Controlled human infection models as a tool for malaria and schistosomiasis vaccine research

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CHAPTER 1

General introduction

The controlled human infection model

Controlled human infection (CHI) models are an important research tool, where healthy volunteers are experimentally infected with a pathogen. So far models have been established for a broad range of viruses, bacteria and parasites. CHIs can be used for a variety of objectives and are, at the moment, mainly used to investigate the efficacy of new vaccines and drugs.[1] Generally CHI trials are performed using a randomised controlled trial (RCT) as study design. In these RCTs the intervention group receives the new vaccine before the controlled infection, or the new drug after the controlled infection (figure 1) and the control group receives a placebo. Besides testing new vaccines or drugs, CHIs also give the opportunity to study the host-pathogen interaction, to profile immune parameters and to test or optimize new diagnostic parameters.

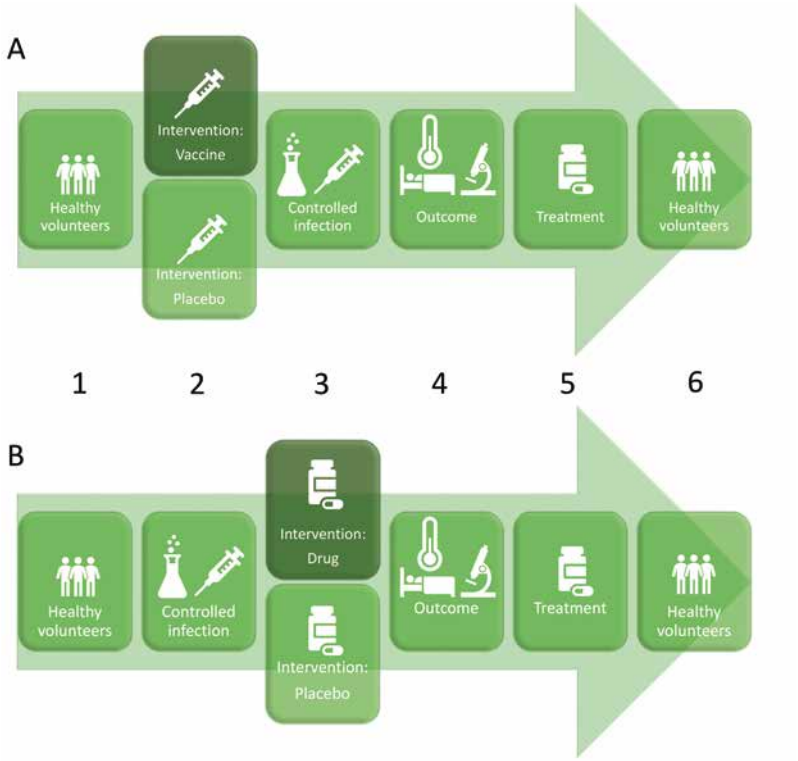

Figure 1. Schematic overview of controlled human infection trials. A. Schematic overview of controlled human infection (CHI) trials for vaccine research or **B.** drug research. Columns represent the different trial stages. **1.** Population, a group of volunteers is being screened to participate in a CHI. **2A.** Intervention, participating volunteers are given a vaccine or placebo. **2B/3A.** Controlled infection, after a predefined period volunteers are deliberately being infected with a pathogen. **B3.** Intervention, participating volunteers are given a drug or a placebo/established vaccine. **4.** Outcome, volunteers are being tested to see whether they developed the disease, historical data can serve as a control group. **5.** Treatment, all volunteers receive pathogen specific treatment.

Over the last decades CHI models are increasingly used.[2] They have accelerated the drug and vaccine development for several pathogens. This led to the development of novel vaccines,[3] but also contributed to stopping the further development of unsuccessful candidates.[4] CHIs are increasingly being used as proof of principle for vaccines before starting phase 2 studies and often act as gatekeeper before moving to large scale phase 2 or 3 field studies in endemic countries.[5]

This proof of principle has several advantages compared to field trials in endemic areas.[5,6] Generally, a low number of volunteers are needed in CHIs to achieve the endpoint, often between 20 and 100, compared to hundreds to 100,000 volunteers in phase II field trials in endemic areas.[2] As a consequence it reduces the unnecessary exposure of potentially vulnerable populations to interventions that might be ineffective. Another advantage is that multiple products can be tested in parallel and the risk of late clinical failure will be minimised (figure 2). Together, this leads to a reduction in the overall costs and a reduced overall risk during development.

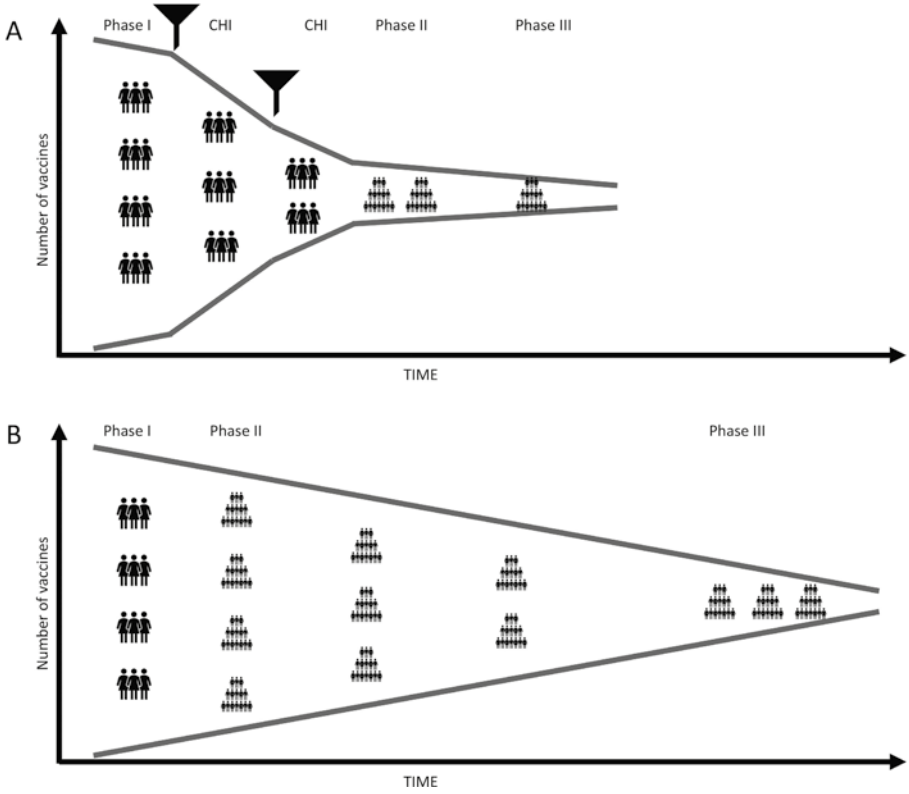

Figure 2. Vaccine development phases. The number of vaccines, the number of volunteers and the time until registration of a vaccine for each phase of clinical development with (**A**) or without (**B**) the use of controlled human infections.

These advantages have led to the development of new CHI models. To be able to design a new model, the specific pathogens should be produced in a controlled process. Preferably this process follows the good manufacturing practice (GMP) guidelines. More important, the expected adverse events in volunteers should be treatable or self-curable and cannot lead to long-term or permanent disabilities. Well-established CHI models can be altered or refined over time, for example when new diagnostic tools to detect the pathogen become available.

Malaria

Malaria is one of the world's most devastating infectious diseases with 219 million cases and over 435,000 deaths in 2017,[7] with most cases and deaths occurring in sub-Saharan Africa. *Plasmodium falciparum* is responsible for most deaths, while the other species, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium knowlesi*, mainly cause morbidity. The non-symptomatic liver stage of malaria precedes the symptomatic erythrocytic stage where patients develop symptoms such as fever, malaise, fatigue, headache, myalgia and arthralgia. In severe cases patients can develop neurological complications, severe anemia, coma and eventually they could die.

Effective treatment is available, though it is not always continuous accessible in remote areas. This may lead to anti-malarial drugs being administered too late. In addition, treatment may fail due to resistance of parasites against specific drugs. As a single infection does not induce complete immunity and multiple infections only induce semi-immunity, the need for a vaccine to prevent disease is high. Currently there is one licensed vaccine, RTS,s which is registered as Mosquirix,[8] which has a limited efficacy of 50% in children between 5-17 months old, 14 months after vaccination.[9] A pilot vaccine program with Mosquirix, for children up to 2 years old, started in Malawi, Ghana and Kenya in 2019.[10,11]

Controlled human malaria infections

The infection of volunteers with malaria for various objectives has been performed since the 1940's.[12] Hereafter, the importance of controlled human malaria infection (CHMI) studies was increasingly recognised.

The most common used CHMI study design is the double blind RCT, where both volunteers and research physicians are blinded. In these studies, the administration of several immunisations to healthy volunteers is followed by a controlled infection, the CHMI. During the CHMI sporozoites are generally administered by the bites of *P. falciparum*-infected laboratory reared *Anopheles stephensi* mosquitoes.[13,14] These mosquitoes become infected after the feeding of a blood meal containing *in vitro* cultured *P. falciparum* in erythrocytes.[13,14] However, the CHMI can also be performed by direct venous inoculation of malaria sporozoites.[15]

The use of CHMIs has resulted in several important scientific advances over the past decades in malaria vaccine research. One of the most important is the first proof of efficacy of the first licensed malaria vaccine, Mosquirix.[8] Due to its limited efficacy which wanes over time, it is important to continue vaccine research aiming at a vaccine with a higher efficacy. Preferably a

protective efficacy of more than 75% against clinical malaria and reduction of transmission of the parasite as is proposed in the malaria vaccine technology roadmap.[16]

Whole sporozoite vaccines

In contrast to the vaccine Mosquirix which targets the circumsporozoite protein on sporozoites,[8] new vaccines based on whole sporozoites are being developed. These whole sporozoite vaccines seem to be potent.[17,18] Most of these vaccines are based on the *P. falciparum* strain NF54, originating from a case of airport malaria in the Netherlands,[19] or its daughter strain 3D7.[20]

In 1999, a ground-breaking milestone was accomplished with whole sporozoite immunisation. Full protective immunity in nearly 100% of volunteers was induced after immunisation with radiation attenuated sporozoites (RAS).[17] Another type of whole sporozoite immunisation appeared to be even more potent. Exposure of volunteers to infected mosquito bites under chloroquine chemoprophylaxis (chemoprophylactic sporozoites, CPS) resulted in 100% protection. This potency is probably related to a longer exposure time to parasites.[21] Alongside chemoprophylaxis and RAS-based vaccines, a new type of whole sporozoite vaccines has recently been introduced, based on genetically attenuated parasites (GAPs). With the deletion of parasite genes which are crucial to develop within the liver or crucial to release merozoites into the blood stream, parasite development will arrest in the liver.[22]

Schistosomiasis

Worldwide 252 million people are infected with *Schistosoma*. Infections are mainly caused by *Schistosoma mansoni* and *Schistosoma haematobium*, but can also be caused by *S. japonicum, S. mekongi, S. intercalatum* or hybrid forms. Schistosomiasis is listed by the WHO as one of the main neglected tropical diseases.[23,24] The endemicity of the disease is focal and is determined by the presence of the intermediate host, the fresh water snail. Each of the *Schistosoma* species infects another species of freshwater snails. The infected snails release cercariae, which can penetrate the skin of the definite host. In their host they mature and migrate through several tissues. Adult worms generally reside in the mesenteric venules (*S. mansoni*) or venous plexus of the bladder (*S. haematobium*), where they mate and start producing eggs. The eggs travel through the lumen of the intestine or the bladder of their host and are released with urine of feces. When adult worms accidentally reside in other veins, their eggs will travel through other tissues. When the eggs get trapped in tissue they promote inflammatory responses at their deposition site, which can induce formation of granulomas and fibrosis.[25] Depending on the site this can result in various forms of pathology, such as liver cirrhosis and portal hypertension.[25]

Treatment of schistosomiasis relies on one drug only, praziquantel, of which the reported efficacy varies between 42 and 91% in endemic areas.[26-28] Current control programs rely on mass drug administration (MDA) with praziquantel and are being used to reduce the burden of infection.[29] There is a delay in regular treatment as people develop disease only after years of infections and re-infection. As a consequence of this suboptimal treatment, a vaccine would be an essential tool in the control of schistosomiasis. The WHO suggests that a vaccine should

fully prevent from infection in 40% of people or reduce the worm burden by 40%.[30] However, a more stringent goal of 75% reduction in worm burden has been proposed by the research community.[31] Currently three vaccines against *S. mansoni* are in various stages of (pre-) clinical development: Sm-TSP-2, rSm14/GLA-SE, and Sm-p80.[32-34]

Controlled human *Schistosoma* infections

The development of a controlled human schistosome infection (CoHSI) model would enhance the schistosomiasis vaccine development. It can be used as a tool to select vaccine candidates early in clinical development and consequently prevent late clinical failure.[1,2] Also, this model would allow characterisation of the immune response against schistosomes in the human host. This could give insight in symptoms involved in an acute schistosomiasis, also known as Katayama syndrome, and allows for optimisation of diagnostic assays.

To be able to develop this new infection model it is important that the schistosome life cycle is available. The maintenance of this life cycle and production of cercariae should be regulated in accordance with the principles of good manufacturing practice (GMP). Furthermore, it is important to be able to ensure the safety of volunteers at all times by optimising the infection (cercarial) dose, frequent follow-up control visits, availability of a research physician at all times, adequate diagnostic tools with the possibility to determine whether treatment has been sufficient, and the availability of adequate drug treatment.

Optimizing controlled human infections

The use of healthy volunteers is common in CHI studies. The ethical principles of CHIs are comparable to those of phase 1 trials, where there is a minimal risk, without direct benefit to the volunteers.[6] However, there is a delicate balance between the acceptance of CHI studies by researchers and the community. The central ethical issue seems to lie in the risk of harm to volunteers.[35] It is important to realize that the aim of CHI studies is not to induce harm, but to gain scientific benefit or benefit to society. As a consequence, this model should only be used under strict conditions.[35,36]

An ethical framework, mainly based on the Nuremberg code and declaration of Helsinki,[37,38] has been proposed to evaluate the ethics regarding CHI studies.[5,6,39] In short: 1) clear scientific rationale 2) minimize risks and discomfort to volunteers 3) exclude vulnerable populations 4) clear and thorough informed consent procedure 5) financial compensation for required time and suspected discomfort 6) compensate research-related injury 7) protect the public 8) potential benefit of the research should outweigh harm of the volunteers, and 9) right to withdraw from research.[5,6] It is the ethical obligation of the researcher to ensure the safety of his volunteers.

Scope and outline of this thesis

In this thesis new *P. falciparum* strains were investigated and introduced in CHMI studies. In addition, the CHMI model is used to test the efficacy of a new GAP vaccine, PfSPZ-GA1 vaccine. Besides the use of this well-established CHI model, the design of a new model, the CoHSI model is introduced and the results of the first study performed with this model are presented. Ultimately, we describe how the use of historical controls in CHIs can be used to improve the safety of CHI studies.

The first part of the thesis focusses on CHMI studies.

Chapter 2 provides insight in the liver stage and blood stage development of the new strains, NF135.C8 and NF166.C10, compared to the generally used strain NF54. From previous CMHI studies with NF54 it is known that five infected mosquito bites result in 100% infection rate in non-vaccinated malaria naïve volunteers. In **Chapter 3** the number of infected mosquito bites that are necessary to guarantee a 100% infection rate in non-vaccinated malaria-naïve volunteers after exposure to one of the two new *P. falciparum* strains, NF135.C8 and NF166. C10 are tested. Additionally, in **Chapter 4** the results of the first phase I and CHMI trial with a genetically attenuated parasite (GAP) malaria vaccine administered by direct venous inoculation are shown. At first the safety of the vaccine was tested in a phase I study. When these results were positive a CHMI study followed, where, three immunisations with a GAP vaccine, PfSPZ-GA1 Vaccine, based on NF54 were followed by a homologous, NF54, mosquito bite CHMI.

The second part of the thesis focusses on the development of the CoHSI model

In **Chapter 5** the establishment of male cercarial production for a controlled human schistosome infection model is described. Based on this production the CoHSI model was implemented. In **Chapter 6** we describe the development of Katayama syndrome in two of our volunteers after CoHSI. **Chapter 7** describes the results of the first CoHSI study, including the safety and optimal dose for infection for future studies.

The last part of this thesis focusses on the improvement of implemented CHI models.

In **Chapter 8** we give suggestions to optimize CHI models and to reduce their risk with the use of historical controls.

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