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(54) **VACCINE COMPRISING LACTOBACILLI FOR TREATING PROSTATE INFLAMMATION AND BENIGN PROSTATE HYPERPLASIAS**

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(57) **ABSTRACT**

The invention relates to vaccines for treating prostate inflammation and benign prostate hyperplasias (stages I and II) comprising *Lactobacillus* strains in an inactivated form and carriers and/or excipients commonly used in vaccine preparations.

# VACCINE COMPRISING LACTOBACILLI FOR TREATING PROSTATE INFLAMMATION AND BENIGN PROSTATE HYPERPLASIAS

## FIELD OF INVENTION

**[0001]** The present invention relates to a vaccine comprising *Lactobacillus* strains useful in treating prostate inflammation and benign prostate hyperplasias (stages I and II).

## BACKGROUND OF THE INVENTION

**[0002]** The pathogenecity of certain *Lactobacillus* strains has been reported in 1938 [F. Marshall: Der Döderleinische *Bacillus vaginalis* als Endokarditiserreger, *Zentr. Bact. Parasit. Kde. I. Abt. Orig.,* 141:153-159 (1938); E. Biocca és A. Sepilli: Human infections caused by lactobacilli, *J. Inf. Dis.,* 81:112-115 (1947); W. Sims: A pathogenic *Lactobacillus, J. Path. Bact,* 87:99-105 (1964); B. Rosan and B. F. Hammond: Toxicity of *Lactobacillus casei, J. Dent. Res.,* 44:783-787 (1965); M. E. Sharpe, L. R. Hill and S. P. Lapage: Pathogenic lactobacilli, *J. Med. Microbiol.,* 6, 281-286 (1973).

**[0003]** G. Wied reported in 1952 [Zbl. Bact., 160:413 (1952)] that certain *Lactobacillus* strains show mucous membrane damaging activity. Rosan and Hammond [1965, ibid.] reported that, with *Lactobacillus* strains strongly pathogenic to mice, intradermal inoculation of bacteria both in living and in thermally inactivated states causes necrosis on the back of rabbits.

**[0004]** K. Újhelyi has found that necrosis can be induced also by *Lactobacillus* strains cultivated from vagina. Based on his observation, it can be stated that the body of the bacterium contains a toxin which is responsible for damaging the epithelia [Újhelyi K. et al.: Role of *Lactobacillus* in urogenital inflammations and their treatment with vaccination, Symposium cum participations internationalis de Biocenosi Vaginae, Smolenie, 1983]. Certain strains, injected intradermally to the back of rabbits, cause necrosis of smaller or larger areas, while others cause necrosis only in higher concentration or do not cause necrosis at all. K. Újhelyi has found that rabbits can be immunized by vaccination against the necrotic effect. He vaccinated rabbits intramuscularly with vaccine produced from certain *Lactobacillus* strains. Six weeks later, he intradermally administered cell-suspensions prepared from strains that have been shown previously to be necrotic, and observed that necrosis was not caused or was only caused in a lesser degree than in the case of non-vaccinated rabbits.

**[0005]** Furthermore, K. Újhelyi has found that *Trichomonas vaginalis* contributes to the rise in vaginal pH by consuming lactic acid produced by *Lactobacillus* strains in the vagina, thereby promoting the over-proliferation of *Lactobacillus* strains. Consequently toxin is present in higher concentration which, by damaging the mucous membrane, causes cell necrosis.

**[0006]** Furthermore, it is known that *Lactobacillus* strains, because of their receptor inhibiting and antibiotic activity as well as pH-modifying effect, are antagonistic to pyogenic microorganisms [Reddy et al.: Natural antibiotic activity of *Lactobacillus, Dairy Prod. J* 18:15-22 (1983); Salminen et al.: Lactic acid bacteria in the gut in normal and disordered states, *Dig. Dis.,* 10:227-238 (1992)].

**[0007]** Recently, it has been shown that *Lactobacillus* strains can bind directly to T-lymphocytes since both the T-helper and T-killer cells have specific receptors for *Lactobacillus* strains. Furthermore, *Lactobacillus* strains promote the gamma-interferon production of the lymphocytes and the cytotoxic activity of the natural killer cells [De Simone C., et al.: Enhancement of immune response of murine Peyer's pothes by a diet supplemented with yoghurt, *J. Immunopharmacol.,* 1:87-95 (1987)]. It has been shown that *Lactobacillus* strains aspecifically increase the production of IgM and IgG [Blocksma et al.: Adjuvant activity of lactobacilli, different effects of viable and killed bacteria, *Clin. Exp. Immunol.,* 37:367-373]. Additionally, under experimental conditions, *Lactobacillus* strains show antitumour and macrophage-activating activity [Kato I. et al.,: Antitumor activity of *Lactobacillus casei* in mice, Gann, 72:517-523 (1983); Oda M. et al.: Antitumor polysaccharide from *Lactobacillus* sp., *Agric Biol. Chem.,* 47:1623-1627 (1983)]. H. Rüttgers has found that immunostimulation by *Lactobacillus* strains causes a significant long-lasting rise in secretory immunoglobulin level in the vagina [Bacterial vaginitis: Protection against infection and secretory immunoglobulin levels in the vagina after immunization therapy with Gynatren, *Gynecol. Obstet. Invest.,* 26:240-249 (1988)].

**[0008]** Újhelyi et al. [1983, ibid.] used parenterally administered *Lactobacillus* strains for aspecific immunostimulation and observed that the *Lactobacillus* strains used, in contrast to other aspecific immunostimulation (e.g. by BCG, endotoxins etc.), show protective effect against certain bacterial toxins. This applies especially to toxic *Lactobacillus* strains.

**[0009]** In trials carried out with vaccines (Gynevac®, Gynatren®, Solco Trichovac®) made of strains cultured by Újhelyi it has been demonstrated that immunostimulation by *Lactobacillus* strains, in contrast to other therapeutic treatments, restores the biological balance of the vagina, normalizes the pH, decreases the number of pathogenic bacteria, and contributes to the propagation of Döderlein-flora (a mixed population of *Lactobacillus* strains capable of being cultivated from vaginal specimens). It is an accepted fact that inflammatory diseases of the vagina caused by bacterial and Trichomonas infections can be cured in this way more successfully than by other therapy and that such inflammatory conditions are a major cause of premature births. Therefore, the frequency of premature births can also be decreased by such therapy [see e.g. in Genitalinfektion der Frau (SolcoTrichovac/Gynatren), *Geburtsch. u. Frauenheilk,* 44:311 (1984); E. Lázár, Gy. Varga, I. Institoris and K. Újhelyi: Investigating the factors, especially vaccination with lactobacilli, influencing the premature births, in Kazincbarcika (in Hungarian), *Magyar Nöorvosok Lapja* (Journal of Hungarian Gynaecologists), 51:353-356 (1986); E. Lázár, Gy. Varga, I. Institoris and K. Újhelyi: Decreasing the ratio of neonates with small weight by lactobact vaccination of pregnant women (in Hungarian), *Orvosi Hetilap* (Physicians Weekly), 37:2263-2268 (1981), Rüttgers, 1988, ibid., K. Újhelyi, Gy. Philipp, Gy. Plank and V. Sági: The Trichomonas syndrome I (in Hungarian), *Magyar Nöorvosok Lapja* (Journal of Hungarian Gynaecologists), 36:433-442 (1973); Sharon et al., *New England Journal*, Dec. 28, 1995.].

**[0010]** More than 50% of men aged 50 or more suffer from prostate hyperplasia and/or prostate inflammation. In spite of numerous known and utilized therapies, treatment is often unsuccessful. Taking into consideration the known and generally accepted pathogenesis, it could not be supposed that such diseases can be healed with vaccines comprising *Lactobacillus* strains successfully.

**[0011]** The inventors of the present invention have, however, found that conditions in the prostate are favorable to the proliferation of *Lactobacillus* strains and that pathogenic lactobacilli can often be cultivated from patients suffering from chronic prostate inflammation and/or prostate hyperplasia. On this basis, therapeutic utilization of vaccines comprising *Lactobacillus* strains for treating such patients has been achieved.

## DISCLOSURE OF THE INVENTION

**[0012]** The invention relates to vaccines for treating prostate inflammations and benign prostate hyperplasias (stages I and II) comprising *Lactobacillus* strains in inactivated form and carriers and/or excipients commonly used in vaccine preparations.

**[0013]** In another aspect, the invention relates to the use of *Lactobacillus* strains for producing vaccines capable of treating prostate inflammation and benign prostate hyperplasias (stages I and II).

**[0014]** In a further aspect, the invention relates to the use of *Lactobacillus* strains for treating patients suffering from prostate inflammation and benign prostate hyperplasias (stages I and II).

**[0015]** Furthermore, the invention relates to a method of treating patients suffering from prostate inflammation and benign prostate hyperplasias (stages I and II) comprising administering an effective dose of a strain-suspension of *Lactobacillus* strains intramuscularly to a patient in need of such treatment.

**[0016]** In an embodiment of the method of the invention, the strain-suspension of *Lactobacillus* strains comprises a mixed population of the said strains in inactivated form.

**[0017]** The lactobacilli used in the vaccine of the invention are *Lactobacillus* strains used in the above-said vaccines Gynevac®, Gyantren® and Solco Trichovac® that previously have been cultivated from women suffering from gynecologic inflammations of bacterial origin. The single cultivated strains can be use per se or in the form of a blend of the strains.

**[0018]** The vaccine of the invention can be produced by methods commonly used for preparing vaccines. Advantageously, the cultivated strains are stored in lyophilized form, then, before use, they are propagated by culturing in Man-Rogosa-Sharpe medium at 45° C.

**[0019]** The composition of the said medium and the preparation method are set forth below.

**[0020]** To 2300 ml of sterile water the following components are added sequentially, after dissolving the previously added component:

| | |
|---|---|
| Bactotripton (Raenal) | 30 g |
| Lablemko (Reanal) | 30 g |
| $K_2HPO_4$ | 6 g |
| triammonium citrate | 6 g |
| sodium acetate | 15 g |
| glucose | 30 g |
| lactose | 30 g |
| maltose | 9 g |
| yeast extract (Reanal) | 15 g |
| Tween 80 | 3 ml |
| Salt solution (composition see below) | 15 ml |

**[0021]** The obtained solution is adjusted to 3000 ml by the addition of sterile water, filtered on G4 filter, bottles in smaller volumes and sterilized at 121° C.

**[0022]** The composition of the above-said salt solution is as follows: 28.75 g of $MgSO_4$-$7H_2O$, 6 g of $MnSO_4$-$2H_2O$ and 1.7 of $FeSO_4$-$7H_2O$ dissolved in 250 ml of sterile water.

**[0023]** After culturing, the cells are harvested by centrifuging and are suspended in physiological saline solution and treated with formaldehyde. The inactivated cells are harvested and resuspended in physiological saline solution. The level of dilution is adjusted on the basis of the protein content of the suspension. The protein content of the vaccine (suspension) of the invention is at least 0.08 mg/ml, and may be up to 1 mg/ml or more, preferably from about 0.08 to about 0.32 mg/ml, more preferably about 0.16 mg/ml.

**[0024]** The dosage of the vaccine of the invention and the frequency of the administration depend on the conditions of the patient and the severity of the symptoms to be treated. The precise dose and frequency of administration should be specified by the practicing physician. During treatment, it is advantageous if the vaccine is administered intramuscularly in a volume of 1 ml, once a week for five weeks.

**[0025]** The following example is given for the purpose of illustration of the invention without the intention of limiting of the scope claimed.

## EXAMPLE

**[0026]** Investigations were carried out with the vaccine of the invention by administering same to patients with a diagnosis of prostate inflammation and/or prostate hyperplasias (stages I and II). The patients were administered intramuscularly 1 ml of a vaccine comprising *Lactobacillus* strains of the invention once weekly for 5 weeks, without any other medical treatment. The results of the control examination carried out after this cure are summarized in the following Tables.

Number of the treated patients: 127

Diagnosis: prostate hyperplasia stages I and II

| Condition of the | Time elapsed after the treatment | | |
|---|---|---|---|
| patients | 4 to 8 weeks | 2 to 4 months | 6 months |
| Healed | 52 (40.94%) | Worsening of the | 60% of 94 |
| Improved | 47 (37.0%) | condition was not | examined patients |
| Unchanged | 28 (22.0%) | observed in any of | were symptom- |
| Worsened | 0 | the patients. | free. |

Number of the treated patients: 168

Diagnosis: prostate inflammation

| Condition of the | Time elapsed after the treatment | | |
|---|---|---|---|
| patients | 4 to 8 weeks | 2 to 4 months | 6 months |
| Healed | 76 (45.23%) | Worsening of the | 70% of 79 |
| Improved | 61 (36.31%) | condition was not | examined patients |
| Unchanged | 31 (18.45%) | observed in any of | were symptom- |
| Worsened | 0 | the patients. | free. |

**[0027]** As can be seen in the above Tables, a significant ratio of the patients were healed or their conditions improved essentially.

**1**. A process for treating a patient suffering from prostate inflammation, benign prostate hyperplasia stage I, or benign prostate hyperplasia stage II, said process comprising vaccinating the patient with a vaccine, said vaccine containing an effective amount of a mixture of *Lactobacillus plantarum, Lactobacillus paracasei* and *Lactobacillus fermentum* in its inactivated form.

**2**. A method of treating a patient suffering from prostate inflammation, benign prostate hyperplasia stage I, or benign prostate hyperplasia stage II, said method comprising vaccinating said patient intramuscularly with an effective dose of a suspension of a mixture of *Lactobacillus plantarum, Lactobacillus paracasei* and *Lactobacillus fermentum* in its inactivated form.

**3**. The method of claim **2**, wherein the patient is suffering from a benign hyperplasia stage I or II.

**4**. The method of claim **2**, wherein said *Lactobacillus* strains previously have been cultivated from women suffering from gynecologic inflammations of bacterial origin.

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