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Influenza vaccine as prevention for cardiovascular diseases: Possible molecular mechanism

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ABSTRACT

Despite plausible evidence for beneficial effects of the vaccination against influenza in cardiovascular diseases (CVD) very limited studies have been carried out to explain the molecular mechanism of this phenomenon. Using the informational spectrum method (ISM), a virtual spectroscopy method for analysis of protein–protein interactions, the bradykinin 2 receptor (BKB2R) was identified as a principal host protein which could mediate molecular processes underlying the cardioprotective effect of influenza vaccines.

Based on this finding we suggest that some antibodies elicited by influenza vaccines act as agonists, which activate a BKB2R-associated signaling pathway contributing to the protection against CVD. The ISM analysis of 14 influenza viruses, which were used as components of seasonal vaccines, revealed four vaccine viruses A/Beijing/262/95(H1N1), A/NewCaledonia/20/1999(H1N1), A/Christchurch/28/2003(H3N2) and A/Perth/16/2009(H3N2), which could be suited best for further studies on the cardioprotective effect of influenza vaccines.

1. Introduction

According to the recently released World Health Organization report more people die annually from cardiovascular diseases (CVD) than from any other cause [1]. It is estimated that 17.3 million people died from CVDs in 2008, representing 30% of all global deaths [1]. It is projected that CVD will remain the single leading cause of death and that by 2030, almost 23.6 million people will die from these diseases [1].

Children and adults with CVD are particularly vulnerable to complications of influenza infections [2–4]. For this reason, immunization against influenza has a critical role in the prevention of serious complications in patients with CVD during the influenza season. Numerous studies confirmed a strong correlation between influenza vaccination and reduction of cardiovascular events [5–12]. Recent systematic review and meta-analysis of randomized controlled trials from the Netherlands, Argentina, Poland and Thailand, conducted between 1994 and 2008 that studied effects of influenza vaccination in patients with and without established CVD revealed about 50% reduction in the risk of having a major cardiovascular event in the year following vaccination [13]. This protective effect of the influenza vaccine was also observed beyond the influenza season [7,11,14]. This convincing evidence of a protective role of influenza vaccines in CVD suggests their possible use as cost-effective primary and secondary preventive intervention in CVD patients, as well as in CVD risk patients (see Ref. [15] and references therein).

Understanding the molecular mechanism underlying this phenomenon is the basis for further studies of influenza vaccines as possible preventive intervention against CVD. Despite numerous publications reporting plausible evidence for beneficial effects of the vaccination against influenza in CVD, rarely studies have been carried out to explain the molecular mechanism behind these protective effects. Recently, Bermudez-Fajardo and Oviedo-Orta, based on results obtained with adapted influenza viruses in mice, suggested that influenza vaccination may protect against CVD by promoting atherosclerotic plaques stabilization and anti-inflammatory responses [16]. However, other authors mainly ascribe this protective effect to the prevention of virus infection per se, as it causes a procoagulant state, representing an important risk

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factor for developing CVD (for review see [17]). A recent most comprehensive and systematic review and meta-analysis of controlled clinical trials investigating the association between influenza vaccination and cardiovascular outcomes in high risk patients between 1946 and 2013, revealed that between 3238 CVD patients who received a flu vaccine and 3231 patients in the control or placebo group the difference in absolute risk is 1.74%, which means 58 people would have to be vaccinated to prevent one major CV event [18]. However, a separate analysis in this study of patients with the acute coronary syndrome (ACS) showed that the difference in absolute risk is 12.9%, meaning that vaccinating just eight people would prevent one cardiovascular event [18]. According to a variety of studies, the incidence of infection during the influenza season is 4–5 flu cases per 100 adults. On the other hand, the flu vaccine effectiveness is about 50% [19]. Thus, the vaccine prevents about 2 flu cases per 100 persons. This raises the question how it is possible to prevent 1 case of serious cardiovascular complication for every 8 vaccinated persons, if the vaccine only protects 2 of 100 persons from infection. These data, together with the finding that influenza vaccine protects against CVD beyond the influenza season [7,11,14], suggest that prevention of infection is not the only mechanism underlying this phenomenon. The small amount of antigen in a vaccine shot and the prolonged protective effect against CVD implies that the key role in this phenomenon is mediated indirectly by antibodies elicited by the influenza vaccine. This in turn raises the question, why antibodies induced by natural infection are not also protective against CVD, but rather during the influenza season the frequency of CVD diseases is increasing. A possible explanation may be that the protective role of antibodies elicited by influenza virus infection is masked by CVD caused by different pathogenic viral components (e.g. NS1, PB1, PB2 proteins [20–23]).

Here we hypothesized that some antibodies elicited by influenza vaccines act as agonists, which activate a BKB2R-associated signaling pathway contributing to the protection against CVD. We also propose four vaccine virus strains that could be suited for future investigations to further elucidate the role of influenza vaccines as primary and secondary prevention against CVD.

2. Material and methods

2.1. Databases

Hemagglutinins from influenza A viruses were retrieved from the GenBank and GISAID data bases.

Human proteins (66,623 sequences) were retrieved from the UniProt database.

2.2. Informational spectrum method

The informational spectrum method (ISM), a virtual spectroscopy method was developed for a fast and simple structure analysis of proteins and their functionally important domains. Physical and mathematical basis of the ISM is described in detail elsewhere (for review see Refs. [24,25]) and here the method is only presented briefly.

A sequence of N amino acid residues is represented as a linear array of N terms, with each term given a weight. The weight assigned to a residue is the electron–ion interaction potential (EIIP) (Table 1) [26,27], determining the electronic properties of amino acids, which are responsible for their intermolecular interactions [28]. In this way the alphabetic code (Fig. 1a) is transformed into a sequence of numbers (Fig. 1b). The signal obtained is than decomposed in periodical function by Fourier transformation. Thus, the initial information defined by the sequence of amino acids can now be presented in the form of an informational spectrum (IS), representing the series of frequencies and corresponding amplitudes (Fig. 1c). The IS frequencies correspond to the distribution of structural motifs with defined physico-chemical characteristics determining long-range interaction properties of the protein.

Table 1
The electron–ion interaction potential (EIIP) used to encode amino acids.

Amino acid	EIIP [Ry]
Leu	0.0000
Ile	0.0000
Asn	0.0036
Gly	0.0050
Glu	0.0057
Val	0.0058
Pro	0.0198
His	0.0242
Lys	0.0371
Ala	0.0373
Tyr	0.0516
Trp	0.0548
Gln	0.0761
Met	0.0823
Ser	0.0829
Cys	0.0829
Thr	0.0941
Phe	0.0946
Arg	0.0959
Asp	0.1263

The primary structures of interacting proteins or proteins interacting with a common interactor encode the common information, which is represented by the same code/frequency pair(s) in their informational spectra (IS). This common informational characteristic of sequences is determined by cross-spectrum or consensus informational spectrum (CIS). Peak frequencies in CIS represent common information and are characterized by the amplitude and the signal-to-noise ratio (S/N, ratio of the amplitude value on particular frequency and the sum of amplitudes on all frequencies in IS). The amplitude values in CIS determine the efficacy of protein–protein interaction and parameter S/N determines the specificity of a particular protein–protein interaction.

3. Results

All influenza vaccines which exerted protective effects against CVD were trivalent vaccines that encompassed two viruses of type A (H1N1 and H3N2) and one virus of type B. Because the protective effect of the influenza vaccines against CVD is prolonged during the second year after vaccination [7] and because antibodies against type A viruses are known to persist beyond the influenza season [7,11,14], in contrast to antibodies against type B viruses, which significantly wane over the second year [29], we directed our analysis toward the type A influenza viruses. This decision was supported by literature data showing that only type A viruses influence the human cardiovascular system [30–33].

Influenza A viruses contain two major virus-coded surface antigens, the hemagglutinin (HA), encompassing two subunits HA1 and HA2, and neuraminidase (NA), which are the antigens primarily involved in the induction of specific humoral immunity against influenza viruses. Vaccination with conventional, inactivated influenza virus vaccines, containing both HA and NA, stimulates immunity against both antigens, although the immunologic response to NA is severely suppressed in primed subjects through HA–NA antigenic competition [34,35]. For this reason, we concentrated on HA for further analysis.

The protective effect against CVD was observed with vaccines which contained diverse influenza viruses [5–12,16]. This suggests that all these vaccine viruses have some common property which is connected with this phenomenon. Previously, the ISM analysis

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