Review article
UDC: 616.981.45-618.14-006:616.24-006:615.371

The use of tularemia live vaccine in clinical oncology

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The review of literature on the use of tularemia live vaccine in clinical oncology is presented. The results of the investigation of tularemia live vaccine as a non-specific stimulator of natural immune and antitumor resistance of patients with endometrial cancer and inoperable lung cancer and its influence on the immunological status of these patients after immunization, during the process of combined treatment, and on long-term (up to 5 years) follow-up after vaccination are shown. A single immunization with tularemia live vaccine increases the main immunological indices in patients with endometrial cancer and inoperable lung cancer already in 2 to 3 weeks after vaccination and they remain during the whole term of follow-up. Moreover, the vaccine shows not only immunostimulating but immunomodulating effect as well and does not give any serious local or general complications. It is shown that due to these effects immunization of patients with tularemia live vaccine in 15 to 20 days before surgery or chemotherapy is followed by a significantly decreased frequency of postoperative complications and better survival. On the basis of the data obtained the authors recommend to use tularemia live vaccine in the complex treatment of those cancer patients who have deep decrease of immunological reactivity.

KEY WORDS: *Tularemia; Vaccines; Medical Oncology; Endometrial Neoplasms; Lung Neoplasms; Adjuvant, Immunologic; Immunization*

INTRODUCTION

One of the methods of the active nonspecific antitumor immunity stimulation of cancer patients is application of bacterial antigens, from which in the past BCG vaccine was the most widely adopted (1). However, this vaccine has rather variable and short-term effects (2) that need the revaccination, whereas multiple applications of the vaccine undesirably increases the activity of suppressors and breaks the ratio of the immunoregulatory cells (3). Moreover, the multiple applications of the vaccine often exhaust the resources of the immune system (4) and even stimulate tumor growth (5). At present, due to possible numerous side effects of this vaccine, including tubercular meningitis, the vaccine is not used even for prophylaxis of tuberculosis in the USA (4), and the WHO recommends to use it only in developing countries (6). Therefore, the search for new bacterial nonspecific stimulators of the immune and anti-tumor resistance is fairly justified. Application of tularemia live vaccine (TLV) as a nonspecific stimulator of natural and antitumor resistance of the organism during the treatment of patients with endometrial cancer (EC) and inoperable lung cancer (LC) has for the first time been suggested for clinical use in Cancer Research Center, Yerevan, Republic of Armenia (7). This vaccine has been chosen for application based on the results of an experimental research carried out in the Laboratory of Carcinogenesis of the Center. The experimental results established that preliminary immunization of tumor-bearing animals helps to decrease the toxic action of cytostatics, increases the radioresistance and the antitumor effect of radiotherapy, and prolongs the mean life span (MLS) (8). Besides, it has been established that TLV also has anticarcinogenic, antitumor, and antimutagenic effects (8). The research carried out by other authors revealed that upon vaccination of mice with TLV it is possible to detect TNF-alpha, IFN-gamma, IL-alpha, IL-2, IL-4, IL-6, IL-10, and IL-12 (9-12), meanwhile the indices not only of T-cell (13), but B-cell (14) immunity raise as well. In most cases, antibodies appear 2 to 4 weeks after vaccination, reach highest titers in 1 to 4 months and can be traced even 1.5 years after vaccination (15). Experiments carried out on humans showed the similar results - examination of immunized people revealed

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The manuscript was received: 24. 02. 2003

Provisionally accepted: 02. 04. 2003

Accepted for publication: 08. 12. 2003

increase in titers of TNF-alpha, IFN-gamma and IL-2 in 2 weeks after vaccination (16).

TLV used in the former USSR and currently in Russia has somewhat different characteristics. Antibodies in the organisms of rats, guinea pigs and the Armenian hamsters develop in 3 to 5 days after vaccination, reach the highest titers in 15 days and stay at that level about 10 more days. After that period, the titers of the antibodies slowly go down but they can still be detected by immunological methods even after 60 days (8). One of the reasons why TLV has been chosen for application in clinical oncology is that people can be revaccinated only in 5 years, since during that period it maintains its antigen activity. Meanwhile, 5 years is also the period specified for active observation of cancer patients and particularly with EC (17).

The purposes of our paper were the following: 1. The review of the literature concerning the use of TLV in clinical oncology; 2. The analysis of the impact of TLV on the main immunological indices in patients with EC and LC, and the examination of possible side effects of the immunization; 3. The analysis of dynamics of the immunological indices of EC patients during combined treatment (surgery + radiotherapy) and after it (up to 5 years), and in case of LC patients after each course of polychemotherapy; 4. The analysis of immediate, short-term and long-term results of the treatment of patients with EC, and tumors regression and MLS of LC patients, immunized with TLV before the combined treatment and chemotherapy, correspondingly.

The research was carried out by randomized method on 97 hospital patients (Cancer Research Center, Yerevan, Armenia) with histologically verified (mostly with adenocarcinoma of high and moderate differentiation) diagnosis of EC (76 patients with disease stages I and II , and 21 with stages III and IV) (FIGO, 1988), and on 29 patients with inoperable LC. All patients had been immunized with TLV by scarification method according to instruction in 2 to 3 weeks before they received special treatment (main groups) (18, 7). There were no cases of any local or general complications of vaccination, except for hyperemia and little swelling on the place of cuttings 4 to 5 days after vaccination (8.7% of patients) or a little swelling and pain in armpit lymph nodes in 8 to15 days (40%), that would disappear within 2 to 3 days after vaccination (18). EC patients stage IV of the disease had metastases in lungs, liver, and lymph nodes. All EC patients received combined treatment: radical hysterectomy with adnexectomy or Wertheim's operation, depending on the stage of disease, tumor's differentiation and its localization in uterus, which were 15 to 20 days later followed by distant gamma therapy on the areas of small pelvis and the zones of regional metastasization at dose of 40 to 50 Gy (19). All LC patients underwent through courses (from 2 to 5) of polychemotherapy (Adriablastin [doxorubicin hydrochloride], methotrexate, and Endoxan [cyclophosphamide] combination in average therapeutic doses) (7). As control groups 97 EC patients with the same stages of disease (18, 19) and 25 patients with inoperable LC (7), who had received the same treatment as patients of main groups, but without immunization, were observed.

EC patients of the main group were immunologically examined before vaccination with TLV, 2 to 3 weeks later (at the same time with patients of control group, that is immediately before operation), 10 to 15 days after the operation (before radiotherapy) and just after the latter, as well as 2 to 3 and 4 to 5 years after the end of treatment (19). Indices of immunological reactivity of the main group of LC patients were studied before vaccination, 2 to 3 weeks later (with control group just before chemotherapy) and after each course of it (7).

Patients' general, theophylline-sensitive (ThS) and theophylline-resistant (ThR) erythrocytes - rosette-forming cells (E-RFC), the reaction of blast-transformation of leukocytes (RBTL) on phytohaemagglutinin (PhHA), and index of macrophagic transformation of mononuclears (IMTM) according to the routine methods were studied. At the same time the phagocytic activity of blood neutrophiles to staphylococcus (strain 200 R) by used methods was investigated and functional activity of T-suppressors by method of double reaction of blast transformation (20) was determined. For a norm control of all the above-mentioned immunological indices the group of 50 not vaccinated and practically healthy women of the same age were observed (18,19).

The immediate results of treatment - healing of the operation wound of the front abdominal wall (divergence of skin sutures, phlegmona) and the vaginal stump (marked necrosis, parametritis), short-term and long-term results (relapse and metastasis frequency, recurrence-free period, 2-, 3- and 5-year survival, lethality) and MLS obtained by method of life-table construction in both groups of EC patients were studied (21-24). Complete or partial tumor regression and MLS in all patients with LC were studied as well (7). Statistic meaning of obtained data was determined by means of chi-square and Student's *t* tests.

Results of the research showed that vaccination of EC patients with TLV in all stages of disease already in 2 to 3 weeks increases both the indices of T lymphocytes of the immune system (except for RBTL on PhHA at III-IV stage) and the nonspecific antitumor resistance of the organism (IMTM and phagocytic activity of blood neutrophiles). At stage I-II of the disease they all reach their normal physiological levels compared with control (18). Within this period of time not only the quantity of general and active E-RFC in blood of patients with disease stage I-II reaches control levels, but ThS/ThR ratio gets normalized and proliferative function of T lymphocytes reaches control level (healthy women) as well. However, despite the statistically proven ($p<0.05$) increase in the percentage of general E-RFC in

comparison to their initial level, it does not reach the control level in disease stages III-IV ($p<0.05$). Authors do not exclude that this, to a certain extent, could be determined by the immunodeppressive serum factors revealed particularly at late stages of tumor process (25). By analogy, ThS/ThR E-RFC ratio in disease stages III-IV does not become normal too. In authors' opinion, the fact that in these stages RBTL on PhHA indices do not raise in comparison to the initial level can indicate that at last stages of EC T-lymphocytes might loose their proliferative activity as well as their sensitiveness to PhHA (18).

It is especially important that TLV immunization does not lead to total stimulation of all populations of T lymphocytes. Thus, if the quantity of ThR lymphocytes after vaccination increases, the quantity of ThS lymphocytes decreases, which results in statistically proven decrease of KThS/ThR E-RFC ratio in comparison to the initial level in all stages of the disease, although complete normalization takes place only in disease stages I-II compared with control. Thus, TLV vaccination eliminates ThS cells and neutralizes the immunosuppressive effects of T lymphocytes, which can be observed in the organism of EC patients. Consequently, TLV has an immunomodulating effect (18). In the same way TLV affects the indices of nonspecific antitumor resistance of the organism of these patients - IMTM and indices of phagocytic activity of blood neutrophiles (18). So, results of the research indicate that vaccinated patients have completely rebuilt the immunoactivity of the organism at early stages and considerably increased it at late stages (18). Meanwhile, in case of nonimmunized patients the immunoactivity of the organism has distinctly decreased in comparison with control (18).

Besides the task to rebuild the immunocompetence of the organism and activate antitumor immune mechanisms, one of the main goals of adjuvant immunotherapy of cancer patients is to compensate the immunodeppressive effect of the main methods of tumor treatment - surgery, radio- and chemotherapy (17). The work devoted to this problem (19) aimed to study how TLV affects the indices of natural immune and non-specific anti-tumor resistance of the organism of EC patients during the process of combined treatment (after surgery followed by radiotherapy), as well as in the long-term perspective (in 2-3 and 4-5 years). The research was carried out on the same 97 EC patients of the main group and 97 patients of the control group. Same indices were studied by the same methods (18,19). In 2 to 3 years after the treatment of tumor process in stages I-II and stages III-IV the indices of the immunized patients being decreased especially after radiotherapy returned to normal physiological levels compared with control, while in case of non-immunized patients they being decreased much more reached only the initial level (as before surgery), i.e., lower than control (p from <0.05 to <0.001) (19).

EFFECT OF CONTRASUPPRESSION IN PATIENTS IMMUNIZED WITH EC

Experiments carried out on mice have revealed a new level of immunoregulation - contrasuppression (26). However, so far only few works have been devoted to this phenomenon in humans (27-29). Meanwhile, the possibility of induction of this effect in case of different pathological conditions and, in particular, in case of tumor growth is not studied at all. This phenomenon was studied in case of EC patients (30). Particularly, it has been shown that in the population of mononuclears in humans there are some cells that play an important role in immunological homeostasis. It is also established that there is a certain interaction disorder in the suppressor-contrasuppressor system in the organisms of these patients. The long-term results of the combined treatment of EC patients depending on the effect of contrasuppression of blood lymphocytes before treatment are studied. It has been concluded that in case of EC patients the effect of contrasuppression, as well as the stage of the disease, can serve as a criteria for how far the process has gone and what the prognosis will be (21). The effect of TLV on contrasuppression of EC patients during the process of combined treatment (31) and on the long-term results (up to 5 years after treatment) (31, 24) has also been studied.

It has been established that already 2 to3 weeks after vaccination of EC patients with TLV in all stages of the disease the percentage of patients with detected level of contrasuppression effect increases up to the normal physiological level and is maintained during the process of combined treatment (except for stages III-IV), a long after that (more than 3 years) (31). Since the fact of contrasuppression effect before treatment is an indication for favorable prognosis for EC patients (21, 31), authors assume and show that immunization of these patients improves this index and, consequently, should increase the chances for recovery at early stages of disease and prolong the MLS in late stages (31, 24).

IMMEDIATE, SHORT-TERM, AND LONG-TERM RESULTS OF COMPLEX TREATMENT OF PATIENTS WITH EC PRELIMINARY IMMUNIZED WITH TLV

In order to clarify finally this very important question a research has been carried out regarding the immediate, short-term and long-term results of the treatment of these patients (21-24). The research shows that preliminary (2 to 3 weeks before beginning of combined treatment) TLV immunization of EC patients the major part of which suffers from strong obesity and diabetes (17) considerably decreases the frequency of suppurative complications of operation wounds (Table 1).

Taking also into account that preliminary TLV vaccination considerably improves almost all indices of short-term and long-term results of the treatment of these patients at all stages of the

Table 1. The influence of TLV on the healing of operation wound of the front abdominal wall and the vaginal stump in patients with EC

Groups and the number of patients	The operation wound healing											
	No complications		Divergence of skin sutures		Phlegmon of abdominal wall		Parametrial infiltrates		Marked necrosis of vaginal stump		Coupled complications	
	No	%	No	%	No	%	No	%	No	%	No	%
1 (n=76)	56	3.7	15	19.7	-	-	9	11.8	7	9.2	11*	14.5
2 (n=76)	49	64.5	20	26.3	1	1.3	15	19.7	13	17.1	22	28.9
3 (n=21)	15*	1.4	6*	28.6	-	-	3	4.3	5*	23.8	6*	28.6
4 (n=21)	7	33.3	14	66.7	2	9.5	5	23.8	13	61.9	14	66.7

1 - The patients with EC in I-II stage immunized with TLV
2 - The patients with EC in I-II stage treated without vaccination
3 - The patients with EC in III-IV stages immunized with TLV
4 - The patients with EC in III-IV stages treated without vaccination
* - p<0.05 in comparison with not vaccinated patients at the same stages of disease

Table 2. The influence of TLV on the end-results of combined treatment of patients with EC

Groups and number of patients	End-results of treatment										
	Relapse and metastasis frequency		Recurrence-free period (in months)	Survival 2-year		3-year		5-year		Lethality rate	Mean life-span (in years)
	No	%	M±m	No	%	No	%	No	%	%	
1 (n=76)	14*	18.4	20.6±2.9*	71	93.4	71*	93.4	62*	81.6	18.4*	4.7± 0.4*
2 (n=76)	26	34.2	11.7±3.3	63	82.9	60	78.9	50	65.8	34.2	7±0.3
3 (n=21)	11	52.4	8.8± 2.3*	15*	71.4	14*	66.7	10	47.6	52.4	2.5± 0.3*
4 (n=21)	16	76.2	3.1±1.7	8	38.1	6	28.6	5	23.8	76.2	1.7±0.2

1 - The patients with EC in I-II stage immunized with TLV
2 - The patients with EC in I-II stage treated without vaccination
3 - The patients with EC in III-IV stages immunized with TLV
4 - The patients with EC in III-IV stages treated without vaccination
* - p<0.05 in comparison with not vaccinated patients at the same stages of disease

process (21-24) (Table 2) that corroborates the previous assumption of the authors (19,31) and that TLV practically has no contraindications and does not cause any complications (18), the authors come to conclusion that obtained results allow to recommend it for application in combined and complex medical treatment of these patients. This opinion has been confirmed in case of the treatment of patients with inoperable LC as well (7).

Study of short-term (complete or partial tumor regression) and long-term (MLS) results of treatment of 29 patients with inoperable LC shows that single TLV vaccination ensures normal level of functioning of their immune system also in 2 to 3 weeks, and this has a positive impact both on short-term and long-term results of their chemotherapy (7). So, the 50% regression of tumors in LC patients was noticed in 17 from 29 (58.6%) of those vaccinated with TLV whereas only in 8 from 25 (32%) of not vaccinated patients ($p<0.05$). MLS in the main group was 14.3 ± 2.2 months versus 9.0 ± 1.4 months in control (not immunized) group ($p<0.05$).

CONCLUSION

Reviewing all the above-mentioned, it is possible to conclude that single TLV immunization ensures steady increase of main immunological indices of EC and LC patients already 2 to 3 weeks after vaccination, which continues during the whole period of observation (up to 5 years). The vaccine has not only immunostimulating, but also immunomodulating effects and does not give any serious local or general complications. This phenomenon warrants further investigations, and if similar results are obtained in other cancer patients, in our opinion, TLV will be recommended for application as an immunostimulator and immunomodulator of natural immune and non-specific anti-tumor resistance of the organism in complex treatment of those cancer patients, who suffer from a distinct decrease of immunological reactivity.

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