

Whole Parasites as Promising Malaria Vaccines: Previous Efforts and New Developments

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Abstract

Malaria is an important infectious disease in the world that it is the cause of million deaths each year, especially in pregnant woman and child. Different individual preventive strategies have been applied in endemic regions to reduce the rate of infection, but those were not effective totally. Consequently, it seems that development of potent vaccines would be the best solution for blocking the parasite life cycle. According to the non-promising results of sub-unit vaccines for malaria, whole parasite vaccines have been reintroduced to overcome the present obstacles. This review describes the different types of whole parasite malaria vaccines based on their preparation methods.

Keywords: Malaria; Chemoprophylaxis and Sporozoites; Genetically attenuated parasites; Chemically attenuated parasites; Killed parasites; Radiation-attenuated parasites

Abbreviations: WHO: World Health Organization; CPS: Chemoprophylaxis and Sporozoites; GAPS: Genetically Attenuated Parasites; RASs: Radiation-Attenuated Parasites; CpG-ODN: CpG-Oligo Deoxy Nucleotide; MVRG: Malaria and Vector Research Group; PII: Pasteur Institute of Iran.

Introduction

Malaria is an ancient vector borne infectious disease that is caused by the six known *Plasmodium* species in human. With regard to broad scope of its impacts on different nations in the world, especially its mortality and morbidity on pregnant women and child, it has been considered as one of the priorities of the world health organization (WHO) from previous years. Based on recent WHO report, malaria has been the cause of more than 216

million clinical cases and claims about 445,000 lives per year which most of them are related to African countries and their child (WHO World Malaria Report, 2017). According to the scientific researches, different intervention and individual protective approaches such as different insecticides, insecticide-treated bed nets and repellents have been introduced and applied in malaria endemic regions [1]. Despite the applied strategies and protective measures for mosquito control, elimination of the disease was not reached. From the previous years, vaccines have been one of the main tools to combat and control infectious diseases and it seems that it should be considered as one of the main objects to reach malaria elimination and eradication in the future. According to the WHO road map, malaria should be eliminated in the world till 2050 [2]. Consequently, scientific findings in different

subjects should be guided and managed in specific direction to reach this goal as soon as possible.

Many efforts have been done for developing an effective malaria vaccine in these decades, but this goal has remained elusive yet. Several elements such as: complicated life cycle of *Plasmodium* parasite; different and somewhat unknown interactions between the parasite and human; genetic and immune response diversity between the different nations, genetic diversity of the parasite and host immune system escaping of the parasite; distinct parasite and vector species for each geographical region and limited knowledge for stimulating a robust sterilizing immune response are the main causes of this failure.

Improvements in molecular cloning, genetic engineering approaches and recombinant protein production led to the shift in vaccine research from whole organism to sub-unit vaccines. This change of direction has positive impacts against the several infectious diseases [3]. But, acquired results after challenges have been discouraging about malaria and its advanced sub-unit vaccine [4]. Consequently, whole parasite vaccines have considered again as a promising tool to combat against malaria by research community.

First attempts for vaccine production in medicine included the targeted microorganism isolation, culture of the isolated strain for large scale production of vaccine, killing or attenuation of the whole microorganisms and their injection. Interestingly, the first malaria vaccine researches were started by whole parasite organism in the 1940 on killed *Plasmodium lophurae* and *Plasmodium knowlesi* and their efficacy was evaluated on ducks and monkeys [5,6]. After that, killed *P. falciparum* parasites were used for immunization of monkeys [7]. The results of these experiments were promising but the negative points of these studies were using the complete Freund's adjuvant for stimulating the immune system. With regard that this adjuvant is not applicable for human, in the next experiments several compatible adjuvants with human use were applied, but the immunization process were not protective against the infection [6]. As mentioned before, according to the disappointing results of sub-unit vaccines in clinical trials, for example RTS, S as a pioneer and advanced malaria sub-unit vaccine, malaria vaccines based on whole parasite persuade the attentions as a new promising solution [4].

These vaccines can be divided to different types based on their preparing approaches or their target step on parasite life cycle. In this paper, we review them based on

their preparing methods. According to this criterion, those are divided to five groups:

- a) Chemoprophylaxis and Sporozoites (CPS)
- b) Genetically attenuated parasites (GAPs)
- c) Chemically attenuated parasites (CAP)
- d) Killed parasites
- e) Radiation-attenuated parasites (RASs)

Chemoprophylaxis and Sporozoites (CPS)

This method was introduced for the first time by Beaudoin RL, et al., Golenser J, et al. & Orjih AU, et al. on rodent malaria model and chloroquine prophylaxis [8-10]. In this group of vaccines, ant malarial drugs; which are against the erythrocytic stage of parasite's life cycle; are administered with Sporozoites? In this situation, Sporozoites enter the liver and complete the liver stage and then merozoites are released to blood stream. Now, asexual stage parasites are killed by the administered prophylactic drug. Therefore, killed merozoites and Sporozoites, which could not enter the liver in previous step, are exposed to immune system. Consequently, different stages of parasites provoke the immune system without permission of creating the clinical manifestations. This type of vaccine was tested on human in 2009 for the first time with *P. falciparum* and Chloroquine prophylaxis [11]. Acquired results were hopeful and a sterile immunity with prolonged immune response was seen [11,12]. Chloroquine and Mefloquine that block the blood stage replication of parasite are the first choices for CPS. Furthermore, those can be used in combination with anti-liver stage drugs for more assurance and potent sterility [13,14].

Genetically Attenuated Parasites (GAPs)

By introducing the new genetic engineering methods for gene rearrangements in different organisms, these methods were applied for free-living forms of unicellular parasites as well [15]. According to the importance of malaria and necessities for understanding the function of genes and creating specific mutant strains, gene replacement and deletion methods were presented for *P. berghei* as a model by van Dijk MR, et al. & Menrad R, Janse C for the first time [16,17]. Determination of genome sequence of different *Plasmodium* species and profiling the parasite transcriptome during the distinct stages of infection have been very beneficial for finding the important target genes which are necessary for infectivity during the each stage of parasite life cycle. By targeting the genes which are vital for late liver infection steps, a broad array of antigens are presented to immune system and more potent immunity would create. Another

advantage of this type of vaccines is that those are safe for production staff, because those cannot start the blood stage infection. Gene targeting is done by homologous recombination and recently CRISPR/Cas9 technology has been used for more efficient gene replacement in different studies [18-20]. The acquired results from challenges in rodent model were acceptable and promising view is predicted for this type of vaccine according to their programming capability [21,22]. In addition, several studies performed on creating the GAPs which are related to blood stage of parasite life cycle. Most of these studies have focused on critical parasite metabolic process [23-26]. The most important difference between the pre-erythrocytic and blood stage GAPs is that the pre-erythrocytic types cannot replicate in the liver altogether and the other type can replicate in blood stream but under a specific controlling system.

Chemically Attenuated Parasites (CAP)

Using the DNA binding chemical compounds that can block the DNA replication is another strategy to create chemically attenuated Sporozoites. Centanamycin has been used for production of *P. berghei* and *P. yoelii* sporozoites that their liver stage completion had been arrested completely [27,28]. It seems that these drugs alkylate the parasite's DNA in poly-A rich regions. Tafuramycin-A and Centanamycin have been used for production of attenuated blood stage parasites in rodent model and their challenge tests have been promising [29]. Furthermore, a new strategy is inhibition of the protein synthesis by apicoplast genome that can lead to the loss of apicoplast function. With regard that apicoplast is necessary for intra-erythrocytic development; parasite life cycle would be blocked in this stage. Tetracyclines such as Doxycyclines have been used for induction of delayed death phenotype in *P. falciparum* successfully [30].

The only concern about this type of vaccines is the probable and potential toxicity of the remained drugs for human. Although, it has been mentioned that the probable free drugs can be washed before vaccine administration in production process and residual drug molecules have been attached covalently to the parasite's DNA and cannot affect the host DNA [28].

Killed Parasites

As mentioned before, administration of the killed parasites or parasite lysate with complete Freund's adjuvant was protective in rodent and monkeys [5,6,31]. A recent study has been performed by low dose of killed

P. c. chabaudi and *P. yoelii* in combination with CpG-oligodeoxynucleotide (CpG-ODN) as adjuvant [31]. Their challenge results on rodent model were hopeful and their vaccine could generate prolong and cross-strain protection. The most important points about this type of vaccines for their future applying are: possibility of scaling up to produce large number of parasites and finding a potent and compatible with human adjuvants.

Radiation-Attenuated Sporozoites (RAS)

To produce effective RASs, infected mosquitoes should be exposed to an enough dose of irradiation (usually 100 kilorad) to ensure complete attenuation [4,32]. By this high dose irradiation, non-specific damages are created in parasite's DNA and these damages prevent the parasite replication completely. Therefore, the attenuated parasites can enter to hepatocytes, but cannot continue their life cycle and start the blood stage infection. There is an important difference between the GAPs and RASs. RASs cannot continue their development when DNA replication is necessary but GAPs development is under the control and those are programmable by deleting the critical specific genes. Furthermore, there is a concern about this type of vaccines and it is the reversion of the irradiated and attenuated Sporozoites. This phenomenon has been seen in a study on *P. falciparum* in 1983 that reversion was observed after sub-inoculation of the attenuated parasites into the culture [33]. According to the recent report, the PfSPZ which is related to this type of vaccines is in phase II of clinical trial [1].

Conclusion

With regard to the importance of malaria in the world and its social and economic impacts on involved and non-involved countries, development and introducing a potent vaccine with durable and sterile immune response is crucial. According to the non-promising results of clinical evaluation of sub-unit vaccines, a refocusing has been done on whole parasite vaccines. Whole parasite vaccines expose a broad array of natural antigens to immune system and it can lead to the homologous and heterologous protection. Nevertheless, antigenic diversity is one of the most important problems for weak efficacy of the sub-unit vaccines. Furthermore, more durability of the observed immune responses and sterile protection are another advantages of the whole parasite vaccines.

However, some points should be considered for improving the efficacy and practical properties of this type of vaccines. For producing the *plasmodium* parasites and in vitro culture, human erythrocytes are used.

Therefore, screening methods should be considered for avoiding the transmission of probable contaminations. Furthermore, specific checkpoints should be involved in the production process as well. With regard to the mentioned limitations in production process, improvements should be considered in immunization protocols that those be done with low dose of parasites. Another important factor is designing the potent and human compatible adjuvants for final vaccine formulation. Moreover, considering the cryopreservation capability would improve and solve many problems that are related to transportation and administration of the produced vaccines in endemic area in the future.

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