cells after treatment with IFN α . Some years ago we started the study of the 2'5' system in cell lines, experimental animals and finally in human beings. We have found a very good correlation between the induction of this enzyme and the clinical response to IFN α of the patients with a large spectrum of different cancers. This spectrum consists of patients with renal cancer, thyroid cancer, multiple myeloma, penile cancer and hepatitis C. The results show a very reliable and distinguished values of this marker. From this point of view, we measured the induction of this enzyme in patients with bladder cancer which were enrolled in protocols with INTRON-A instillation after the removal of the tumor by TUR.



CONCLUSION

We could conclude that this marker is very good for monitoring the patients which are in regional chemotherapy with Interferon α -2b. In cases of increased levels of the enzyme, a clinical response is expected to this cytokine, which is a very well-known agent for its antineoplastic and antiviral effects. The number of false positive results is low and probably suggests the use of the IFN α -2b, probably in different schedules. In these cases, the use of different amounts of IFN α , in different schedules including alterations of time, biological response modifiers or chemotherapeutic substances, could give better results.

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