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(54) Title: A VACCINE COMPRISING LACTOBACILLUS STRAINS FOR TREATING PROSTATE INFLAMMATION AND BENIGN PROSTATE HYPERPLASIAS

(57) Abrégé/Abstract:
The invention relates to vaccines for treating prostate inflammation and benign prostate hyperplasias (stages I and II) comprising lactobacilli in an inactivated form and carriers and/or excipients commonly used in vaccine preparations.

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(54) Title: A VACCINE COMPRISING LACTOBACILLI FOR TREATING PROSTATE INFLAMMATION AND BENIGN PROSTATE HYPERPLASIAS

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

A vaccine comprising *Lactobacillus* strains for treating prostate inflammation and benign prostate hyperplasias

FIELD OF THE INVENTION

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

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).

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, in case of *Lactobacillus* strains strongly pathogenic to mice, intradermal inoculation of bacteria both in living and in thermally inactivated state causes necrosis on the back of rabbits.

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 participatione internationalis de Biocenosi Vaginae, Smolenie, 1983]. Certain strains injected intradermal to the back of rabbits cause necrosis of smaller or larger area, while others cause necrosis only in higher concentration or do not cause necrosis at all. K. Újhelyi has found that rabbits can be immunised by vaccination against the necrotic effect. He vaccinated rabbits intramuscularly with vaccine produced from certain *Lactobacillus* strains, 6 weeks later he administered intradermally 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 case of non-vaccinated rabbits.

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

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 disorded states, *Dig. Dis.*, **10**: 227-238 (1992)].

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, furthermore, 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 raise of 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)].

Újhelyi *et al.* [1983, ibid.] used parenterally *Lactobacillus* strains for aspecific immunostimulation and observed that the used *Lactobacillus* strains, 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.

In trials carried out with vaccines (Gynevac (R), Gynatren (R), SolcoTrichovac (R)) 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 vagina). Nowadays 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, on the one hand, and that such inflammatory conditions are the most important reason of premature births, on the other hand. 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. Làzàr, 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. Làzàr, 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. Sàgi: The Trichomonas syndrome I (in Hungarian), *Magyar Nöorvosok Lapja* (Journal of Hungarian Gynaecologists), **36**: 433-442 (1973); Sharon *et al.*, New England Journal, December 28, 1995.]

More than 50 % of men aged 50 or more suffer from prostate hyperplasia and/or prostate inflammation. In spite of numerous kind of known and utilized therapies these patients are in need of medical treatment which is often unsuccessful. Taking into consideration the known and generally accepted pathogenesy, it could not be supposed that such diseases can be healed with vaccines comprising *Lactobacillus* strains successfully.

The inventors of the present invention have, however, found that the conditions in prostate are favourable 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 worked out.

DISCLOSURE OF THE INVENTION

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.

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).

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).

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

In a preferable embodiment of the method of the invention the strain-suspension of *Lactobacillus* strains comprises a mixed population of the said strains in inactivated form.

The lactobacilli used in the vaccine of the invention are *Lactobacillus* strains used in the above-said vaccines Gynevac$^{(R)}$, Gyantren$^{(R)}$ and SolcoTrichovac$^{(R)}$ that previously have been cultivated from women suffering from gynaecologic inflammations of bacterial origin. The single cultivated strains can be used *per se* or in the form of a blend of the strains.

According to one aspect of the present invention, there is provided *Lactobacillus* strains as used in the vaccines Gynevac®, Gynatren®, and Solco Trichovac® in inactivated form together with a vaccination acceptable carrier, excipient or a combination thereof, for use in treatment of prostate inflammation and benign prostate hyperplasias stages I and II.

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

The composition of the said medium and the preparation method are set forth below.

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

Bactotripton (Reanal)	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

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

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

After culturing the cells are harvested by centrifuging, the pellet is 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.

The used dose 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 practitioning physician. According to our experiences it is advantageous if during a cure

the vaccine is administered intramuscularly in a volume of 1 ml, once weekly, altogether 5 times.

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

EXAMPLE

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 summarised in the following Tables.

Number of the treated patients: 127

Diagnosis: prostate hyperplasia stages I and II

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

Number of the treated patients: 168

Diagnosis: prostate inflammation

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

As can be seen in the above Tables, a significant ratio of the patiens were healed or their conditions improved essentially.

Claims

1. *Lactobacillus* strains as used in the vaccines Gynevac®, Gynatren®, and Solco Trichovac® in inactivated form together with a vaccination acceptable carrier, excipient or a combination thereof, for use in treatment of prostate inflammation and benign prostate hyperplasias stages I and II.

2. *Lactobacillus* strains of claim 1 as strain-suspension for intramuscular administration.

3. *Lactobacillus* strains of claim 2, wherein the strain-suspension comprises a protein content of at least 0.08 mg/ml.

4. *Lactobacillus* strains of claim 3, wherein the strain-suspension comprises a protein content of from 0.08 mg/ml to 0.32 mg/ml.

5. *Lactobacillus* strains of claim 3 or 4, wherein the strain-suspension is for intramuscular administration in a volume of 1 ml, once weekly, altogether 5 times.

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