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Research Article

Immunogenicity and safety of inactivated Influenza Split-Virion vaccine administered via a Transdermal Microneedle System

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Abstract

The purpose of the study was to evaluate the immunogenicity and safety of an inactivated influenza split virion vaccine administered via a transdermal microneedle system.

In this Phase 1, single-center, randomized, controlled study, 90 subjects aged 18 to 40 years received influenza vaccine (strains (A/H1N1, A/H3N2, and B) either via a transdermal microneedle system ("patch"; 10 µg) for 5 or 15 minutes or by Intramuscular (IM) injection (15g). Influenza antibody titers were measured by the hemagglutinin inhibition method and compared to EMEA guidelines for influenza vaccines (seroconversion rate, mean increase in hemagglutinin inhibition titer, and percentage of seroprotected subjects). Safety was assessed through local and systemic adverse events, and specific application site events in the transdermal groups.

At Day 21, the EMEA criteria were met in all treatment groups for all three influenza strains. The immunogenicity response was similar between all three groups and increased antibody levels persisted to Month 6. The transdermal microneedle system was generally well tolerated, although pinpoint red spots, edema, and erythema were noted after patch removal in most subjects.

Influenza vaccination administered via a novel transdermal microneedle system was generally well tolerated and provided similar antibody response using a lower dose than IM injection.

Abbreviations

APC: Antigen Presenting Cell; CI: Confidence Interval; dil: dilution; EMEA: European Medicine Agency; HA: Hemagglutinin; HI: Hemagglutinin Inhibition; IM: Intramuscular; TD: Transdermal; VAS: Visual Analog Scale

Introduction

The influenza virus belongs to the Orthomyxovirdae family and is separated into three types of strains according to antigenic differences: influenza A, associated with severe and widespread epidemics and pandemics; influenza B, responsible for widespread regional epidemics; and influenza C, that causes sporadic or small localized outbreaks.

Vaccination represents the major preventive measure against influenza. It is recommended in various countries in populations at high risk such as the elderly, young children unprimed to influenza, subjects with chronic respiratory, cardiac, and /or renal disease, metabolic diseases such as diabetes, asthma and immune-comprised subjects and also for all individuals who are particularly exposed to the disease or likely to transmit influenza to susceptible individuals [1-4].

Three types of vaccines delivered intramuscularly (IM) are commercially available for protection from influenza: i) whole virus vaccines, which contain intact virions inactivated by treatment with formaldehyde, ii) split vaccines, iii) subunit vaccines. The split and subunit vaccines are composed of purified influenza antigens, of which Hemagglutinin (HA) predominates. These vaccines are classically considered to be less reactogenic than previous whole virus vaccines and are the most commonly used vaccines in developed countries.

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The route of administration of vaccines plays an important role both in patient compliance, and, as has increasingly been shown, for producing a robust immune response. Parenteral delivery through Intramuscular (IM) and Subcutaneous (SC) routes using a needle is the most common route of vaccine administration [5,6]. Despite being established methods, IM and SC vaccination has several drawbacks, including: 1) requiring medical personnel; 2) causing pain; 3) and often inducing an inadequate immune response [7-10]. Additionally, these methods are associated with the risk of needlestick injury. Treatment of these injuries are associated with a cost estimated to be $535 million per year worldwide [11]. Given the limitations and liabilities of SC and IM delivery, there is a significant need to identify alternative delivery methods to enhance vaccination practices.

The epidermis and dermis have an abundance of antigen-presenting cells that are key to generating a protective immune response making it an ideal site for vaccine delivery. What makes the dermis so unique is the panoply of immune cell types that are present in the epithelium, including dendritic cells, T lymphocytes, natural killer cells, macrophages, and mast cells. In particular, the cells that are responsible for triggering the inflammation cascade in the skin are the Langerhans cells, which make up 2-4% of epithelial cells. Langerhans cells are a specific subset of dendritic cells that migrates into the lymph nodes following antigen capture and facilitate the initiation of an adaptive immune response. Langerhans and dermal dendritic cells present antigens to both T and B lymphocytes, resulting in the activation and establishment of both mucosal and systemic immunity. The same cells are also efficiently stimulated by Pathogen-Associated Molecular Patterns (PAMPs) using germline encoded Pattern Recognition Receptors (PRR), including Toll-Like Receptors (TLR) and langerin (CD207). Dermal mast cells stimulate the innate immune response through the release of granules containing a variety of inflammatory mediators [12-15].

Clinical studies have supported the potential of ID delivery to provide an enhanced immune response compared with SC and IM delivery of various agents [5]. In general, ID immunization generates greater immune responses than IM injection, while SC and IM immunizations induce very similar responses. The skin being the body's largest immunocompetent organ and the first-line of defense for the body contributes to the enhanced effects for the ID route. Additionally, the dermis with a higher proportion of Dendritic Cells (DCs) can facilitate the capture of antigens, and local inflammations inducing maturation of the DCs and their migration into draining lymph nodes, which can lead to vaccine dose-sparing. A recent clinical study supported the use of ID delivery of a vaccine to provide for an appropriate induced immune response using lower levels of vaccine [16]. A major challenge of ID delivery is correct placement of the needle using commercially available syringes and needles. A potential solution is to use ID delivery devices to enable more targeted and consistent ID delivery such as microneedle-array system [17]. In addition to providing improved vaccine efficacy, the enhanced immune response resulting from ID delivery enables dose-sparing approaches (a benefit not available with IM and SC delivery), namely: 1) reduced costs per-injection; and 2) increased vaccine availability from extending the supply. The ability to stretch the vaccine supply is of importance for vaccines that are limited by manufacturing capacity, such as influenza [6]. Despite the advantages of ID delivery, it is not widely used for vaccine delivery aside from administration of Bacille Calmette-Guerin and rabies vaccines, and is used in patients that do not respond well to IM injection (e.g. HBV vaccine in dialysis patients) [6,18]. The limited use of ID delivery is due to the challenges in accurate delivery without the use of an appropriate device and, until the development of microneedle delivery systems, the lack of reliable delivery devices to enable more accurate ID delivery [6]. Given the advantages of ID delivery over standard IM and SC methods, validation of novel approaches to provide reliable and accurate ID delivery is necessary to accelerate patient benefit from the enhanced immune protective responses provided by ID delivery [6].

These factors led to the development of a novel transdermal microneedle system for the delivery of trivalent influenza vaccine [19-21]. The transdermal microneedle system consists of titanium microneedles (~1,300 microneedles per 2 cm^2) attached to the center of a 5 cm^2 adhesive backing (patch) with vaccine formulation coated onto the tip of each microneedle. The patch is seated in a retainer ring and is applied (pressed onto the skin) with a hand-held reusable applicator. The drug-coated microneedles penetrate through the superficial skin barrier layer into the epidermal/dermal layers (50-150 micrometers in depth), where the vaccine formulation rapidly dissolves and is released into the skin.

This paper presents the results of a study comparing the immunogenicity and safety of inactivated influenza vaccine administered by the transdermal microneedle system and by IM injection.

Materials and methods

This was a phase 1, single-center, randomized, controlled study, conducted at University Hospital Lyon Sud (Lyon, France) between May 2004 and January 2005. The study was open-label for route of administration (transdermal versus IM) and double-blind for transdermal product (placebo vs active) administration. The study protocol (version 3.0, 25 March 2004) and informed consent (version 3.0, 25 March 2004) form were approved, before study start on 11 May 2004, by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, the local independent ethics committee. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, International Conference on Harmonization [22,23] and applicable national and local requirements and regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research. After being informed of the nature of the study and the potential risks and obligations, the subjects gave written informed consent before being included in the study. Trial registration was not required for this single-center, phase 1 study.

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Subjects

Male and female Caucasian subjects, 18 to 40 years of age, who were registered with the French file of healthy volunteers in clinical studies were recruited. Major exclusion criteria were known hypersensitivity to egg proteins, chick proteins, or any other vaccine component; chronic illness (such as cardiac or renal insufficiency, diabetes, or auto-immune disorders); congenital or acquired immunodeficiency; influenza symptoms within the past 6 months or influenza vaccination within the past 18 months; vaccination with any live-attenuated vaccine within the previous 28 days or with any other vaccine within the previous 14 days; and pregnancy or breast feeding. Ninety subjects were randomly assigned in a 1:1:1 ratio to receive the influenza vaccine administered via patch (patch wear time of 5 minutes or 15 minutes) or IM injection. Subjects in the patch groups received both the influenza patch and a placebo patch. All three groups received the IM injection at the six-month mark. More details regarding the study arms are discussed in the Vaccines and Dosing section.

Transdermal microneedle system

The formulation, preparation and characterization of the transdermal microneedle system has been described in detail elsewhere [24]. Briefly, monovalent bulk vaccine was diafiltered, concentrated, and lyophilized. The lyophilized powder of three monovalent vaccines was separately reconstituted and combined into a trivalent vaccine formulation for coating onto the microneedles. Coating was conducted in GMP facility. The coated patch system is preservative-free and can be stored at room temperature. The placebo systems were coated with sucrose and polysborate 20 and formulated to match coating load of total solids on vaccine patches, 45 mg. The transdermal microneedle systems were made under low bioburden environment and each system had less than or equal 10 CFU for total aerobic microbial count.

Vaccines and dosing

The inactivated influenza split-virion (with octoxinol-9) vaccine (2003-2004 formulation) was composed of the following strains: A/New Caledonia/20/99 (H1N1); A/Moscow/10/99 (H3N2) (analogous strain A/Panama/2007/99); and B/Hong Kong/330/2001 (analogous strain B/Shandong/7/97).

The vaccine was administered on Day 0 either by the transdermal microneedle system (patch), containing 10 µg hemagglutinin (HA) of each influenza strain, or by IM injection containing 15 µg HA of each strain. Subjects were randomly allocated to 1 of 3 treatment groups. The transdermal groups received an influenza patch on one arm and a placebo patch on the other arm; the patches remained in place for 5 minutes (TD5 group) or 15 minutes (TD15 group). The IM group received a single IM injection into the deltoid area.

All subjects also received a single IM injection of inactivated influenza split-virion (with octoxinol-9) vaccine (2004-2005 formulation) at Month 6. This vaccine contained 15 µg HA of each of the following strains: A/New Caledonia/20/99 (H1N1); A/Fujian/411/2002 (H3N2) (analogous strain A/Wyoming/23/2003); and B/Shanghai/361/2002 (analogous strain B/Jiangsu/10/2003).

Safety

The primary endpoint for this study was safety which was assessed based on local and systemic adverse events. Immediate safety, local and systemic adverse events occurring within 30 minutes of vaccine administration, was assessed by the Investigator (a medical doctor). Solicited local and systemic events were also recorded from Day 0 to Day 7 and unsolicited events were recorded until Day 21. Local events included administration site pain and pruritus with at least mild severity and administration site erythema, induration, edema and bruising ≥0.5 cm in diameter. Systemic events included pyrexia (oral body temperature >37.5°C) and, at least a mild severity of, asthenia, headache, arthralgia, myalgia, rigors, sweating, and malaise. Adverse events were categorized as mild (aware of symptom but well tolerated), moderate (hindering enough to interfere with normal daily activity), and severe (preventing normal daily activity).

In the transdermal groups, the presence and extent of pinpoint red spots were assessed visually using a ruler 2 minutes after patch removal. Affected areas of erythema and edema were determined by dividing the patch application site into 4 quadrants and visually approximating spread of erythema/edema within each quadrant. The presence of edema (wheal thickness and extent) and erythema (color intensity and extent) were rated at 15 minutes after patch removal and on Days 1, 2, and 3 and using a 4-point scale: none, noticeable/barely perceptible, well-defined (with clear boundary delineation), and severe (beet red for erythema) [25].

Pain and transdermal microneedle system acceptability

The secondary safety endpoints assessed pain associated with vaccine administration using a 100 mm VAS scale (0 = no pain, 100 = extreme pain) on Day 0 along with acceptability. Acceptability of the transdermal microneedle system was assessed by a questionnaire completed by the on Day 0, which included pain after patch removal and preferred choice of vaccine administration method.

Immunogenicity

Secondary endpoints also evaluated the immunogenicity of the inactivated influenza vaccine in the three study groups. For each vaccine strain, antibody titers against influenza HA were measured in serum samples obtained on Day 0 (before vaccination), Day 21, and Months 2, 3, and 6 using the Hemagglutinin Inhibition (HI) referenced technique [26]. Changes in antibody titers were evaluated based on the EMEA Guidance on the harmonization of requirements for influenza vaccines [27]. The EMEA criteria for evaluation were: (i) a > 40% seroconversion rate (seroconversion was defined as the conversion from a pre-vaccination titer <10 l/dil to a post-vaccination titer ≥ 40 l/dil or at least a 4-fold increase in post vaccination titer when the pre-vaccination titer was < 10 l/dil);

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(ii) a geometric mean increase in titers from Day 0 to Day 21 of at least 2.5 l/dil; and (iii) a seroprotection rate of at least 70% (seroprotection was defined as a titer of ≥ 40 l/dil post-vaccination).

Specific anti-HA IgA and IgG antibodies against the A/H1N1 strain and total IgE titers were measured on Days 0 and 21.

Statistics

Descriptive statistics were performed using SAS® software version 8.2. For categorical immunogenicity data (seroprotection and seroconversion), the number and percentage of subjects and 95% Confidence Intervals (CIs) were calculated. For continuous immunogenicity data (antibody titers), the geometric mean and 95% CIs were determined. The 95% CIs were calculated using the exact binomial method [28]. Adverse events considered possibly, probably, or definitely related to all treatment were summarized. No sample size calculation was performed.

Results

Subject disposition and demographics

Ninety subjects were enrolled, 82 subjects were included in immunogenicity analyses at Day 21, and 79 subjects completed the study. Reasons for non-inclusion in the immunogenicity analyses were inclusion or exclusion criteria not met (5 subjects), missing measurements at Day 21 (2 subjects), and administration error (1 subject). Reasons for withdrawal from the study were missed study visits (3 subjects), pregnancy (2 subjects), shingles (1 subject) and receipt of vaccinations outside (vaccinations obtained by patients for personal medical needs) of the clinical trial during the study period (5 subjects).

The mean (SD) age at enrollment was 27.5 (6.9) years (range 18-41 years) and 64 of 90 subjects were females. Ten of 90 subjects had previously received an influenza vaccination between 1986 and 2002. Demographic characteristics (Table 1) were similar for all treatment groups, except for a higher proportion of females (24 of 29 subjects) in the TD15 group than in the TD5 (20 of 32 subjects) and IM (20 of 29 subjects) groups.

Safety

Immediate events: All subjects in the transdermal groups and 16 of 29 subjects in the IM group had vaccination site events within 30 minutes after vaccination on Day 0. The most commonly reported events in the transdermal groups were erythema, pinpoint red spots, induration, and edema. Erythema, induration, and edema all occurred in the IM group but the incidence was lower than in the transdermal groups. In the IM group 19 of 29 subjects experienced erythema and induration and 8 of 29 subjects experienced edema while, in the transdermal groups, 60 of 61 subjects experienced erythema and induration and 50 of 61 subjects experienced edema. There were no immediate systemic events. Refer Table 2 for data tabulation.

Table 1: Demographics of Subjects.

Treatment Group	Patch (5 min wear time)	Patch (15 min wear time)	Intramuscular Injection	Total
Number of subjects	32	29	29	90
Mean Age (± SD)	26.5 ± 7.3	26.9 ± 5.9	29.3 ± 7.1	27.5 ± 6.9
Number of Subjects (%) by Gender:				
Male Female	12 (37.5) 20 (62.5)	5 (17.2) 24 (82.8)	9 (31.0) 20 (69.0)	26 (28.9) 64 (71.1)
Number of Subjects with Previous Flu Vaccination (%):				
Yes No Unknown	5 (15.6) 27 (84.4) 0 (0)	1 (3.4) 27 (93.1) 1 (3.4)	4 (13.8) 25 (86.2) 0 (0)	10 (10.11) 79 (87.8) 1 (1.1)
Number of Reaction to Previous Flu Vaccine (%):				
Yes No	0 (0) 5 (100)	0 (0) 1 (100)	1 (25.0) 3 (75.0)	1 (10.0) 9 (90.0)
Number of Subjects with Previous Flu Symptoms Last Winter (%):				
No	32 (100)	29 (100)	29 (100)	90 (100)

Table 2: Number (Percentage) of Subjects with Local Adverse Events within 7 days after Influenza Vaccine Administration.

Solicited Events	TD5 Active (n=32)	TD5 Placebo (n=32)	TD15 Active (n=29)	TD15 Placebo (n=29)	Intramuscular Injection (n=29)
Any solicited local event	31 (96.9)	32 (100)	29 (100)	29 (100)	27 (93.1)
Application site erythema	31 (96.9)	32 (100)	29 (100)	29 (100)	19 (65.5)
Application site induration	31 (96.9)	10 (31.3)	29 (100)	10 (34.5)	19 (65.5)
Application site edema	31 (96.9)	14 (43.8)	29 (100)	7 (24.1)	8 (27.6)
Application site bruising	1 (3.1)	0	1 (3.4)	2 (6.9)	5 (17.2)
Application site pain	12 (37.5)	1 (3.1)	11 (37.9)	4 (13.8)	17 (58.6)
Application site pruritus	23 (71.9)	4 (12.5)	23 (79.3)	5 (17.2)	9 (31.0)
Unsolicited Events	**TD5 Active (n=32)**	**TD5 Placebo (n=32)**	**TD15 Active (n=29)**	**TD15 Placebo (n=29)**	**IM (n=29)**
General disorders and administration site conditions	29 (90.6)	32 (100)	27 (93.1)	29 (100)	0
Pinpoint red spots at Application site	28 (87.5)	32 (100)	24 (82.8)	29 (100)	0
Application site bruising	0	1 (3.1)	0	1 (3.4)	0
Application site burning	4 (12.5)	3 (9.4)	0	1 (3.4)	0
Application site desquamation	15 (46.9)	0	12 (41.4)	0	0
Application site dryness	1 (3.1)	0	2 (6.9)	0	0
Application site pigmentation changes	1 (3.1)	0	1 (3.4)	0	0
Application site scar	1 (3.1)	1 (3.1)	0	0	0
Application site warmth	4 (12.5)	3 (9.4)	4 (13.8)	2 (6.9)	2 (6.9)
Nervous System Disorders	5 (15.6)	10 (31.3)	4 (13.8)	5 (17.2)	0
Paresthesia	5 (15.6)	10 (31.3)	4 (13.8)	5 (17.2)	0

TD5: Transdermal Microneedle System, 5 minutes; TD15: Transdermal Microneedle System, 15 minutes; IM: Intramuscular Injection.

Local events: During the first 7 days after vaccination, solicited application site events of erythema, induration, and edema were reported at nearly all active sites in the transdermal groups, and edema and induration were reported

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for a majority of subjects in the IM group (Table 2). Most solicited local events were mild or moderate. There was 1 event of severe pruritus at the active site in the TD5 group; and 4 events of severe erythema, and 1 event each of severe bruising, induration, and edema in the IM group. The majority of local events appeared within the first 3 days. In the transdermal groups, most events lasted for less than 8 days, except for erythema where most episodes lasted more than 8 days and the mean time to resolution was 32 days. In the IM group, most events lasted no more than 3 days.

The most frequently reported unsolicited local events within the first 21 days in the transdermal groups were pinpoint red spots at the application site and desquamation (sloughing of upper dead skin layers and most noticeable at time of patch removal); the only unsolicited local event in the IM group was injection site warmth. Most unsolicited local events occurred within 7 days of vaccination (Table 2) and lasted no more than 3 days. None of the events were severe.

Systemic events: Solicited systemic events within the first 7 days were reported in 21 of 32, 11 of 29, and 15 of 29 subjects in the TD5, TD15, and IM groups, respectively. The most frequently reported events in all groups were asthenia, headache, and myalgia. The higher overall incidence of systemic events in the TD5 group was mainly due to a higher incidence of asthenia and headache (Table 3). Most events were mild or moderate, started within 3 days of vaccination, and lasted no more than 3 days. Severe systemic events were reported for 4 subjects in the TD5 group (3 episodes of asthenia and 1 of headache), 1 subject in the TD15 group (asthenia), and 2 subjects in the IM group (sweating, asthenia, and myalgia).

Specific local events in the transdermal groups

At 2 minutes after transdermal patch removal, pinpoint red spots were observed at 28 of 32 active sites in the TD5 group and 24 of 29 active sites in the TD15 groups. More than 80% of those subjects with pinpoint red spots had spots covering less than 25% of the patch area, and no subject had spots covering more than 50% of the area.

At 15 minutes after patch removal, 22 of 32 subjects in the TD5 group and 21 of 29 subjects in the TD15 group had noticeable (barely perceptible) erythema at the active site, and 9 of 32 subjects and 8 of 29 subjects, respectively, had well-defined erythema. On Days 1, 2, and 3, a majority of subjects (71.9% to 93.5%) had well-defined erythema at the active site. Erythema covered more than 50% of the application site for nearly all (>95%) active sites at all evaluation times.

Noticeable/barely perceptible edema was present at 15 minutes after patch removal for 14 of 32 active sites in the TD5 group and 18 of 29 active sites in the TD15 group, and well-defined edema was observed for 10 of 32 and 8 of 29 active sites, respectively. At least 80% of active sites in both groups had well-defined edema on Day 1, but that proportion decreased to fewer than 30% on Day 3. The edema covered more than 50% of the application area for a majority (75.0% to 100%) of active sites at all evaluation times.

Table 3: Number (Percentage) of Subjects with Systemic Events within 7 Days after Influenza Vaccine Administration.

Treatment	Transdermal Microneedle System 5 Min (n=32)	Transdermal Microneedle System 15 Min (n=29)	Intramuscular Injection (n=29)
Any solicited event	21 (65.6)	11 (37.9)	15 (51.7)
Asthenia	20 (62.5)	8 (27.6)	11 (37.9)
Headache	13 (40.6)	6 (20.7)	8 (27.6)
Arthralgia	5 (15.6)	1 (3.4)	4 (13.8)
Myalgia	8 (25.0)	6 (20.7)	8 (27.6)
Rigors	7 (21.9)	3 (10.3)	0
Sweating	3 (9.4)	0	2 (6.9)
Malaise	1 (3.1)	0	0

Events following second vaccination at 6 months

Following the IM injection at Month 6, 22 of 32,12 of 29 and 12 of 29 subjects in the TD5, TD15, and IM groups, respectively, experienced immediate adverse events. The frequency of most immediate injection site events was similar for each group, with injection site erythema being the most common event. Only 2 subjects reported immediate systemic events (arthralgia, asthenia, and headache).

At 21 days after the second vaccination, 26 of 32, 28 of 29, 26 of 29 subjects in the TD5, TD15, and IM groups, respectively experienced local events and 20 of 21, 19 of 29 and 13 of 29 subjects, respectively, had systemic events.

Pain score and transdermal microneedle system acceptability

The mean (SD) VAS pain score was low for all treatment groups: 5.2 (7.3) mm, 15.2 (18.5) mm, and 7.7 (20.5) mm for the TD5 (active patch), TD15 (active patch), and IM groups, respectively.

Pain after patch removal was reported by 6 of 32 subjects in the TD5 group and 7 of 29 subjects in the TD15 group. On Day 0, most subjects (28 subjects in the TD5 group and 18 subjects in the TD15 group) considered patch application and removal to be less painful that the classical IM injection. On Day 21, most subjects (20 in the TD5 group and 21 subjects in the TD15 group) considered the patch as the first choice for mode of administration.

Immunogenicity

The three EMEA criteria were met for all three influenza strains at Day 21 in each treatment group. The immunogenicity results for both transdermal groups were generally similar to the IM group. In general, the system wearing time did not appear to affect the degree of antibody response (Table 4). Nearly all subjects were seroprotected against all three influenza strains at Day 21, although there was a decrease in antibody titers between Day 21 and Month 6 in all groups (Table 5). Many subjects were seroprotected against the A/H3/

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N2 strain pre-vaccination, indicating possible recent exposure to this strain.

Total IgE (non-specific) titers were similar for all three groups at Day 0 ranging between 24.7 and 41.6 kU/L and at Day 5 the IgE was similar to Day 0, ranging from 24.6 to 44.5 kU/L. IgA and IgG (against the A/H1/N1 strain) titers were similar for all groups at Day 0 and increased 5- to 11-fold between Day 0 and Day 21 (Table 6).

Discussion

Influenza continues to be a significant cause of morbidity and mortality, with an estimated 490,600 hospitalizations and 34,200 deaths in the United States during the 2018-2019 flu season [29]. Despite recommendations for universal vaccination [30], the rate of flu vaccination remains low [31]. Drawbacks to current vaccination methods include the requirement for cold-chain storage, the suboptimal immune

Table 4: Immunogenicity Results at Day 21 Compared to EMEA Guidelines.

Treatment	EMEA Requirements[a]	Transdermal Microneedle System 5 min	Transdermal Microneedle System 15 min	Intramuscular Injection
No. of subjects		27	27	28
Strain A/H1N1				
Seroconversion rate[b] or significant increase in HI titer[c]	>40%	81.5 (61.9, 93.7)	96.3 (81.0, 99.9)	89.3 (71.7, 97.7)
Geometric mean of titer increase[d]	2.5	18.0 (10.5, 30.7)	50.1 (31.3, 80.2)	27.2 (15.3, 48.4)
% of seroprotected subjects[e]	>70%	88.9 (70.8, 97.6)	100 (87.2, 100)	92.9 (76.5, 99.1)
Strain A/H3N2				
Seroconversion rate[b] or significant increase in HI titer[c]	>40%	44.4 (25.5, 64.7)	51.9 (31.9, 71.3)	71.4 (51.3, 86.8)
Geometric mean of titer increase[d]	2.5	4.91 (2.61, 9.24)	4.10 (2.56, 6.57)	8.72 (4.45, 17.1)
% of seroprotected subjects[e]	>70%	100 (87.2, 100)	96.3 (81.0, 99.9)	100 (87.7, 100)
Strain B				
Seroconversion rate[b] or significant increase in HI titer[c]	>40%	74.1 (53.7, 88.9)	74.1 (53.7, 88.9)	67.9 (47.6, 84.1)
Geometric mean of titer increase[d]	2.5	13.7 (8.81, 21.3)	13.9 (8.41, 23.0)	10.4 (6.15, 17.5)
% of seroprotected subjects[e]	>70%	81.5 (61.9, 93.7)	77.8 (57.7, 91.4)	75.0 (55.1, 89.3)

[a]EMEA Guidance Committee for Proprietary Medicinal Products (CPMP) (16)
[b]Proportion of subjects with pre-vaccination titer <10 (I/dil) to post-vaccination titer ≥40 (I/dil)
[c]Proportion of subjects with titers <10 (I/dil) and ≥4-fold increase in titer
[d]Mean geometric increase between Day 0 and Day 21
[e]Proportion of subjects achieving post-vaccination titer ≥40 (I/dil)
All data with 95% confidence interval
HI: hemagglutinin inhibition

Table 5: Geometric Mean (95% CI) Antibody Titers (I/dil) for Each Influenza Vaccine Strain.

Strain Type	Strain A/H1N1			Strain A/H3N2			Strain B		
	TD5	TD15	IM	TD5	TD15	IM	TD5	TD15	IM
Day 0	14.3 (7.53, 27.2)	7.94 (5.11, 12.3)	8.73 (5.35, 14.2)	53.1 (33.5, 84.1)	71.3 (40.5, 125)	26.9 (16.7, 43.3)	7.35 (5.75, 9.38)	7.64 (5.98, 9.75)	6.73 (5.27, 8.59)
Day 21	257 (140, 473)	398 (276, 573)	238 (130, 435)	261 (170, 399)	293 (200, 427)	235 (159, 347)	101 (66.2, 153)	106 (64.7, 174)	69.8 (43.6, 112)
Month 2	209 (111, 395)	316 (209, 476)	209 (111, 392)	248 (170, 364)	255 (181, 359)	198 (133, 294)	61.3 (40.2, 93.4)	82.2 (49.8, 136)	62.1 (37.5, 103)
Month 3	136 (75.1, 245)	217 (141, 336)	171 (97.7, 298)	138 (92.1, 206)	169 (113, 252)	144 (101, 207)	48.7 (31.5, 75.2)	73.9 (47.8, 114)	58.8 (35.5, 97.4)
Month 6	118 (63.1, 219)	164 (103, 263)	156 (81.7, 297)	162 (107, 246)	194 (131, 288)	162 (104, 252)	46.7 (28.7, 75.7)	66.8 (41.1, 109)	60.5 (33.9, 108)

Number of subjects for each calculated mean ranged from 23 to 28
TD5: Transdermal Microneedle System, 5 minutes; TD15: Transdermal Microneedle System, 5 minutes; IM: Intramuscular Injection

Table 6: Geometric Mean (95% CI) IgA and IgG Titers Against A/H1N1 (ELISA units).

Group	IgA		IgG	
	Day 0	Day 21	Day 0	Day 21
TD5 (n=27)	165 (103, 265)	1332 (890, 1994)	12259 (8630, 17415)	60128 (43125, 83833)
TD15 (n=27)	193 (121, 306)	2924 (1842, 4640)	14443 (10951, 19049)	92946 (74632, 11574)
IM (n=28)	199 (141, 281)	2141 (1369, 3348)	13538 (10993, 16764)	69875 (53237, 91713)

N = total number of subjects in immunogenicity analysis set
TD5: Transdermal Microneedle System, 5 minutes; TD15: Transdermal Microneedle System, 5 minutes; IM: Intramuscular Injection

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response of IM formulations [1,2], pain from the injection, the need for preservatives, and the use and disposal of needles and syringes. These drawbacks have led to the search for improved vaccine delivery methods, including intradermal administration, which has been shown to be effective for flu vaccines [16,32-34].

This study evaluated the immunogenicity and safety of a novel transdermal microneedle system for the delivery of trivalent influenza vaccine. The transdermal microneedle system consists of titanium microneedles with vaccine formulation coated onto the tip of each microneedle [22]. The drug-coated microneedles penetrate through the superficial skin barrier layer into the epidermal/dermal layers, where the vaccine formulation rapidly dissolves and is released into the skin. This system has the advantages of penetrating the superficial skin barrier without pain or inconvenience [17,19,35]; it is also preservative-free and can be stored at room temperature [22].

The immunogenicity of the trivalent influenza vaccine administered by the transdermal microneedle system met all three EMEA criteria for influenza vaccines (seroconversion, increase in antibody titers, and seroprotection) at 21 days after vaccination. The length of time the transdermal system was in place (5 or 15 minutes) did not affect the antibody response. The immunogenicity response to all three vaccines strains was equivalent to the IM injection response, with similar antibody titers achieved at all evaluation times from 21 days to 6 months after vaccination. Importantly, the response to the transdermal administration was achieved with lower doses of all three influenza strains (10 µg each) than those used for the IM injection (15 µg each). Thus, transdermal administration using this system is dose-sparing, providing significant benefits to patient safety and cost savings.

The inactivated influenza vaccine was generally well tolerated following transdermal administration. Local adverse events immediately following the first vaccination, notably erythema, induration, and edema, were slightly more frequent with transdermal than IM administration. Pinpoint red spots were also noted in a majority of transdermal application sites, although these covered less than 50% of the application area. Solicited local and systemic events within 7 days of vaccination were similar for both transdermal and IM administration, although more unsolicited events were reported in the transdermal groups. The IM vaccination at 6 months after the initial vaccination was well tolerated with similar adverse event profiles for subjects who initially received transdermal or IM vaccination. Pain associated with transdermal administration was low and similar to IM injection, and most subjects preferred the transdermal vaccine administration to IM administration.

The Covid-19 pandemic has shown us that traditional vaccine delivery risks cross-infection as hospitals are potentially a primary source of viruses; thus, minimizing the number of people to be vaccinated in a hospital, Doctor's office would reduce the spread of the virus, protect health care workers, and conserve personal protective equipment utilization. Transdermal microneedle coated with a vaccine that can be mailed to each household and is patient administered could be a solution to global distribution and delivery.

Conclusions

Influenza vaccination administered by a novel transdermal microneedle system was effective, safe, and well tolerated. The system provides three key advantages over the currently available formulations; it is preservative-free, can be stored at room-temperature storage, and is dose sparing.

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