Review article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Current status of human papillomavirus vaccines

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Cervical cancer is a malignant neoplasm arising from cells that originate in the cervix uteri. It is the second most prevalent cancer among women. It can have several causes; an infection with some type of human papillomavirus (HPV) is the greatest risk factor for cervical cancer. Over 100 types of HPVs have been identified, and more than 40 types of HPVs are typically transmitted through sexual contact and infect the anogenital region. Among these, a number of HPVs types, containing types 16 and 18, are classified as "high-risk" HPVs that can cause cervical cancer. The HPVs vaccine prevents infection with certain species of HPVs associated with the development of cervical cancer, genital warts, and some less common cancers. Two HPVs vaccines are currently on the global market: quadrivalent HPVs vaccine and bivalent HPV vaccine that use virus-like particles as a vaccine antigen. This review discusses the current status of HPVs vaccines on the global market, clinical trials, and the future of HPVs vaccine development.

Keywords: Papillomavirus vaccines, Virus-like particle vaccines, Uterine cervical neoplasms, Clinical trial

Introduction

Cervical cancer is the second most prevalent cancer among women, accounting for 500,000 cases per year [1]. There have been continuous efforts to determine the causes of the onset of cervical cancer. Since 1977, when Zur Hausen [2] discovered that the infection of human papillomavirus (HPV) is the major causative agent of cervical cancer, various research has been conducted and is in progress. As a result, it has been determined that the relationship between cervical cancer and the HPV infection is higher than the relationship between lung cancer and smoking, and also higher than the relationship between liver cancer and the hepatitis B virus [3]. Globally, approximately 100 different types of HPV have been reported; types 16 and 18 have caused 70% of cervical cancer onsets worldwide [4-8].

A new vaccine was developed using the non-infective recombinant virus like particle (VLP) for the antigen of the vaccine. In 1991, Zhou et al. [9] developed VLP technology, which was the crucial opportunity to develop the cervical cancer vaccine. Seventy-two of the capsomers, each comprising five L1 proteins, are assembled into a VLP, and such VLP exhibits a virus-like structure; therefore, it has higher antigenicity while being safe due to the absence of foreign DNA [10].

Currently, there are two cervical cancer vaccines used globally on the market: quad-

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rivalent HPV (qHPV) vaccine, produced by Merck & Co. Inc. (Gardasil®, Merck, Rahway, NJ, USA); and bivalent HPV vaccine, produced by GlaxoSmithKline plc. (Cervarix™, GSK, Middlesex, UK). qHPV vaccine contains HPV type 6, 11, 16, and 18 L1 proteins for its antigen in the VLP form and uses aluminum hydroxyphosphate sulfate as an adjuvant. Bivalent HPV vaccine contains the VLP form antigen of HPV type 16 and 18 L1 proteins and uses an adjuvant called AS04, which is a combination of aluminum hydroxide and monophosphoryl lipid A (MPL) [11-14].

qHPV Vaccine

The early developments of HPV L1 VLPs leading to VLP-based prophylactic vaccines were the work of the University of Queensland (Brisbane, Queensland, Australia), the US National Institute of Health's National Cancer Institute (NCI), Georgetown University (Washington DC, USA) and the University of Rochester (Rochester, NY, USA). In 1991, Jian Zhou and Ian Frazer at the University of Queensland discovered a non-toxic and infectious VLP that induces cellular immune response. Subsequently, in 1993, researchers at the NCI discovered a synthesis method of VLP that has the same structure as HPV type 16; this VLP was used as a constituent of Merck's qHPV vaccine. The University of Queensland licensed its patent to CSL Limited (Melbourne, Australia) and then again licensed it to Merck. Merck had a cooperative development with CSL Limited to develop a therapeutic HPV vaccine. At present, Merck owns the majority of patents related to the development of the HPV vaccine [15-17]. In 2005, Merck and GSK agreed to cross-license HPV patents. In addition, in 2006, the US Food and Drug Administration (FDA) approved Merck's Biologics License Application (BLA) for the production of qHPV vaccine. qHPV vaccine is currently available on the global market [18].

qHPV vaccine comprises four antigens produced from the yeast *Saccharomyces cerevisiae* expression system. Each gene is expressed using the yeast expression vector pGAL110, induced by galactose, followed by harvesting cells. The harvested cells are lysed with break buffer and then the primary sample is obtained with cation exchange chromatography, followed by size exclusion chromatography as the final purification step. Purified VLPs are adsorbed onto the aluminum hydroxyphosphate sulfate and formulated with sodium chloride, sodium borate, L-histidine, polysorbate-80, and water for injection [19-21]. These produced antigens are combined with the adjuvant to be developed as an HPV vaccine. The detail combination of the vaccine is 20 μg of HPV type 6 L1 protein, 40 μg of HPV type 11 L1 protein, 40 μg of HPV type 16 L1 protein, 20 μg of HPV type 18 L1 protein, and 225 μg of aluminum hydroxyphosphate sulfate [22].

The phase I clinical trial of the qHPV vaccine was designed to investigate the dose responses of monovalent HPV VLP serotypes 11, 16, and 18, respectively. The results showed that the higher-dose vaccination yielded higher immune responses: groups injected with doses of 20 μg, 40 μg, and 50 μg exhibited higher immune responses compared to that of 10 μg. However, there was not a particular advantage of the immune response with 80 μg dose.

Approximately 6,000 people from North America, Latin America, Australia, and Europe were involved in the phase II clinical trial to test safety and immune response. Each antigen quantity of HPV types 6, 11, 16, and 18 is determined as 20, 40, 40, and 20 μg/dose, respectively. The phase III clinical trial was conducted to confirm the efficacy and safety of qHPV vaccine and to evaluate the degree of onset of external genital sessions induced by the vaccine; overall, it involved 17,500 subjects from North America, South America, Latin America, Asia, Australia, and Europe [19,23,24]. Between 2006, when it gained FDA approval, and 2011, qHPV vaccine has been approved in 121 countries and has supplied over 74,000,000 doses globally. In 2012, qHPV vaccine recorded sales of $1.631 trillion [25,26].

Bivalent HPV Vaccine

GSK acquired the right to develop its HPV vaccine through an agreement with MedImmune, LLC (Gaithersburg, MD, USA), which obtained the right to HPV vaccine development in 1998 and the cross-license of HPV patents with Merck in 2005 [18]. In 2007, GSK submitted the BLA to FDA for the production of bivalent HPV vaccine; in 2009, GSK received FDA approval. Its HPV vaccine is currently on the global market.

Unlike qHPV vaccine, bivalent HPV vaccine is composed of only two antigens: HPV types 16 and 18. For the synthesis of its L1 proteins, genes are cloned into the baculovirus expression vector and the manufactured vector is infected into the insect cell, *Trichoplusiani* cell (High Five), followed by incubation in the serum-free media for two days; then the cells are harvested. HPV type 16 and 18 L1 proteins are purified from the cells through the following steps: 1) concentration through centrifugation; 2) extraction using hypotonic buffer;

and 3) clarification by tangential flow filtration. Crude products are further purified through anion exchange chromatography and hydrophobic interaction chromatography for HPV 16 and 18 L1 proteins, respectively. Purified L1 proteins are then adsorbed on the aluminum hydroxide, followed by adding AS04 adjuvant system to produce bivalent HPV vaccine [27-29].

Bivalent HPV vaccine includes the self-developed adjuvant system called AS04, which is composed of MPL, which activates cellular and humoral immune response, and general adjuvant aluminum hydroxide adsorbed form [30,31]. MPL, the major constituent of AS04, is a modified diphosphoryl lipid A derived from *Salmonella Minnesota* R595 that removes the phosphate and fatty acid groups. Synthesized 3-O-desacyl-4´-monophosphoryl lipid A induces the activation of antigen presenting cell (APC); it also induces Th1 and Th2 cellular immune responses by emerging from toll-like receptor activation [11,32,33].

Bivalent HPV vaccine confirmed its safety and degree of immune response induction against monovalent/bivalent HPV VLP types 16 and 18 through phase I clinical study; the phase II clinical trial was conducted to evaluate the safety of vaccine composition and immunogenicity; phase III was conducted to verify the efficacy of the vaccine on HPV infected and/or non-infected populations by varying the age group as follows: 15-25 years, 10-25 years, 10-14 years, and 15-55 years [34,35].

In addition, the latest clinical follow-up study of the long-term immunogenicity of bivalent HPV vaccine—which examined females aged 15-26 years from the United States, Canada and Brazil for 8.4 years—determined that no new infection or lesions associated with HPV type 16/18 occurred in the vaccine group. Vaccine efficacy over the entire follow-up (up to 8.4 years) was 95.1% for incident infection, 100% for 6-month persistent infection, 100% for 12-month persistent infection, and 100% for cervical intraepithelial neoplasia of grade 2 or worse (CIN 2+) associated with HPV type 16/18. All females with the vaccination exhibited the positive antibody against HPV type 16/18, which were at least several folds higher titers of total antigen and neutralizing antibody than would be found after natural infection. This study is the longest follow-up study of the approved cervical cancer vaccine [36].

Based on the above-mentioned clinical trials, bivalent HPV vaccine obtained FDA approval in 2009; as of 2011, it has been licensed in 100 countries and approved in more than 60 countries. Tens of millions of doses have been sold globally, and in 2012, sales reached approximately 270 million euros [37,38].

Comparative Clinical Trials of Quadrivalent and Bivalent HPV Vaccines

Among the two commercially available vaccines, qHPV vaccine, which entered the market earlier, holds the dominant position in the market sector. However, GSK emphasizes its superiority over the qHPV vaccine in terms of duration of protection. GSK conducts various clinical trials to compare the antibody titers of qHPV vaccine and bivalent HPV vaccine to prove the superiority of bivalent HPV vaccine.

A two-year clinical experiment conducted by the UK Department of Health from 2009 to 2011 demonstrated that both vaccines showed high levels of antibody titers against HPV types 16 and 18 when using three different doses for each vaccine. In addition, both vaccines showed a higher reaction rate for HPV type 16 than 18. However, the distinct efficacy of the two vaccines was clear hours after the vaccination. Bivalent HPV vaccine exhibited higher levels of antibody titers compared to qHPV vaccine, and also offered higher titers of antibody against HPV types 31 and 45, which are not the intended targets [14].

In a 24-month observer-blind study, women (n=1,106), stratified by age (18-26 years, 27-35 years, 36-45 years), were randomized (1:1) to receive either bivalent HPV vaccine for 0, 1, and 6 months or qHPV vaccine for 0, 2, and 6 months. The seropositive rates of neutralizing antibodies were then analyzed for these two groups. For the entire vaccinated cohort, across all age strata, the result was that geometric mean titers were 2.4- to 5.8-fold higher for HPV type 16 and 7.7- to 9.4-fold higher for HPV type 18 with bivalent HPV vaccine than with qHPV vaccine ($p<0.0001$). Similar results were obtained using enzyme-linked immunosorbent assay. At month 24, analysis of $CD4^+$ T-cell responses and memory B-cell responses also demonstrated that the bivalent HPV vaccine is relatively similar to or better than the qHPV vaccine [39].

The immunogenicity of the two vaccines is expected to be different, due to the effect of the adjuvants. Bivalent HPV vaccine uses AS04 for its adjuvant, which includes aluminum hydroxide and MPL. MPL is an agonist molecule against Toll-like receptor 4 (TLR4) that facilitates innate immune response by stimulating TLR4. Both *in vitro* and *in vivo* data suggest that the addition of MPL to aluminum hydroxide enhances vaccine-induced immune response by rapidly triggering a local cytokine response, leading to the optimal activation of

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APCs [40]. Hence, GSK uses this fact in its marketing.

The Other HPV Vaccines

At present, various companies are conducting research on HPV prophylactic vaccines. Since 2002, Innovax YST Biotech Co. Ltd. (Haicang, Xiamen, China) has developed HPV type 16 and 18 bivalent vaccine using *Escherichia coli* expression system to produce its antigens. This vaccine comprises HPV type 16 and 18 VLP adsorbed in alum-adjuvant. The product is in clinical trial phase III [41,42].

Another company investigating the HPV vaccine is Takeda Pharmaceutical Company Ltd. (Takeda, Osaka, Japan). In 2010, Takeda obtained exclusive worldwide patent rights to the Kanda HPV vaccine (invented by Dr. Tadahito Kanda) through a license agreement with the Japan Health Sciences Foundation. The Kanda HPV vaccine has neutralizing activity against six variants of high-risk HPV. In addition, in 2012, Takeda acquired US-based LigoCyte Pharmaceuticals, Inc. (Bozeman, MT, USA), a biotechnology firm specializing in the development of new vaccines using proprietary VLP technology. Currently, this vaccine is in the pre-clinical study stage [43,44].

A third company, Eyegene Inc., Seoul, Korea, developed a new HPV prophylactic vaccine, EG-HPV, using antigens of HPV type 16 and type 18; its clinical phase I trial is in progress in Korea. Eyegene Inc. uses yeast expression system to produce and purify antigens of the vaccine. These two antigens are combined with CIA06 as an adjuvant, which is a combination of aluminum hydroxide and CIA05; this is a novel adjuvant to be developed as a new HPV prophylactic vaccine by Eyegene Inc. CIA06 has the same concept as GSK's AS04: one constituent of the cocktail, CIA05, has the same mechanism as MPL and shares a high level of similarity in terms of structure. CIA05 is a nontoxic *E. coli* lipopolysaccharide derivate that has the short carbohydrate chain; it is also a TLR4 agonist. CIA05 consists of a core oligosaccharide lacking the terminal glucose residue, a glucosamine disaccharide with two phosphate groups, and two N-linked acyl groups. CIA05 induces Th1-, Th2-, and Th17-type immune responses in a dose-dependent manner and has been shown to enhance immunity when there is a low concentration of antigen. From the detoxification process, decreased secretions of tumor necrosis factor and low toxicity in the acute toxicity tests have been reported [45-47]. It has also been reported that the EG-HPV vaccine has twice the efficacy of bivalent HPV vaccine *in vivo* system of mice when using type 16 and 18 L1 VLP-specific serum IgG antibody titer, memory B-cell levels in splenocytes, and interferon-γ secretion levels in splenocytes as measurement indexes [45].

Adverse Events Associated with HPV Vaccines

qHPV vaccine

From 2006 to 2008, there were 12,424 reported cases from the study of adverse events following immunizations (AEFIs) of qHPV vaccine for women aged 9 to 26 in the United States, according to the Vaccine Adverse Event Reporting System (VAERS). This represents a 53.9/100,000 ratio of adverse effect onset. Of these cases, 772 (6.2%) were reported as serious AEFIs. When examining the occurrence ratio of serious AEFIs from the 100,000 qHPV doses in detail, there were 8.2% of syncope; 7.5% of local site reactions; 6.8% of dizziness; 5% of nausea; 4.1% of headache; 3.1% of hypersensitivity reaction; 2.6% of urticaria; 0.2% each of venous thromboembolic events, autoimmune disorders, and Guillain-Barre syndrome (GBS); 0.1% each of anaphylaxis and death; 0.04% of transverse myelitis and pancreatitis; and 0.009% of motor neuron disease. A review of the 12,424 reports of AEFIs following receipt of qHPV after licensure revealed that most did not meet the FDA definition of serious, including GBS [48].

GBS has been recently recognized the adverse event caused by HPV vaccines. GBS is a rare neurological disorder that induces muscle weakness. The FDA and Centers for Disease Control and Prevention (CDC) reexamined reports and concluded that qHPV vaccine vaccination has no relationship with GBS onset increment; however, the study of additional reports continues [49].

Bivalent HPV vaccine

From 2009 to 2011, according to the report of Rijksinstituut voor Volksgezondheid en Milieu, there were 647 cases of spontaneous adverse events from the mass vaccination campaign study of bivalent HPV vaccine for girls aged 15 to 18 in the UK. This is a ratio of 11.6/10,000. Of these reported cases, 61% are the results of causality assessments that are normally reported. Examples of generally unreported cases are complicated migraine (5 cases); GBS (1 case); Bell's palsy (1 case); anaphylaxis (1 case); severe anemia (1 case); viral meningitis (1 case); severe pain in the back (1 case); hematuria (1 case); loss of strength (1 case); and sensitivity disorder (1 case). The most

frequently occurring AEFIs were fever (115 cases, 18%), local reaction (105 cases, 16%), exanthema/erythema (47 cases, 7%), menstruation problems (44 cases, 7%), headache (44 cases, 7%), and malaise (38 cases, 6%). Reported AEFI onset cases of bivalent HPV vaccine related to convulsions and neuralgia were noticeably lower than those of qHPV vaccine [50]. However, the adverse effects of the two vaccines are not serious compared to those of other general vaccines; therefore, their vaccinations are continuously recommended to prevent cancer [51,52].

The Future of HPV Prophylactic Vaccine

Currently, there are different strategies to increase the rate of HPV prevention from various angles. Researchers have been looking into ways to increase cross-protections against various types of HPV, to enhance the half-life and decrease the number of vaccinations [51,52]. A novel nonavalent vaccine under phase III clinical trial has the goal of establishing 15-30% higher anti-cancer effect by adding the new carcinogenic HPV types 31, 33, 45, 52, and 58 to Merck's existing quadrivalent vaccine (HPV type 6, 11, 16, and 18) [53]. However, the value of the nonavalent vaccine as a next-generation HPV vaccine is doubtful, because there are no studies that have analysis of cost effectiveness and comparative efficacy of the novel vaccine compared with the conventional vaccines. Other attempts to reduce the manufacturing costs that are under investigation include the generation of L1 VLPs in alternative vectors such as the yeast *Pichia pastoris* [54] or in plants [55]. Alternatively, improvements in the methods of storing the vaccine, or easier administration methods such as nasal spray or patch, may result in easier and cheaper accessibility of the vaccines.

At least 40 countries had implemented HPV vaccination in their national immunization programs (NIPs) by the beginning of 2012. Among these countries, the United States, the UK, Canada, and Australia were the first countries to execute the implementation. In 2007, only 3 European countries had introduced HPV vaccine which then increased to 22 countries who had introduced the vaccine into their NIPs. While the target of most country programs is young adolescent girls, defined age groups vary by country to country. Such various implementation strategies are resulted from different health care infrastructure and systems. In general, HPV vaccinations are recommended for females aged minimum 11 to maxmimum 26 years [56]. In addition, by means of a new policy-based approach, HPV vaccines are also recommended for males in several countries [57].

Most importantly, the elimination of cervical cancer can be accelerated through the effect of herd immunity, by developing cheaper, next-generation vaccines, providing education on HPV infections and sexually transmitted diseases, and advertising the availability of vaccinations in low-income countries.

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