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Trivalent influenza vaccine and febrile adverse events in Australia, 2010: Clinical features and potential mechanisms

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ABSTRACT

Introduction: Increased numbers of children presenting with febrile adverse events following trivalent influenza vaccine (TIV) were noted in Australia in 2010. We describe the epidemiology and clinical features of the adverse events and explore the biological basis for the adverse events using an in vitro model.

Materials and Methods: Children presenting to a tertiary paediatric hospital in 2010 with adverse events within 72 h of TIV were retrospectively reviewed. Demographics, clinical features, physiological variables and outcomes were examined. Plasma cytokine and chemokine levels were examined in a subgroup of children with vaccine-related febrile convulsions. Peripheral blood mononuclear cells of age-matched children were stimulated with different TIV preparations. Inflammatory cytokine and chemokine analysis was performed on cultured supernatants.

Results: Vaccine-related febrile adverse events were identified in 190 children. Most occurred in healthy children (median age: 1.5 years) within 12 h of vaccination. Twenty-eight (14.7%) required hospital admission. High temperature ≥39.0 °C (101/190; 53%), vomiting (120/190; 63%) and convulsions (38/190; 20%) were common. All children presenting had received Fluvax® or Fluvax Junior®.

In the in vitro model, IFN-α, IL-1β, IL-6, IL-10, IP-10 and MIP-1α levels were significantly higher when measured at 6 and 24 h in cultures stimulated with Fluvax® compared with alternative 2010 TIV preparations.

Conclusions: Numerous febrile adverse events (including febrile seizures) were observed following Fluvax® or Fluvax Junior® in 2010. Clear differences in cytokine production were observed when peripheral blood mononuclear cells were stimulated with Fluvax® compared with alternate TIV preparations. Increased awareness of these potential adverse events is required to ensure earlier detection and prevention in the future.

1. Introduction

The increased risk of severe influenza in preschool children[1] has prompted the use of seasonal influenza vaccines in this age group. Routine influenza vaccination of healthy young children using trivalent influenza vaccine (TIV) is recommended in many countries including the United States and Canada.[2,3] Large case series have shown this to be well tolerated and rarely associated with serious adverse events including seizures.[4,5] Within Australia, Western Australia (WA) is the only state recommending routine immunization of children aged 6 months to 5 years with TIV. Established in 2008, this program has been associated with decreased severity of illness and hospitalizations.[6]

Abbreviations: TIV, trivalent influenza vaccine; WA, Western Australia; PBMCs, peripheral blood mononuclear cells; ACIR, Australian Childhood Immunisation Register; LPS, lipopolysaccharide; SEB, staphylococcal enterotoxin B; PMH, Princess Margaret Hospital; IQ, interquartile.

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Vaccines from three manufacturers were used in the WA vaccination program in 2010. All vaccines contain the three haemagglutinin antigens recommended by the World Health Organization for the 2010 southern hemisphere influenza season (A/California/7/2009 (H1N1)-like virus, A/Perth/16/2009 (H3N2)-like virus and B/Brisbane/60/2008-like virus).[7] Fluvax® (CSL Biotherapies, Parkville, Australia), Influvac®, (Solvay SA, Brussels, Belgium) and Vaxigrip®, (Sanofi Pasteur, Lyon, France) contain 15 μg of haemagluttinin from each of the three vaccine strains per dose. Children 6 months to 3 years are administered a half dose (0.25 ml) and children three and older are administered an adult dose (0.5 ml). In addition, Fluvax Junior® is distributed for children 6 months to 3 years and contain 7.5 μg of haemagluttinin from each of the three vaccine strains per dose. Despite similarities in their composition, differences exist in their manufacturing processes.

These vaccines were made available to vaccine providers from the 8th March 2010. Safety concerns were raised in Australia after an apparent increase in adverse events, particularly in young children. Increased numbers of children presenting with severe febrile reactions post-TIV were noted in April 2010, a number of whom developed febrile convulsions. Investigation of these reports resulted in suspension of the WA preschool influenza vaccination program on the 22 April 2010 and the national influenza immunization program for children less than 5 years of age the following day.[8]

Following suspension of the program, the rates of febrile convulsions in WA children < 5 years following Fluvax® and Fluvax Junior® were calculated to be 4.4 convulsions per 1000 doses administered (>14,000 doses administered; Armstrong et al.,[23] Manuscript in review, BMJOnline). No febrile convulsions were observed following administration of Influvac® and Vaxigrip® (<5000 doses administered). The rate of febrile convulsions following Fluvax®/Fluvax Junior® was significantly higher in 2010 than that observed with all TIV preparations in 2009 (0.3 febrile convulsions per 1000 doses administered). Likewise, the rate of adverse events in 2010 following Fluvax®/Fluvax Junior® were significantly higher compared with the rate of adverse events for all other TIVs.[8]

To date, despite extensive analyses, the biological basis for the excess cases of febrile adverse events after the administration of Fluvax® and Fluvax Junior® remains unclear.[8] This report explores the biological basis for the reactions by describing the clinical features of children with convulsive and non-convulsive febrile adverse events and examining inflammatory cytokine profiles in a group of children with adverse events. Based on these data, we hypothesise that excessive pyrogenic cytokine production contributed to the adverse events. We explored this hypothesis using an in vitro stimulation model in which peripheral blood mononuclear cells (PBMCs) from young children (12–36 months of age) were challenged with different TIV preparations and cytokine responses assessed.

2. Material and methods

2.1. Clinical data

Princess Margaret Hospital is the only tertiary paediatric hospital in the state of WA (population 2.26 million) with more than 65,000 emergency paediatric presentations per year. Admission or discharge diagnoses are available through the WA Emergency Department Information System. Children presenting from the 8th March 2010 to 25th April 2010 with possible immunization-related adverse events were identified retrospectively using ICD-10 diagnostic codes: R56.0 (febrile convulsion), T88.8 (other vaccination complication), R50.9 (febrile illness of unknown origin), B34.9 (viral illness), G40.3 (generalized idiopathic epilepsy and epileptic syndromes), T80.5 (anaphylactic shock due to immunization) and T80.6 (vaccination complicated by allergic reaction).[9]

A vaccine-related febrile adverse reaction was defined as any child presenting within 72 h of TIV with a documented fever (>37.5 °C as recorded by health-care providers or parents) where an alternative source of fever was not identified clinically and/or microbiologically. Febrile convulsions were identified using published definitions.[10] All adverse events were reviewed by one author (CCB) to ensure consistency of data collection.

Medical records of all children identified were reviewed. Receipt of TIV in the previous 72 h was confirmed by examining the Australian Childhood Immunization Register (ACIR) and case notes. Demographics, symptoms, physiological parameters, results of any investigations performed, treatment and outcome data were recorded. Where clinical data and immunization status were uncertain, parents and/or immunization providers were contacted.

2.2. Immunological experiments

Clinical samples were routinely stored by our pathology service at 4–8 °C for 7 days following collection. Plasma samples were collected into tubes containing lithium heparin (BD Vacutainer®, Becton Dickenson, Franklin Lakes, NJ) and centrifuged as per manufactures instructions. Following suspension of the program, any remaining plasma samples from children sustaining vaccine-related febrile adverse events were identified and stored at −20 °C.

IL-6, IL-10, IP-10, MIP1α, TNFα and IFNγ levels in samples were determined using an in-house multiplex bead-based assay. In brief, primary antibodies (IL-6, IL-10, IP-10, MIP1α, and IFNγ: Becton Dickinson; TNFα: BioScientific Pty Ltd, Australia) were covalently conjugated to carboxylated microspheres (Bio-Rad Laboratories Inc, Hercules, CA). Samples were diluted in PBS/0.05% Tween/2% FCS. Microspheres and samples were incubated at room temperature on an orbital shaker. After 30 min, biotinylated secondary antibodies were added for another 30 min. After washing (PBS, 1% BSA, 0.05% Tween, 0.001% Sodium azide), streptavidin-PE conjugate (Becton Dickenson) was added for 15 min. Samples were washed again and fluorescence in each specific bead region was measured on the BioPlex® 200 System (Bio-Rad). Data was acquired electronically in real-time and analysed using BioPlex Manager 5.0 software. Data in pg/ml was generated from a 5-PL standard curve of median fluorescent intensity against a standard curve of recombinant cytokines. IL-1β, IL-8 and IFNα levels were measured using a commercially available ELISA Kit (Bender MedSystems, eBioscience, Inc, San Diego, CA) according to the manufacturer's instructions.

No baseline values for cytokine levels in plasma from healthy children have been reported for this age group. Cytokine levels from clinical samples were therefore compared with baseline 'normal' values established in plasma from healthy preschool children participating in a study investigating ear disease.

2.3. Potential mechanisms for the reaction were explored using an in vitro model

PBMCs (1.25×10^5) from 22 donors aged <36 months were cultured in 125 μl of RPMI 1640 Medium + L-Glutamine (Invitrogen, Victoria, Australia) with 10% foetal calf serum (SAFC-Biosciences, Victoria, Australia) and stimulated with either 12.5 μl of neat Fluvax® 2010 (CSL Biotherapies, Parkville, Australia), Influvac® (Solvay SA, Brussels, Belgium), Vaxigrip® (Sanofi Pasteur, Lyon, France), lipopolysaccharide (LPS; 1 ng/ml) or *Staphylococcal* enterotoxin B (SEB; 1 μg/ml) and incubated at 37 °C/5% CO_2. Culture supernatants were collected after 6 or 24 h and stored at −20 °C until cytokine analysis was performed (as above). Responses to fur-