

IMMUNOMODULATING EFFECT OF LYPRINOL IN HUMANS  
VACCINATED WITH LIVE INFLUENZA VACCINE

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## 1. Introduction

Live cold-adapted reassortant influenza vaccine (LAIV) is an effective means of controlling influenza among children, adults and elderly [1-3]. In comparison with inactivated influenza vaccines, LAIV has the advantages of stimulating the mucosal as well as cellular immune responses whereas it is slightly less effective in stimulating the serum antibody responses [4]. The possibility of using Lyprinol to raise serum antibody as well as cellular immune responses in human after vaccination with live attenuated influenza vaccine were studied. Lyprinol is a natural product with potent anti-inflammatory properties comprising marine lipids

containing omega-3 polyunsaturated fatty acids (PUFAs), extracted from stabilized New Zealand Green-Lipped Mussel (*Perna canaliculis*). Lyprinol is safe, gastro-protective and has no contraindications. Clinical studies have demonstrated very significant anti-inflammatory activity in patients with osteoarthritis, rheumatoid arthritis and asthma [5,6]. The immunomodulating properties of Lyprinol and its effect on the immune and hemopoietic systems were studied.

## **2. Materials and method**

### *2.1. Preparations*

Russian live attenuated influenza vaccine was prepared using cold-adapted master strains A/Leningrad/134/17/57 (H2N2) and B/USSR/60/69 and contain the following strains: A/17/Beijing/95/25 (H1N1) (6.5LgEID<sub>50</sub>/0.2ml), A/17/Sydney/95/25 (H3N2) (6.5LgEID<sub>50</sub>/0.2ml), and B/60/St.Petersburg/95/20 (6.0LgEID<sub>50</sub>/0.2ml). 0.5 ml of LAIV was administered by intranasal spray. 0.5 ml placebo vaccine comprising lyophilized reconstituted uninfected allantoic fluid was administered by intranasal spray [4]. Lyprinol is a stabilized marine lipid extract rich in a select group of unique eicosatetraenoic acids. Lyprinol is produced by Biomex Pty, Ltd. (Australia) in capsular form (150 mg per capsule) and it taken orally as a food supplement. Placebo Lyprinol was capsulated (150 mg per capsule) pure olive oil.

## *2.2. Study design*

The study comprised four groups of 10 adult volunteers aged 18-48 years (median 34 years): group (I) taken four 150 mg capsules Lyprinol daily during 28 days after one dose intranasal placebo-vaccine; group (II) taken Lyprinol for same scheme after one dose intranasal trivalent live attenuated vaccine; group (III) taken four placebo-lyprinol capsules during 28 days after one dose of intranasal LIV; group (IV) taken placebo-lyprinol for the same scheme after one dose of intranasal placebo-vaccine.

## *2.3. Clinical studies*

Individuals were monitored for symptoms of diseases throughout the study. All participants had no history of egg allergy and previous influenza vaccination. Once a week during the study all participants were examined by a physician. No adverse clinical reaction was observed in any volunteers during the study.

## *2.4. Laboratory studies*

Venous blood samples and nasal swab samples were collected from each volunteer at 3 time points: (i) prior to study, (ii) 28 days after vaccination, and (iii) 84 days after vaccination (56 days after taking last Lyprinol capsules). The titres of specific serum antibodies (HI), specific mucosal IgA-antibodies (ELISA), total IgA-, IgG-, IgM- antibodies in serum (ELISA), functional activity of neutrophils (NBT-test), proliferative

activity of lymphocytes stimulated *in vitro* by PHA or influenza antigen and total and differential white blood cells count were studied by standard procedures as described [4,7]. For *in vitro* assays the same influenza antigens as in the vaccine preparations were used.

### 3. Results

In those individuals who had taken Lyprinol after vaccination with LIV (group II) the number of seroconversions and GMTs of HI antibodies were 1.5-2.2 times ( $P<0.01-0.001$ ) higher than respective values for those who received LIV without Lyprinol (group III) (Table.1). Geometric mean titres (GMT) rises after vaccination in individual from group II were 2.7-3.2 ( $P<0.001$ ) and were 1.8-2.2 ( $P<0.01$  and  $P<0.05$ ) in those from group III. Likewise, the lymphocyte proliferative activity after vaccination (net CPM) in individuals from group II were 3.3 times higher when stimulated *in vitro* with A(H1N1) antigen and 2.7 times higher ( $P<0.001$ ) when stimulated *in vitro* with PHA than in individuals from group III (Table. 2). Lyprinol (group I) did not influence the total and differential blood count, functional activity of neutrophils, total IgA-, IgG-, IgM- antibodies concentrations in serum and specific nasal IgA antibodies response (data not shown) when compared with placebo-only volunteers (group IV).

#### **4. Discussion**

The results obtained showed that Lyprinol possesses significant prolonged immunomodulating effects on postvaccination systemic and cellular immune responses in humans, vaccinated with live influenza vaccine. There was no indication of Lyprinol being immunosuppressive. It was established by previous data that live influenza vaccine stimulates secretory antibodies production, T-cell response and production of Th1-cytokines to a greater extent than inactivated vaccine, but less effective in serum HI antibodies production [4,8-10]. The above data indicates the possibility of utilizing Lyprinol as an active and safe stimulant of serum antibody and cell-mediated immune responses in humans vaccinated with live influenza vaccine. Lyprinol's mode of action was not the subject of this study but may be a matter of future investigation. It was shown that Lyprinol treatment did not lead to systemic and local immunity suppression and did not alter the white blood formula. Moreover, using Lyprinol may be recommended for elderly patients with age-related immune disorders. In which case Lyprinol treatment may have a double action: treatment of autoimmune disorders including asthma, rheumatoid arthritis, osteoarthritis in addition to the immunomodulating effect in respect of annual influenza vaccination.

## References

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**Abstract**

Live trivalent cold-adapted reassortant influenza vaccine is an effective preparation for the control of influenza among children, adults and elderly. In comparison with inactivated influenza vaccines it has advantages in the stimulation of mucosal and cell mediated immune responses, but has no advantages in induction of serum antibodies. Therefore the study of approaches to raise of immune response in human after vaccination with live attenuated vaccine is desirable. In this study we evaluate the possibility of using Lyprinol, a safe and active food supplement as an immunostimulator of human serum antibody immune response after vaccination with live influenza vaccine. The study has shown that Lyprinol possesses significant immunostimulating effect with a prolonged action on post-vaccination antibody and cell mediated immune response to live influenza vaccination. Lyprinol treatment did not lead to any suppression of systemic or local immunity and did not alter total or differential white blood counts. It is conceivable that Lyprinol could be useful to stimulate the immune response to live influenza vaccine, particularly in the elderly with age-related immune disorders.

Key words: live attenuated influenza vaccine, lyprinol, immune response



Table 1. Influence of Lyprinol on serum HI antibody response in humans vaccinated with live attenuated influenza vaccine.

Group	Preparation	No. In Group	% with 4-fold or greater rise of HI antibodies			GMT <sup>b</sup>								
			A(H1N1)	A(H3N2)	B	A(H1N1)			A(H3N2)			B		
						Pre	Post <sup>a</sup>	Rise	Pre	Post <sup>a</sup>	Rise	Pre	Post <sup>a</sup>	Rise
I	Lyprinol + Placebo Vaccine	10	0	0	0	17	20	1.2	16	19	1.1	19	24	1.3
II	Lyprinol + Vaccine	10	60.0	60.0	60.0	16	43	2.7	11	33	3.0	11	35	3.2
III	Placebo Lyprinol + Vaccine	10	30.0	40.0	30.0	9	20	2.2	8	16	2.0	9	16	1.8
IV	Placebo Lyprinol + Placebo Vaccine	10	0	0	0	12	12	1.0	9	9	1.0	16	13	0.8

<sup>a</sup> – samples collected at 56 days after last Lyprinol taking

<sup>b</sup> – geometric mean antibody titres

Table 2. Influence of Lyprinol on cell mediated immune response in humans vaccinated with live attenuated influenza vaccine.

Group	Preparation	No. in group	Proliferative immune response PBMC stimulated with in vitro ( Net CPM <sup>b</sup> )					
			A(H1N1) <sup>c</sup>			PHA		
			Pre	Post <sup>a</sup>	Rise	Pre	Post <sup>a</sup>	Rise
I	Lyprinol + Placebo Vaccine	10	227	379	1.7	13707	10506	0.8
II	Lyprinol + Vaccine	10	498	5092	10.2	2386	12575	5.3
III	Placebo Lyprinol + Vaccine	10	339	1526	4.5	2241	5085	2.3
IV	Placebo Lyprinol + Placebo Vaccine	10	440	555	1.3	8230	7456	0.9

<sup>a</sup> samples collected at 56 days after last Lyprinol taking

<sup>b</sup> average of individual values counts per minute (cpm) <sup>3</sup>H-thymidine uptake in stimulated cultures subtracted cpm in media control cultures

<sup>c</sup> A/17/Beijing/95/29 (H1N1)

## DECLARATION

We, authors of paper “Immunomodulating effect of Lyprinol in humans vaccinated with live influenza vaccine” hereby confirm that above paper has not being submitted for publication elsewhere.

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