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Hepatitis B needle-free vaccines: a step closer

Olga Borges[†1,2] & Gerrit Borchard[3]

"...investigation of new needle-free hepatitis B vaccines relies on the development of adjuvants/new formulations with additional capability to increase the immunogenicity of the antigen."

[1]Center for Neuroscience & Cell Biology, University of Coimbra, Pólo das Ciências da Saúde Azinhaga de Santa Comba 3000–548, Coimbra, Portugal
[2]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
[3]School of Pharmaceutical Sciences, University of Geneva, 1211 Geneva, Switzerland
[†]Author for correspondence:
Tel.: +351 239 488 428
E-mail: olga@ci.uc.pt

Since the time of Edward Jenner and his discovery of the smallpox vaccine, and during the 200 years that have followed, vaccination has been successfully applied to protect against some of the most important infectious diseases. Moreover, in the case of smallpox, the dream of eradication of a pathogen causing a potentially deadly disease, especially in infants, has come true, with the last case of natural contraction of smallpox observed in 1977 in Somalia [101]. The only other eradicated disease is rinderpest, as will be officially announced by the UN in 2011 [1].

In campaigns to eradicate smallpox, the vaccine was administered using a bifurcated needle or a needle-free jet injector. Both devices deposit the antigen in the subcutaneous tissue. Curiously, before Jenner, a Chinese medical text described several ways of inoculation against smallpox practiced in China. In one of the methods, the nose was plugged with powdered scabs on cotton wool [102]. In another, white cow fleas were ground into powder and made into pills. These may have been the first attempts to formulate a vaccine to be applied by mucosal routes (intranasal and oral, respectively). We may therefore consider that needle-free vaccines were developed even before injectable vaccine formulations, possibly caused by the lack of technology available.

At present, the term 'vaccination' is generally considered to be equal to 'injection'. This conception is due to the fact that vaccines are typically given by intramuscular injection. By analyzing some of the exceptions in the market, such as the polio and rotavirus oral vaccines, it can be observed that both contain the live attenuated virus, which does explain, at least in part, the strong immune response observed after oral administration of these vaccines. In the new era of vaccine development, with the emergence of subunit vaccines, the formulation of needle-free vaccines is undoubtedly more challenging. Novel vaccines obtained by recombinant technology are, in principle, safer with regards to toxicity; however, they are also less immunogenic, making it mandatory to include adjuvants in the formulation of such vaccines. The hepatitis B vaccine, licensed in 1981, was the world's first recombinantly expressed plasma-derived subunit vaccine. Despite being on the market for over 30 years, the European Centre for Disease Prevention and Control estimates a prevalence of 8000 newly diagnosed cases of hepatitis B in the EU alone [103].

Numerous efforts made by the scientific community to develop needle-free vaccine formulations are justifiable by several distinct advantages. An obvious one is the possibility of painless self administration of the vaccine. Moreover, vaccine delivery via mucosal surfaces elicits mucosal immune responses at the site of pathogen entry as well as enhanced cellular immunity through Toll-like receptors stimulation [2], thus improving overall effectiveness. Since the hepatitis B virus can be transmitted perinatally or by exchange of body fluids (e.g., blood, semen and vaginal fluid), the design of new hepatitis B vaccines with the additional possibility to induce mucosal

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antibodies (e.g., secretory IgA) is particularly attractive. The only available hepatitis B vaccines to date are injectable formulations, adjuvanted with aluminum salts, which are evidently not appropriate for oral or intranasal administration owing to two main reasons. One, antigens mucosally administered will be exposed to enzymatic degradation, and second, the adjuvant is not adequate for application at mucosal surfaces. Therefore, formulations with enhanced adjuvant properties are needed for the application at mucosal surfaces in order to reduce the high antigen doses normally required, increase the low immune response and decrease the variability of the individual immune responses frequently observed.

Although various needle-free formulations of hepatitis B vaccine have shown promise in a preclinical setting [3], only very few have progressed to the clinical stage, with less than a dozen clinical studies published so far. Promising approaches to develop an oral vaccine include the design and use of recombinant-attenuated bacteria and edible transgenic plants, both with good capacities to produce a hepatitis B virus surface antigen (HBsAg). In the 1990's, Nardelli-Haefliger *et al.* described the use of attenuated *Salmonella typhimurium* strains expressing the hepatitis B nucleocapsid as an orally applied vaccine formulation. The live vector was tested in female adult volunteers for its ability to elicit a systemic as well as a mucosal immune response [4]. Contrary to what would be expected, oral immunization induced seroconversion against the bacterial lipopolysaccharide in six out of seven volunteers; however, it did not induce immune responses against the HBsAg.

Attenuated bacterial vectors are theoretically suitable vectors for the administration of oral vaccines as their natural properties may be utilized to more efficiently deliver antigens. For example, *Salmonella* spp. is able to penetrate the gastrointestinal epithelial barrier and infect macrophages within the lamina propria, from where they spread to other organs [5]. An example of the utilization of *Salmonella* spp. already on the market is a vaccine against typhoid fever, *Salmonella Typhi Ty21a*, which is considered as safe, thus opening the door for ensuing clinical trials with salmonella-based vectors. Although live-attenuated vectors have been widely accepted, the future of needle-free vaccines will rely on the development of artificial vectors, such as particles, which are safer.

The second approach for the development of oral vaccines already in clinical trials are edible vaccines. One of the first edible vaccines for hepatitis was developed with the expression of HBsAg in lettuce, as described by Kapusta *et al.* [6]. The transgenic lettuce leaves were fed to three human volunteers twice. Results obtained from the sera of the volunteers after the first feeding showed no relevant levels of HBsAg-specific IgG. However, sera collected after the second feeding revealed HBsAg-specific antibodies and in two volunteers these levels were superior to 10 IU/l, indicative of sufficient protection against the antigen in humans. In this study, however, the response was not sustained, as specific antibodies were no longer detectable after an additional 2 weeks. Although this very small study suggested that humans might be immunized using edible vaccines, the expression levels of the antigen in lettuce were shown to be very low and the plant was not adequate for large-scale vaccination since the leaves needed to be processed immediately after harvesting.

A more recent study evaluated the immunogenicity of a HBsAg expressed in potatoes [7]. Phase I clinical trials were conducted with 42 healthcare workers previously parenterally vaccinated with hepatitis B vaccine, resulting in antibody titers of <115 mIU/ml. The study described that 62.5% of the volunteers that ate three doses of transgenic potatoes had serum anti-HBsAg titers increased by up to 56-fold. However, approximately 40% of the volunteers were nonresponders to the oral HBsAg vaccine. Indeed, doubt remains whether edible vaccines are capable of priming an immune response or inducing the production of secretory IgA in the common mucosa.

Plant-based vaccines can be a simple technological solution, accessible to most if not all countries, especially those that have biotechnological platforms with the potential to render them self suppliers of the hepatitis B vaccine. This feature is an important advantage that justifies an additional investment in this class of vaccines, which obviously will be subject to evaluation by the US FDA and EMA with regard to medicinal products regulations. On the other hand, it may prove difficult in the near future for a plant-based vaccine to show a higher efficacy and superiority when compared with the injectable vaccines already on the market. One concern is the increased reported number of nonresponders to edible hepatitis B vaccines compared with the conventional vaccines. The development of safe mucosal adjuvants may help to decrease this number, and are therefore an important and exciting subject of research in the new era of vaccines. The second concern is related to the induction of antibodies in vaginal secretions, which would be desirable in the case of hepatitis B, however, may not be produced at sufficient amounts after oral administration [8]. Finally, a major obstacle to oral-vaccine delivery is the possibility of tolerance developing against the antigen delivered [9]. It is well known that tolerance can be induced in humans by feeding antigens that were never previously present systemically [10].

Other obstacles to oral vaccination are caused by the interference from existing gut flora, age, nutritional deficits, breastfeeding and the presence of maternal antibodies. The last factors should be seriously taken into account considering that the hepatitis B vaccine is to be applied at an early age in several vaccination schedules.

A promising alternative to an oral hepatitis B vaccine is the nasal administration of vaccines. This route of administration is not fraught with some of the previous difficulties, and like the gut-associated lymphoid tissue, enables antigens to access specialized mechanisms for antigen sampling, including uptake by M cells. Moreover, intranasal vaccination in humans results in antibody responses in the upper airways and the cervicovaginal mucosa [8], which is a good indication that a more efficacious nasal vaccine may eventually be obtained. The reason why this is not yet the case is simple; lack of good adjuvants for the nasal mucosa and pharmaceutical formulations combining vaccine and adjuvants are major challenges that need to be addressed. The results of a Phase I clinical trial of a nasal vaccine candidate, containing recombinant HBsAgs and core antigens demonstrated the shortcomings of the present situation [11]. Clinical end points for both antigens were safety and immunogenicity. The nasal vaccine candidate induced anti-hepatitis B virus core and HBsAg antibodies, respectively, in 100% and 75% of the vaccinated subjects. However, five doses of a solution containing 50 µg of each antigen were required to obtain these results. The single nasal formulation was administered using a nasal spray device Accuspray®. The subjects that had never been in contact with neither virus nor vaccine, received a total dose of 0.5 ml by two applications per nostril (15 min apart), each with a fixed volume of 125 µl. Therefore, this study confirms the necessity to apply 'carefully' higher doses of the antigens and more boosters when compared with injectable vaccines. These disadvantages may prevent the immediate entrance of the vaccine into the market. Therefore, preclinical investigation of new needle-free hepatitis B vaccines relies on the development of adjuvants/new formulations with additional capability to increase the immunogenicity of the antigen. To achieve this goal, several strategies are currently being discussed [3,10]. A good example is the development of nanosized carrier systems that adsorb or encapsulate antigens, protect them from proteolytic enzymes present in the mucosa, allow the increase of antigen retention time at the nasal mucosa and finally target antigens to M cells [12]. Lastly, the association of particles, not only the antigen particles, but also immunopotentiators such as combinations of Toll-like receptor ligands [13], cytokines, mast-cell activators or vitamins, may modulate the quantity and the quality of the immune response. The development of new nasal vaccines is currently underway and their introduction into the market largely depends on the demonstration of adjuvant safety in clinical studies and on simplicity of the administration.

Finally, there is currently another group of needle-free vaccines emerging, in which antigens are deposited into the dermis or epidermis. This region of the skin contains antigen-presenting cells, mainly dendritic cells, in abundance, making it an immunologically active site and, therefore, an attractive vaccination route. The deposition of the antigens into one of the skin layers results in the stimulation of Langerhans cells and can be achieved by physical, chemical or vesicular needle-free means [3]. The pursuit of a better hepatitis B vaccine led researchers to exploit some of the mechanisms involving strategies such as nanopatch/microneedles and particle-mediated epidermal delivery (PMED).

In PMED, gold particles coated with antigens or DNA vaccines accelerated to high speed by helium gas are fired into the epidermal layer of the skin, directly into the immune-cell network, stimulating rapid immune responses. Results obtained from several clinical trials performed with different devices suggested that administration of DNA vaccines by this route gave good results [14–16]. Evidence exists that demonstrate the potential of nucleic-acid vaccines to elicit both seroprotective antibody and cell-mediated immune response against hepatitis B [15]. These vaccines may also be able to show effectiveness in chronic hepatitis B recipients, since there are several studies demonstrating a critical role for cell-mediated immune response in the resolution of an already established infection.

Nanopatch technology requires a patch to be pressed onto the skin, with miniaturized arrays two orders of magnitude smaller than standard needles and smaller than current microneedle arrays, coated with antigen, adjuvant and/or DNA payloads. From a rather practical point of view, this second device has the disadvantage, when compared with PMED, to be inappropriately re-usable, bearing the risk of transferring infections. In addition, after application of either nanopatch or microneedle arrays, the application site needs to be covered to achieve occlusive conditions for better vaccine penetration, a procedure that might prove complicated especially in developing countries.

In conclusion, the development of needle-free hepatitis B vaccines may depend on the successful introduction of novel adjuvants boosting the immunogenicity of the vaccine and eliciting an immune response at the ports-of-entry of the pathogen. The most promising approaches in our view are the use of nanosize carrier systems combining vaccine and immunopotentiators in a particulate system, targeting antigen-presenting cells to deliver antigens onto nasal mucosa and the

needle-free DNA vaccine administration using nanopatches. Since hepatitis B virus persistence, observed in chronic hepatitis B virus carriers, is associated with a weak or absent specific immune response to hepatitis B virus, particularly the cellular immune response, DNA vaccines would be useful not only as a prophylactic vaccine but also as a therapeutic vaccine [17], since their ability to induce cellular immune responses has been shown.

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