J PREV MED HYG 2011; 52: 111-115

REVIEW

Utility of Thymosin α-1 (Zadaxin™) as a co-adjuvant in influenza vaccines: a review

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Key words

Thymosin alfa-1 • Zadaxin™ • Influenza vaccine • Elderly • Immunocompromised subjects

Summary

Influenza constitutes a serious problem for healthcare and social services worldwide, owing to its pattern and the severity of its complications in some categories of subjects at risk, such as the elderly and immunocompromised individuals. The only really effective means of combating influenza is vaccination. The elderly and immunocompromised subjects are refractory or low responders to vaccination. The need for ever more immunogenic and efficacious influenza vaccines, especially for subjects at risk, has prompted the development of adjuvated vaccines. With a view to enhancing the immune response in the elderly and in subjects at risk, the possibility of co-administering immunostimulants as Thymosin α-1 (Tα1) with influenza vaccines has been investigated. Tα1 is a biologically active peptide made up of 28 amino acids that can enhance T-cells, dendritic cell and antibody responses, modulate cytokines and chemokines production. Several studies were conducted and showed that Tα1 ameliorate the performance of influenza vaccination in elderly and subjects at risk.
Although further studies on co-adjuvants are necessary, the future prospects of producing ever more efficacious influenza vaccines appear very promising.

Introduction

Influenza constitutes a serious problem for healthcare and social services worldwide, owing to its pattern of recurrent epidemics and pandemics and the severity of its complications in some categories of subjects at risk, such as the elderly, those with pulmonary, cardiovascular, kidney, liver, hematological and metabolic disorders, and immunocompromised patients [1]. Indeed, flu vaccination is offered free of charge to these categories of subjects in all developed countries.
Each year, an average of 5-20% of the population catches influenza and more than 200,000 people are hospitalized for complications related to seasonal influenza in the United States [2]. Sullivan et al. estimated 314,000 hospitalizations per year due to flu syndrome in the USA [3]. In the 30 years between 1976 and 2006, estimates of influenza-associated deaths ranged from a minimum of about 3,000 to a maximum of about 49,000 [2]. During the typical winter influenza epidemics of recent years, the average annual number of deaths attributable to influenza (all causes, all age-classes) is 12,000 in England and Wales [4]. Some categories of subjects, such as the elderly, young children, pregnant women and people with certain medical conditions (immunocompromised individuals, those with chronic diseases, etc.), have an increased risk of developing severe complications as a result of influenza infection. Furthermore, uremic patients are especially vulnerable to infections [5], and it is generally recommended that patients with chronic renal insufficiency should be vaccinated yearly against influenza [6]. In patients on hemodialysis (HD), the influenza vaccination response has been considered suboptimal [7].
About 90% of excess mortality due to influenza and about 50% of excess hospitalizations occur among the elderly [8]. Moreover, as underlined by the US Centers for Disease Control (CDC), the complications of influenza more often affect persons over 64 years of age, especially those aged more than 75 years, as well as children and people of all ages with recognized risk conditions [9].
It is acknowledged that the only really effective means of combating influenza and its possible complications is vaccination [10]. However, the efficacy of a vaccine depends on the concordance between the strains used for immunization and the virus circulating among populations (matching). Vaccine efficacy is strongly influenced by the conditions of immune system and the age of recipients. Many studies support the hypothesis that influenza vaccination significantly reduces the risk of severe complications, e.g. hospitalizations for pneumonia and death among the elderly living in communities [11-13]. In subjects over 64 years of age, vaccination against influenza seems to reduce hospitalizations by 50-60% and mortality by up to 80% [9].
The need for ever more immunogenic and efficacious influenza vaccines, especially for subjects at risk, has prompted the development of adjuvated vaccines. In order to enhance the immune response to influenza vaccines, several adjuvants have been proposed (e.g. alum salts, MF-59®, liposomes, virosomes, etc.) [14, 15].

Adjuvants are agents which, when incorporated into vaccines, enhance the immunogenicity of their antigens by activating and/or prolonging their stimulatory effect.
The reasons for developing adjuvated influenza vaccines are:

- to enhance the immune response in subjects in whom the efficiency of cell-mediated and humoral immunity is reduced, such as elderly or immunocompromised persons;
- to produce a powerful vaccine for subjects at risk, such as those with chronic disorders;
- to improve the immunogenicity of highly purified or recombinant antigens;
- to increase antigen delivery systems for the uptake of antigens by the mucosa;
- to obtain vaccines able to enhance cross-reactivity versus a broad spectrum of strains similar to those contained in the vaccine formulation;
- to reduce the amount of antigens per dose and the number of doses necessary in unprimed children under 9 years;
- to provide cover for the entire influenza season;
- to develop pre-pandemic and pandemic vaccines.

With a view to enhancing the immune response in the elderly and in subjects at risk, the possibility of co-administering immunostimulants with influenza vaccines has been investigated. In this perspective, the potential of Thymosin α-1 (Tα1) (Zadaxin™) to increase the immune response to influenza vaccination has been studied.

Thymosin α-1 (Zadaxin™)

Thymosins are biologically active substances produced by the human organism. Tα1 is a biologically active peptide made up of 28 amino acids. In 1966, Goldstein et al. first isolated and described a lymphocytopoietic factor obtained from calf thymus [16]; they subsequently sequenced Tα1 [17] and studied its activity on the immune, endocrine and central nervous systems [18].
Thymosin is a protein produced naturally by the thymus and in many other cells of the organism. It acts on the maturation of the immune system and the production of T cells and exerts a regulatory effect on many essential biological processes in order to ensure proper functioning of the immune system. Indeed, the main biological properties of thymosin include enhancement of T-cell-dependent specific antibody production, helper T-cell activity and secondary T-cell-dependent IgG, IgM, and IgA antibody responses [19, 20].
Zadaxin™ was provided by Sigma-Tau SpA. Zadaxin™ is approved in Italy as an adjuvant for influenza vaccine in immunocompromised subjects. Zadaxin™ contains Tα1, a 28 amino acid peptide acetylated at its amino terminus. Chemically synthesized Tα1 is identical in amino acid sequence to the Tα1 isolated from Thymosin fraction-5, an extract from the thymus gland.

Mechanism of action

Although numerous studies have aimed at clarifying the immunoregulatory activity of Tα1, the mechanisms through which this activity is carried out have not yet been completely explained. Amhed et al. [21] found that Tα1 enhanced the efficiency of T lymphocyte maturation. Peng et al. [22] demonstrated that Tα1 stimulated the differentiation of stem cells to $CD4^+$ and $CD8^+$ lymphocytes. Yao et al. [23] showed the importance of Tα1 in the balancing of CD3, $CD4^+$ and $CD8^+$ lymphocytes and peripheral monocytes (PBMCs).
Rustgi [24] hypothesized that Tα1 exerts an antiviral action through the direct stimulation of Natural Killers (NK) and cytotoxic T lymphocytes ($CD8^+$).
Leichtling et al. [25] noted that Tα1 induced an increase in the sensitivity of specific cellular receptors for interleukin 2 (IL-2).
Other direct effects on the effector cells of the immune system have been observed. These concern: the direct inhibition of viral replication [26] and an action that renders viral antigens in infected cells more evident, therefore facilitating attack by the cells of the immune system [27].
An immunoregulatory action of Tα1 has also been observed. Garaci et al. [28] hypothesized that Tα1 might directly stimulate the genes of the cytokines, of the cells of the major histocompatibility complex I (MHCI) and of the major histocompatibility complex II (MHCII) or unknown genes that might act as immune system regulators.
Naylor et al. [29] demonstrated that both T lymphocytes and monocytes exposed to Tα1 display greater sensitivity for the genes coding for the proteins of the major histocompatibility complex, for the co-stimulatory molecules, for chemokines and for cytokines. Moreover, Tα1 also enhances the sensitivity of the related cellular receptors.
On investigating the effect of Tα1 on the modalities of intracellular signaling, Sungarian et al. [30] observed a stimulatory effect on the protein kinases that activate the mitogens (MAPK); previously, Zhang et al. [31] had demonstrated the activation of I (Kappa) B kinase (IKK), which represents a signal for the receptor-associated factor 6 (TRAF6). Moreover, Tα1 is thought to stimulate the production of IL-6, IL-10 and IL-12. In addition, Romani et al. [32] showed that Tα1 exerted sensitizing action on the dendritic cells (DC) through the signal on the Toll-like receptor 9 (TLR9) and on factor 88 by means of myeloid differentiation (MyD88). Again with regard to the maturation and differentiation of the DC, Yao et al. [23] underlined the activation by Tα1 of the p38 MAPK and NF-κB pathways.
Activation of the plasmacytoid dendritic cells (pDC) leads to the activation of interferon regulatory factor 7 and promotion of the IFN-α/IFNγ activation pathway [32]. Moreover, the activation of indoleamine 2,3-dioxygenase (IDO) can improve tolerance to transplantation and reduce allergic inflammation [33].

Qin et al. [34] recently found that Tα1 inhibits the proliferation of HepG2 cells, and that this inhibition may be associated to the protein kinase B signal (AKT). Finally, Bonifazi et al. [35] have hypothesized that Tα1 is a regulator of regulators. Specifically, Tα1 is thought to occupy a central and bivalent position of stimulation and regulation. By this token, it would stimulate the DC through the TLRs, inducing the production of cytokines. Moreover, through the TLRs, Tα1 would activate Th17 cells, which would in turn induce a negative effect on allergies and inflammatory syndromes.

In synthesis, Tα1 acts on the immune system in the following ways:

- it stimulates stem cell differentiation;
- it increases the number of T cells that destroy viruses, including CD4, CD8 and Natural Killers derived from the stem cells;
- it slows down the apoptosis of the T cells, which are of primary importance in the immune response;
- it increases the number of helper cells (Th1 cells), which combat chronic viral infection;
- it increases the production of cytokines, which act to raise the number of T cells in the process of the immune response. In particular, it increases the production of interleukin 2 and interferon gamma;
- it reduces the production of interleukin 4 and interleukin 10, which exert an unfavorable effect on resistance to chronic viral infections.

Tα1 acts directly on virus-infected cells in the following ways:

- it increases the number of surface protein marker molecules (MHC I), which are responsible for identifying and eliminating from the organism foreign bodies, such as viruses;
- it slows down viral replication.

Utility of Tα1 in potentiating the influenza vaccine response

Several studies were conducted and showed that Tα1 can improve response in populations of individuals who are refractory or low responders to vaccination.

Influenza vaccination is strongly suggested for the elderly because in this category of subjects there is an increased risk of developing severe complications as a result of influenza infection. Nevertheless, the degree of protection that can be achieved is sometimes reduced as a result of immunosenescence [36]. This problem is also linked to the ageing of the thymus, which results in a lower production of its hormones and a reduction in the availability of T lymphocytes [37].

In 2005, Jefferson et al. [38] published the results of a meta-analysis which indicated a mean efficacy of influenza vaccination of 23% in the elderly. In other studies, vaccine efficacy among elderly subjects has been estimated as 57.11% (1999-2000 season) and 45.74% (2000-2001 season) [39, 40].

In 2005, Goodwin et al. published a paper concerning 31 studies of the immune response to influenza vaccination in elderly subjects; the response seen in elderly subjects (> 65 years) proved to be between 4 and 1.69 times lower than in the young [41].

Several strategies to improve the response to influenza vaccination in the elderly have been examined. Some are based on conjugation of the vaccine with proteins such as diphtheria toxoid [42]; others involve increasing the quantity of antigen or the number of doses [43], while others again utilize new adjuvants, such as liposomes or oil emulsions in water [44-46].

Even for subjects at risk as immunocompromised subjects, the vaccination is strongly indicated.

In this perspective since the yearly 80s, Tα1 has been studied with regard to its potential ability to increase the immune response to vaccination.

Tα1 has been seen to enhance vaccine responses in both animal experiments and human trials [47-49]. These experiments have suggested that the co-administration of Tα1 and vaccine can potentiate antibody production [47, 48].

Studies conducted on a human model [49, 50] have shown that the addition of thymosin to influenza vaccine enhances the production of antibodies. In a study of this kind carried out by Gravestein et al. [51] high levels of antibody production were achieved in 9 elderly subjects who had not responded to vaccination a year earlier. The same author subsequently conducted a double-blind study on 90 elderly subjects vaccinated against influenza with/without simultaneous administration of thymosin. The results confirmed the efficacy of thymosin; indeed, a response to vaccination was recorded in 69% of the subjects who had received thymosin, as against 52% of those who had received the vaccine alone. The difference between these two percentages proved statistically significant ($p = 0.023$) [52].

Similarly, in a study conducted by McConnell on 330 elderly subjects, the administration of thymosin after administration of the vaccine yielded significant improvements in the immune response towards all three influenza virus strains contained in the vaccine [53].

More recently, Tuthill et al. [54] utilized an animal model to investigate different dosage regimes implemented at different times in relation to the administration of an influenza vaccine adjuvated or not adjuvated with MF59® in 23 groups of mice. On the basis of their results, the authors concluded that the best strategy was to administer Tα1 one week before and on the same day as the influenza vaccine. Moreover, these authors also studied the potential of Tα1 in 5 groups of 4 ferrets (with regard to influenza, this animal model is the closest to man) and observed that the best results were obtained by administering Tα1 at a dose of 6.4 mg one week before and on the same day as the influenza vaccine.

Even in immunocompromised subjects, such as those on hemodialysis, the administration of Tα1 can help to improve influenza vaccine responses [55]. The study was conducted by Shen et al. on 97 hemodialysis patients vaccinated with a monovalent preparation (A/H1N1/Taiwan/1/86). The results showed a significant improvement in immune response ($p < 0.002$) in the group of subjects treated with thymosin. The same author has

studied Tα1 in co-administration with anti-hepatitis B vaccine. The subjects treated with Tα1 showed a better response to the vaccine than those who had received the vaccine alone [56, 57].

Conclusions

Forty years after the first description and characterization of Tα1 by Goldstein [16, 17], it is used worldwide with many medical applications. Nowadays, Tα1 has shown a variety of effects on cells and pathways of the immune system, its central role is stimulating T lymphocytes. Furthermore Tα1 has an important capacity of immunoregulatory action. Tα1 also acts directly on virus-infected cells increasing the number of surface protein marker molecules and slowing down viral replication. Finally, Bonifazi et al. [35] have hypothesized that Tα1 is a regulator of regulators.
The goals of influenza vaccination are to elicit a strong and, if possible, broad-spectrum immune response in all the subjects also in those individuals who are refractory or low responders to vaccination. Particularly, it is important that the subjects, whose immune systems are impaired to some degree by concomitant diseases and who are at greater risk of developing severe forms of the influenza disease, are protected. Undoubtedly, adjuvated vaccines offer good prospects. However, further opportunities could be provided by combining immunomodulators with influenza vaccines. In this perspective, the results of the studies on Tα1 were encouraging. Indeed, Tα1 can potentiate the production of specific antibodies that are dependent on the function of the T lymphocytes. Therefore, Tα1 could contribute to reduce the amount of antigen per dose leading to have cheaper and more amount of vaccine doses particularly in emergency massive vaccination programs. Tα1 could ameliorate the performance of vaccination in particular clinical settings.
The findings, obtained in the studies where Tα1 was used as co-adjuvant to vaccination, allow to suppose similar results for other vaccines, especially those for the prevention of infectious diseases caused by pathogens with a wide variety or variability of antigenic phenotypes. Furthermore Tα1 could be used when it is necessary to have a quick immune response such as in traveling and case of pandemia.
Finally the immunoregulatory and anti-inflammatory actions of Tα1 could be useful to reduce severe adverse events in vaccination (e.g. severe allergic reaction, Guillan-Barré syndrome).
Furthermore, immunomodulators could be studied for use in intranasal influenza vaccines.
In conclusion, although further studies on co-adjuvants are necessary, the future prospects of producing ever more efficacious influenza vaccines appear very promising.

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■ Accepted on August 4, 2011.

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