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· Original Article ·

New possibilities in treatment of chronic urinary tract infections—vaccines, autovaccines and beta glucans

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Abstract **Background:** Repeated infections of urinary tract manifest an increasing trend and became the most common infection in developed world. In addition, currently common increase in resistant microbes including uropathogens underlines the need for new ways of urinary tract infection (UTI) treatment. One of the possibilities is vaccination. However, commercially available bacterial vaccines are not always reliable, leading to the proposition of using autovaccines prepared from uropathogens of individual patients. It is a well-known fact that UTIs are often accompanied by depressed immunity. **Methods:** To respond to both problems, we tried to use yeast-derived, insoluble beta glucans for restoration of depressed immune system and acceleration of the effects of autovaccines. In all patients we repeatedly tested bacterial load in urine. In addition, from the same samples we measured the levels of inflammatory proteins, albumin, orosomukoid, IgA, C-reactive protein. Complex evaluation of inflammatory response and cellular immunity in blood was performed, too. **Results:** Immediately after application of the first dose we observed significant improvements of clinical conditions which were persistent throughout the entire study. **Conclusions:** Beta glucan is optimal addition to autovaccines, as it is natural, active, safe and inexpensive. We propose that the possibility to use beta glucans in application of autovaccines is the first step in preparation of qualitative new type of autovaccines against UTI.

Keywords Urinary tract infection; autovaccine; Beta glucan

Infections of respiratory and urinary tract (UTI) represent the most common infections[1]. UTIs in particular show the most increasing numbers worldwide. This trend is noticeable not only in females of all age groups, but also in senior males[2]. UTIs now represent the most common nosokomial infection of females with over 35% of all infections and are second most common reason for bacteremia in hospitalized females[3]. In most cases, UTI starts as a bladder infection and can subsequently proceed to a kidney damage, acute pyelonephritis with additional kidney damage and development of sepsis via epithelial and endothelial barrier leakage[1].

The causes of UTI are infection with various microorganisms. In 60% to 90% cases the pathogens are heterogenous group of *Escherichia coli* (often characterized as uropathogenic *E. coli*)[1-7]. In addition, infection with *Proteus vulgaris*, *P. mirabilis*, *Klebsiella*

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species, *Enterococcus faecalis*, *Staphylococcus* and other pathogens are common[1,3-5,8-9]. UTIs repeated more than three times/year are considered to be repeated infections which are often accompanied by additional complications and request complex treatment in both males and females. The most common treatment is based on antibiotics requiring the exact knowledge of bacterial sensitivity. Suboptimal antibiotic treatment resulted in the majority of European countries in significant increase of multiresistance and subsequent suboptimal treatment[10-11]. Based on these problems, there is a current push to find alternative ways of UTI treatment, particularly via improvement of the immune status of infected individuals.

Clinical immunology is more and more involved in the search for solutions mentioned above. The major tools are complex evaluation of the patient, use of optimal diagnostic and monitoring of the immune system. Lately, vaccination is considered to be a good approach for solving the UTI manifestation. Last 20 years showed significant development of commercial vaccines used in both prevention and treatment of UTI[1-2,4-7,9]. However, not all commercially available vaccines were found useful in clinical practice. Possible solution might be the use of autovaccines prepared from isolated from patient's microorganisms. This approach is not new and was originally used by A.E. Wright more than 100 years ago by use of *Staphylococcus pyogenes* to improve phagocytic activity of patients[12]. However, the optimism caused by the discovery of antibiotics resulted in their overuse and subsequent development of resistance. Due to these worldwide problems, alternative treatment is currently more popular than ever. Current suggestions negate parenteral treatment and use vaccines perorally, sublingually and nasally[1-2,4-5,7,9,13]. An optimal solution of UTI problems depends partly on antibiotics and prevention but also on good immunotherapy[2,5]. Vaccination needs good monitoring of both clinical and laboratory state of patient. Our laboratory uses scheme of initial and subsequent repeated (every three months) control of microbiological, biochemical and immunological parameters.

Based on 30 years' experience with peroral application of autovaccines against UTI we recently switched to nasal application[8]. Initial evaluation of patients includes testing of both humoral and cellular immune reactions led us to evaluation of beta glucan as an additional aspect of autovaccine treatment. Our laboratory has long term good experience with beta glucans in wide spectrum of diseases[14]. Beta glucans are natural polysaccharides. Extensive research of beta glucans and their actions undertaken in last several decades helped to elucidate numerous mechanisms of action and their involvement in improvement of our health, including microbiome, probiotic and prebiotic effects and most of all, immune system[15-22]. Our studies suggested the use of orally-given beta glucan both in prevention and in therapeutical interventions in various types of diseases. The possibility to use beta glucans in application of autovaccines is the first step in preparation of qualitative new type of autovaccines against UTI[23].

We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.3978/j.issn.2095-6959.2021.09.001).

1 Materials and Methods

The experimental group consisted of 5 males with average age 72 years (range, 44–85 years) and 10 women with average age 57 years (range, 26–86 years) with diagnosis of UTI and wide spectrum of immunodeficiencies. In all patients we found repeated treatment with antibiotics with an average of 6 to 12 treatments over last 3 years. In all cases, the results of the treatment were only partial with only short-term effects. The clinical trial was conducted at the Zdravotní ústav se sídlem v Ústí nad Labem, Czech Republic and the study was approved by the Ethics committees of the Public Health Institute. This study was performed in agreement with Helsinki declaration (revised version 2013.09.01) and was in full compliance with the rules of for clinical testing in the Czech Republic. Informed consent was given in all cases.

1.1 Beta glucan

Yeast-derived insoluble beta glucan #300 (>85% dry w/w basis) was purchased from Transfer Point (Columbia, SC, USA). This beta glucan contains 96% carbohydrates and 2.1% proteins. Neutral sugar analysis confirmed 91.3% glucose and 8% mannose. Beta glucan was used at the dose of 500 mg/day per os. The supplementation started 14 days before the beginning of autovaccination and lasted two months.

1.2 Evaluation

In all patients we repeatedly tested bacterial load in urine with the time between sampling and evaluating being 1 to 2 h. In addition, from the same samples we measured the levels of inflammatory proteins, albumin, orosomukoid, IgA, C-reactive protein. Complex evaluation of inflammatory response (CRP, orosomukoid, serum amyloid A) and cellular immunity (NK cell activity) in blood was performed, too.

1.3 Autovaccine

Bacterial immunomodulatory autovaccine was prepared from isolated bacteria individually for each patient. Purified isolates were diluted to 3rd degree of McFarland opacity standard, i.e., 9×10^8/mL. Next, we prepared 60 mL of suspension and added 0.6 mL of 1% mertiolate (final concentration 0.001%) and 0.06 g of methylcellulose. After repeated 30 min inactivation during 24 h in 100 ℃ steam we controlled the sterility according Czech Pharmacopoeia 2005 in both thioglycolate and Sabouraud culture medium. Next, the suspension was divided into three 20 mL doses in spray containers. Prepared vaccines were stored at 2 to 8 ℃. Autovaccine was applicated daily in the morning by applying one dose into each nostril. The applications were controlled by checking the empty containers.

2 Results

Results summarizing the clinical, microbiological and immunological status of patients are shown in Figure 1. The evaluation was done after use of one dose of autovaccine, i.e., in 90–110 days intervals. Immediately after application of the first dose we observed significant improvements of clinical conditions. In addition, microbiological examinations of urine showed significant decrease of microbial counts in all men and in 6 women. In second control examination we found persistent improvements of clinical conditions. The exception being 3 female patients with diabetes mellitus, where low level of clinical manifestation persisted, but microbiological evaluation was consistently favorable. These conditions persisted for next 6 months.

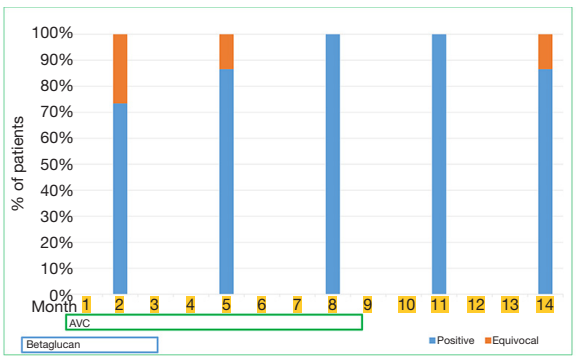

Figure 1 Monitoring of UTI treatment by a beta glucan-autovaccine combination shows index of clinical and subjective condition at various intervals. BG, beta glucan; AVC, autovaccine.

Significant changes were found in case of inflammatory markers. Just after one application, we found significant decrease of CRP and albumin levels. In case of orosomucoid, the decrease of levels was significant in all patients with exception of patients with diagnosis of diabetes mellitus, where the decrease was slow and gradual. In humoral immune responses we observed gradual decrease of CRP and SAA serum levels, three months after application of autovaccines were these levels comparable with controls. An interesting observation was reached in case of NK cells where we found return of depressed numbers of NK cell to normal levels. It is important to note that during our study none of the patients underwent any antibiotic treatment. Most of the patients requested the repeat of autovaccination, as the concerns about return of UTI were high.

3 Discussion

There are no doubts that ever-increasing frequency of UTI represents significant economic burden to the society. Therefore, the search for new and/or improved therapeutic approaches including vaccines and autovaccines is gaining traction[2,9]. First commercial vaccines against UTI appeared 30 years ago as Uro-Vaxom being the mixture of 18 different strains of *E. coli*[4]. Subsequent push for use of vaccines in UTI treatment offered additional approaches how to use microbial components in order to prepare multispectral defense[8]. Urovac, containing much broader spectrum of uropathogens, can serve as an example[1]. However, peroral use of this product did not ensure risk of relapse in our patients, most probably due to the different colonization in individual patients and to the low level of their immune responses. In our clinic we often see patients with wide spectrum of health problems which accompany UTI which was not diagnosed before. It commonly occurs in patients with diabetes, cancer, sclerosis and other diseases, which all are also manifested by significant depression of immune system[4]. Compared to oral application, nasal application of our autovaccines resulted in improved and stronger therapeutic effects, which is in an agreement with other findings[5-6,8]. It shows that even preventive application of autovaccines can have significant effects[1-4], confirmed by both microbiological and immunological evaluations. It is clear that autovaccines can be routinely used not only in treatment, but also in prevention of UTI[13]. Monitoring of UTI from clinical, microbiological and immunological point of view is necessary[3]. Current clinical practice offers various parameters of immune responses which will be of

interest in these cases, the decision which reaction to test is important. The fast and solid information are important for subsequent treatment[3]. Temporary proteinuria is often observed in acute stages and is manifested by presence of numerous proteins in urine, most of all orosomucoid and albumin[7]. Monitoring of these proteins in urine can offer several important information, as microalbuminuria together with the presence of IgG represents a marker of capillary permeability[7]. Similarly, orosomucoid is an indicator of acute inflammatory reaction and has significant immunomodulatory and anti-inflammatory effects. In addition, it also plays a role in keeping optimal barriers against transendothelial permeability of various macromolecules. Increased urine levels of orosomucoid is one of the markers of increased mortality of diabetes type II patients. In our experience, orosomucoid is more important marker of inflammatory response than higher levels of albumin[7,24]. Isoform 1 of orosomucoid can stimulate monocytes and regulate macrophages and results in lower incidence of opportunistic infections[24-25]. The results of evaluation of orosomucoid levels in our patients confirmed these findings. Based on this information, are preparing subsequent studies evaluation the significance of orosomucoid levels not only in UTI patients, but also in patients suffering from additional health problems.

A large study on immunomodulatory effects of beta glucan in children with respiratory problems clearly demonstrated the positive effects of beta glucans mediated by improvements of the immune system[26-27]. In addition, our experiences with beta glucan application in diabetic patients and in cancer patients showed strong immunomodulatory effects[14]. Results of clinical studies together with information on possible use of beta glucans in vaccination[23] led us to the current study which used beta glucan for immune support during autovaccination. Our clinical results are promising, and the therapeutic effects are clear. However, the relatively low number of patients underlines the need for a larger study.

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Footnote

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