Vaccine 35 (2017) 3672–3681

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Deinococcus Mn^{2+}-peptide complex: A novel approach to alphavirus vaccine development

Manoshi Gayen [a,b,1], Paridhi Gupta [a,b,*,1], Elaine M. Morazzani [c], Elena K. Gaidamakova [a,b], Barbara Knollmann-Ritschel [a], Michael J. Daly [a,*,3], Pamela J. Glass [c], Radha K. Maheshwari [a,2]

[a] Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA
[b] Henry M. Jackson Foundation, Bethesda, MD 20817, USA
[c] Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702, USA

ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 17 April 2017
Accepted 4 May 2017
Available online 30 May 2017

Keywords:
Venezuelan equine encephalitis virus
Chikungunya virus
Deinococcus radiodurans
Inactivated vaccine
MnDpPi
Aerosol challenge

ABSTRACT

Over the last ten years, Chikungunya virus (CHIKV), an Old World alphavirus has caused numerous outbreaks in Asian and European countries and the Americas, making it an emerging pathogen of great global health importance. Venezuelan equine encephalitis virus (VEEV), a New World alphavirus, on the other hand, has been developed as a bioweapon in the past due to its ease of preparation, aerosol dispersion and high lethality in aerosolized form. Currently, there are no FDA approved vaccines against these viruses.

In this study, we used a novel approach to develop inactivated vaccines for VEEV and CHIKV by applying gamma-radiation together with a synthetic Mn-decapeptide-phosphate complex (MnDpPi), based on manganous-peptide-orthophosphate antioxidants accumulated in the extremely radiation-resistant bacterium *Deinococcus radiodurans*. Classical gamma-irradiated vaccine development approaches are limited by immunogenicity-loss due to oxidative damage to the surface proteins at the high doses of radiation required for complete virus-inactivation. However, addition of MnDpPi during irradiation process selectively protects proteins, but not the nucleic acids, from the radiation-induced oxidative damage, as required for safe and efficacious vaccine development. Previously, this approach was used to develop a bacterial vaccine. In the present study, we show that this approach can successfully be applied to protecting mice against viral infections.

Irradiation of VEEV and CHIKV in the presence of MnDpPi resulted in substantial epitope preservation even at supra-lethal doses of gamma-rays (50,000 Gy). Irradiated viruses were found to be completely inactivated and safe *in vivo* (neonatal mice). Upon immunization, VEEV inactivated in the presence of MnDpPi resulted in drastically improved protective efficacy. Thus, the MnDpPi-based gamma-inactivation approach described here can readily be applied to developing vaccines against any pathogen of interest in a fast and cost-effective manner.

1. Introduction

Historically, the discovery and commercial development of a licensed lead vaccine candidate such as subunit or recombinant DNA vaccines often require several years of basic research followed by years of pre-clinical and clinical development to ensure safety and efficacy. Recent experiences with pathogens such as Ebola and Zika viruses have highlighted the importance of developing vaccines much more rapidly. A recently developed epitope-preserving but genome-destroying approach using gamma-radiation now presents itself as a promising strategy for speedy deployment of virus vaccines [1].

Abbreviations: CHIKV, Chikungunya virus; VEEV, Venezuelan Equine Encephalitis virus; FDA, Food and Drug Administration; MnDpPi, manganese decapeptide phosphate complex; MRSA, methicillin-resistant *Staphylococcus aureus*; IR, ionizing radiation; ssRNA, single stranded RNA; ROS, reactive oxygen species; IND, investigational new drug; DPBS, Dulbecco's phosphate-buffered saline; $TCID_{50}$, 50% tissue culture infectivity dose; MOI, multiplicity of infection; HRP, Horseradish peroxidase; FBS, fetal bovine serum; CPE, cytopathic effect; VSV, vesicular stomatitis virus; RIDP, residual-infectivity-dependent-pathogenesis.

* Corresponding authors at: Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA (P. Gupta and M.J. Daly).

E-mail addresses: guptaparidhi@gmail.com, paridhi.gupta.ctr@usuhs.edu (P. Gupta), michael.daly@usuhs.edu (M.J. Daly).

[1] Authors contributed equally.
[2] Deceased.
[3] Regarding MnDpPi complex.

http://dx.doi.org/10.1016/j.vaccine.2017.05.016
0264-410X/

The diverse mechanisms of ionizing radiation (IR)-induced damage to cellular macromolecules (proteins, DNA, lipids) are classified into the following categories. 'Direct action' refers to IR energy deposition within or in very close proximity to the macromolecule, and predominates under non-aqueous conditions (deeply frozen or desiccated) [2]. In contrast, under physiological conditions, reactive oxygen species (ROS) generated by water radiolysis produce the vast majority of IR-induced damage through 'indirect action' [3]. Some of these ROS are long-lived (e.g. hydrogen peroxide (H_2O_2) and it's precursor superoxide ($O_2^{\cdot-}$)) and produce more active forms of ROS (e.g. hydroxyl radicals, $HO^{\cdot}$), by reacting with iron through the Fenton reaction) [2]. The most prominent, permanent ROS-mediated protein damages are protein carbonyls [4]. Protein carbonylation and other oxidative modifications alter the structural conformation of the proteins leaving them functionally inactive. For irradiated whole-virus vaccines, IR-induced destruction of a virus' genome is desired, while IR-induced damage to epitopes needs to be prevented to ensure preservation of antigenic potency. However, ROS-mediated damage caused by IR does not normally discriminate between nucleic acids and proteins, and strategies utilizing lower IR doses in order to prevent protein oxidation are invariably compromised by the increasing risk of incomplete inactivation, which can cause infection in vaccine recipients. In the past, incomplete inactivation of whole-virus vaccines has led to several outbreaks [5–7]. Developing inactivated vaccines for viruses with positive, single-stranded RNA (ssRNA) genomes is an even greater challenge since the genome alone is infectious and capable of causing disease and generating live virus particles after entering the host cells [8–10]. Here we present an approach to uncouple IR-induced protein damage from RNA damage for developing second-generation irradiated whole-virus vaccines. A synthetic Mn^{2+}-decapeptide-orthophosphate antioxidant complex named MnDpPi (or MDP [11]) was used to selectively prevent the oxidation of envelope proteins at IR doses which render the genomes of alphaviruses non-infectious.

Recent studies have shown that proteins, but not DNA, in the extremely radiation-resistant bacterium *Deinococcus radiodurans* are extraordinarily resistant to oxidation. Protein protection in *D. radiodurans* is mediated by the accumulation of Mn antioxidants that consist mainly of Mn^{2+}, orthophosphate (Pi) and peptides. These complexes can functionally substitute enzymes such as superoxide dismutase and catalase [12], and scavenge ROS generated by abiotic and biotic processes arising from exposure to gamma and ultraviolet radiation, and desiccation [3,13]. Earlier, Daly et al. reported that when applied *in vitro*, these naturally-occurring Mn^{2+}-peptide-Pi complexes in protein-free cell extract (ultrafiltrate) of *D. radiodurans* protected the activities of enzymes irradiated *in vitro at supralethal doses* of gamma-radiation [14]. This led to the synthesis of a rationally-designed decapeptide DEHGTAVMLK (Dp), yielding MnDpPi, which forms spontaneously when Dp (3 mM), Mn (1 mM $MnCl_2$) and Pi (25 mM potassium phosphate buffer (pH 7.4)) are combined [1]. Under aqueous *in vitro* conditions, MnDpPi protected the structure and function of irradiated proteins exposed to 60 kGy, but did not significantly protect DNA or RNA [1,14]. Manganous ions (Mn^{2+}) in MnDpPi act as a catalytic scavenger of long-lived ROS (e.g. $O_2^{\cdot-}$) which damage proteins primarily, whereas the peptide component of MnDpPi serves to scavenge $HO^{\cdot}$ stoichiometrically [3,12]. We previously reported that MnDpPi was used to develop a highly immunogenic vaccine candidate against methicillin-resistant *Staphylococcus aureus* (MRSA) using a mouse model [1].

In this study, we have extended our earlier studies and successfully applied the MnDpPi-irradiation approach to the development of viral vaccines using V3526 and CHIK 181/25, live-attenuated vaccine candidates for Venezuelan equine encephalitis virus (VEEV) and chikungunya virus (CHIKV), respectively. VEEV, a New World alphavirus, is a potential bioweapon that is highly infectious in an aerosolized form and can result in fatal encephalitis [15–17]. CHIKV, an Old World alphavirus, is an emerging pandemic pathogen that causes severe arthralgia and maculopapular rashes, with outbreaks reported in many Asian countries, France, Italy, Caribbean Islands, and the Americas including hundreds of locally transmitted cases in the USA until last year, making it a virus of significant public health importance [18–20]. At present, there are no FDA-approved vaccines against these two arboviruses. Earlier attempts for VEEV vaccine development have been limited due to high non-responder rate, adverse reactions in recipients and ability of the virus to revert back to its virulent phenotype [21,22]. Although a VEEV DNA vaccine expressing all the viral structural proteins has shown promise in recent clinical trial, it resulted in adverse reactions in 25.3% recipients after vaccination [23]. V3526, a live-attenuated strain of VEEV, was developed by site-directed deletion of furin cleavage site from PE2 glycoprotein and incorporation of a single amino acid mutation in the E1 glycoprotein [24]. Similarly, several approaches have been tested for CHIKV vaccine development of which recombinant vaccine, VLP and live attenuated vaccine approaches have shown promise in the recent clinical trials but are still accompanied with limited seroconversion or systemic adverse reactions in large number of recipients [25–27]. CHIK 181/25, a live-attenuated vaccine candidate for CHIKV, was developed by plaque-to-plaque passaging [28]. Although both CHIK 181/25 and V3526 yielded high-level of immunogenicity in human clinical trials, further development of these vaccines was not pursued due to the development of adverse side-effects in some V3526 vaccinees; and the lack of funding support arising from the lack of military relevance, in the case of CHIK 181/25 [21,27–30].

Live-attenuated vaccines have an inherent risk of reversion to the wild-type as well as transmission potential by the naked genomes themselves as observed with CHIKV vaccine [27,31]. Moreover, the VEEV vaccine candidate (C84), currently under investigational new drug status (IND), causes limited seroconversion and induces a high rate of febrile illness in the recipients [22]. An ideal alphaviral vaccine candidate should be highly immunogenic and safe (unable to revert back to wild type phenotype) [32]. Therefore, inactivation of V3526 and CHIK 181/25 in a manner that renders them non-infectious without compromising their immunogenic potential offers the prospect of rapid production of safe and efficient alphavirus vaccine [27,31,33,34]. In this proof-of-concept study, we used MnDpPi during inactivation of V3526 and CHIK 181/25 by gamma-irradiation to generate highly immunogenic inactivated vaccine candidates. V3526 and CHIK 181/25 irradiated in presence of MnDpPi were found to be safe *in vivo*. Immunization with inactivated V3526 preparation protected mice against the lethal exposure to aerosolized wild-type VEEV.

2. Materials and methods

2.1. Decapeptide

The synthetic decapeptide (Dp) H-Asp-Glu-His-Gly-Thr-Ala-Val-Met-Leu-Lys-OH was custom-synthesized (American Peptide Co. Inc., Sunnyvale, CA) and prepared as described previously [1]. Net peptide content was determined by amino acid analysis at American Peptide Co. Inc., Sunnyvale, CA. Peptide purity was 95%. The radioprotective ability of each batch of Dp was validated by post-irradiation activity assay for *Bam*HI [14].

2.2. Virus preparation

V3526 purification was performed as described previously [1]. Briefly, V3526 was purified from supernatants of baby hamster