Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Experimental preparation of Killed-Rotavirus vaccine and evaluation of immune potential compared to a current available Rotavirus vaccine

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree

In Pharmaceutical Sciences
(Microbiology and Immunology)

By

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Bachelor of Pharmaceutical sciences, 2011
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Ayaa S. M. Hashim

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List of Abbreviations

Ag-Ab: Antigen - Antibody complex.

Alum: Aluminum hydroxide gel.

bp: base pair.

BSA: Bovine serum albumin.

Caco-2: Human colonic adenocarcinoma.

CPE: Cytopathogenic effect.

DMEM: Dulbecco's Modified Eagle's medium with 4.5 g/l glucose and glutamate.

DLP: Double-layered particle.

ds-RNA: Double-stranded RNA molecule.

EDTA: Ethylene-diamine tetra-acetic acid.

ELISA: Enzyme-linked immunosorbent assay.

EM: Electron Microscopy.

G protein: Signify VP7 referring for being a glycoprotein.

HCl: Hydrochloric acid.

hr: hour.

HRP: Horseradish peroxidase enzyme.

IEM: Immune Electron Microscopy.

IgG: Immunoglobulin G (Gamma Globulin).

IRVV: Inactivated Rotavirus Vaccine.

L3: Monooleate/Lauric acid.

MA104 cells: Monkey kidney epithelial cells.

EMEM: Eagle minimum essential medium.

MPL: Monophosphoryl lipid A from *Salmonella minnesota*.

NAMRU-3: Naval Medical Research Unit-3, Egypt.

nm: Nanometer.

NSP: Non-Structural Protein.

OD: Optical density.

P protein: Signify VP4 protein referring to the protease-sensitivity.

PAGE: Polyacrylamide gel electrophoresis.

PPAT: Passive particle agglutination test.

PBS: Phosphate buffered saline solution.

PCR: Polymerase chain reaction

PFU: Plaque forming unit.

RPMI: Roswell Park Memorial Institute medium.

RRV-TV: Rhesus reassortant Rotavirus- tetravalent vaccine.

RT-PCR: Reverse transcription – PCR.

RV: Rotavirus.

RVGE: Rotavirus Gastroenteritis.

RVI: Rotavirus infection.

TLP: Triple-layered particle.

TMB: 3,3′,5,5′-Tetramethylbenzidine liquid Substrate.

VACSERA: The Egyptian Holding Company for production of vaccines, sera and drugs.

Vero: African green monkey kidney.

VLPs: Virus-like particles.

VP: Viral protein.

VRBPAC: Vaccines and Related Biological Products Advisory Committee.

WHO: World Health Organization.

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Abstract

Rotavirus (RV) causes a severe diarrheal illness, mostly in children under five-years of age. RV infection (RVI) is a common illness, affects both developed and developing countries in equal rates, approximately. However, more death resulted in poor/ low-income countries due to the absence of constricted and effective health care.

Nowadays, many attenuated RV vaccines have been approved for immunization against RVI. However, the attenuated vaccination drawbacks of elevated cost; low-efficacy in poor countries; strains reassortment risk; and intussusception events, stimulate a real need for developing of new RV vaccination approaches. That's including the inactivated RV vaccination to avoid the active-virus related concerns.

The current study aimed to prepare a pentavalent, inactivated Rotavirus vaccine (IRVV) using the most prevalent strains, circulating in the Egyptian environment. The vaccine immunogenicity was evaluated, after that, in mice as a representing animal model. The selected immunity marker to be detected was the RV-specific IgG antibodies; owing to their high impact in RV immunization. The trial-inactivated pentavalent vaccine immunogenicity was compared with the Rotarix® attenuated vaccine. That's to evaluate the immunogenicity of the inactivated approach against the attenuated commercially available one.

As result of the variation in the antigenic content and dosing schedule between the trial-IRVV and Rotarix® vaccine, the comparative assessment was dependent on achieving IgG-antibodies level exceeding 1: 6400. That limit implies a less susceptibility of RVI.

The trial-IRVV was developed with Egyptian isolated strains of G1, G2, G3 and G9/ P[8], formulated with 5% sucrose and 2% polysorbate-80 and thermally inactivated at 60°C for 2 hrs. A part of the prepared vaccine was modified with Alum-adjuvant. IRVV and Alum-IRVV trials were injected, subcutaneously, into mice groups at 0, 21, 35 days intervals. In parallel, the reference mice group was vaccinated with Rotarix® vaccine twice on 0 and 28th days, as recommended by the Rotarix® manufacturer.

IgG antibodies elevation was achieved with the pentavalent IRVV/ IRVV-Alum formulas, as well as the Rotarix® reference vaccine. In all vaccine formulas, IgG antibodies were exceeding the limit of 1:6400, indicating a less susceptibility for RVI and a reasonable immunization.

Further intensive research is needed to reveal the heterotopic and intestinal immunization that could be achieved with the inactivated vaccination approach, besides, application of variant adjuvants to best modify the IRVV immunization pattern.

Introduction

Rotavirus (RV) is a 75 nm icosahedral, non-enveloped and double-stranded RNA virus, which is constructed from six structural and five non-structural proteins. It was estimated to be the leading cause of sever gastroenteritis in infants and young children, leading to approximately half millions of deaths each year. In Egypt, RV gastroenteritis (RVGE) was considered as one of the most common causes of severe diarrhea and hospitalization among Egyptian children, as well. Moreover, RV diarrheal-illness was estimated to occur at least once in 40 % of the 2-year Egyptian children, where the susceptibility and severity of infection was estimated to be decreased after the first exposure. By increasing the number of previous infections, RV infection (RVI) severity and related complications was found to be markedly decreased.

There is no specific treatment for RVI. For children with healthy immune system, RVI and associated bowel destruction were illustrated to be a self-limited illness that lasting for few days only. As the natural RVI was found to be partially protective against subsequent reinfections. So, the vaccination is the ideal choice for preventing any mortality and morbidity events associated with RV diarrheal-illness. The attenuated vaccines immunization was found to be equivalent to that developed by natural infections, where the attenuated virus proliferates in the wild virus manner; so producing the desired immunity.

The complete protection against moderate-to-severe diarrhea could be resulted after two infections, regardless of the infection type (symptomatic or asymptomatic). Dependently, a multiple-dosing regimen of the designed attenuated vaccines was described for a reasonable immunization.

Despite of the availability of the attenuated Rotavirus vaccines in the market, global immunization is limited especially in the poor countries as result of variant obstacles. In African countries including Egypt, the most considerable RV attenuated-vaccination obstacles are: (i)- The variation of efficacy as result of the limited virus replication under certain circumstances; (ii)- The high vaccination-dose cost which does not match the individual income in these poor countries; (iii)- The fear from the impact of the attenuated vaccination on the African children, who were suffering mostly from immunodeficiency and nutrition problems; and (iv)- Intussusception and living-virus reassortment concerns. Accordingly, the inactivated-Rotavirus vaccination approach is strongly suggested for current research as an alternative strategy for RV immunization. The inactivated RV vaccine (IRVV) could bypass most of the attenuated vaccines weak points, which mostly related to the active virus presence.

Numerous strains were estimated to circulate in Egyptian environment, where G1, G2, G3, G9 and P[8] epitopes were commonly found in these strains. Accordingly, a special concern should be directed to these pentavalent epitopes.