Bilosomes: the answer to oral vaccine delivery?

Kathryn Senior, Freelance writer

A group of researchers based in Scotland has recently developed drug delivery vehicles called bilosomes, which are vesicles prepared from non-ionic surfactants incorporating bile salts such as deoxycholate[1]. Bilosomes represent a major step forward in vaccine technology because they are resistant to disruption by digestive enzymes and could, therefore, be used to overcome the many problems encountered when delivering antigens via the gastrointestinal tract.

'Protein antigens are prone to substantial degradation by gastric hydrochloric acid and proteolytic enzymes, and gut-associated lymphoid tissue does not absorb antigen efficiently,' says James Alexander (Dept of Immunology, University of Strathclyde, Glasgow, UK), co-developer of bilosomes. As a result, he explains, larger and more frequent doses of antigen are required to achieve levels of immunity comparable with those obtained via systemic administration. This often leads to the induction of specific systemic tolerance to the antigen.

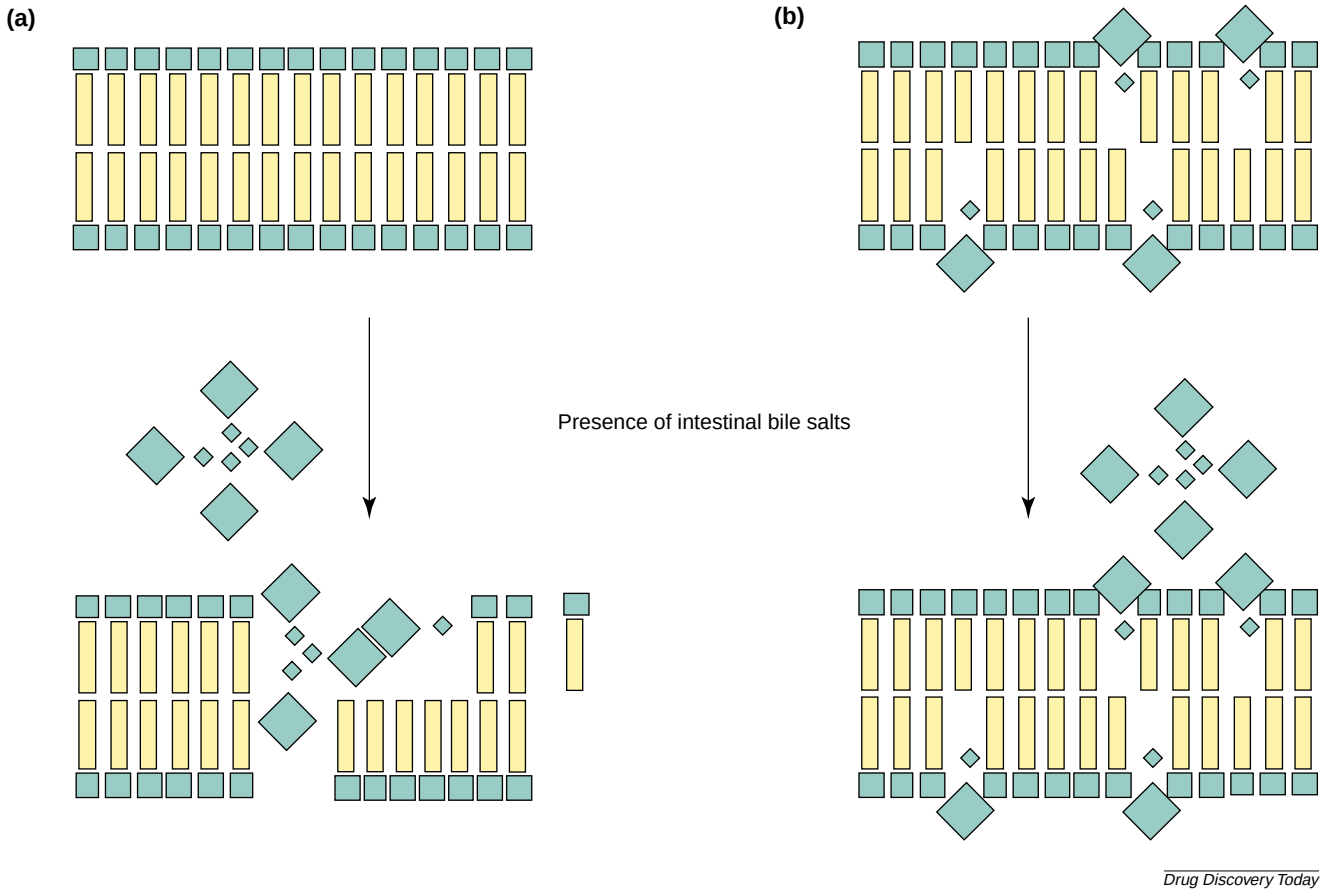

Figure 1. A schematic representation of the relative stability of bilosomes and conventional liposomes and non-ionic surfactant vesicles (NISVs) in the presence of bile salts. **(a)** Lipid vesicles are highly susceptible to the detergent effects of intestinal bile salts, which lead to membrane disruption and vesicle lysis. **(b)** Lipid vesicles can be formed in the presence of bile salts (e.g. deoxycholate) and this renders the membrane stable to the further effects of intestinal bile salts. The hydrophobic regions of the vesicles are shown in yellow, and the hydrophilic regions are shown in green.

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In the face of such problems, why persist in trying to develop oral formulations? 'Most infections – bacterial and viral – initiate infection via mucosal surfaces; therefore, a mucosally administered vaccine is the most effective way to induce a protective immune response,' points out co-author James Brewer (Dept of Immunology and Bacteriology, University of Glasgow, Glasgow, UK). An oral vaccine is generally safer, and also offers other major advantages: 'It can be given by anyone, not just trained staff who can give injections, and so is more cost effective and, because people prefer taking a pill to being given an injection, compliance is higher,' adds Brewer.

Non-ionic surfactant vesicles

Previous work by Brewer and Alexander showed that non-ionic surfactant vesicles (NISV), which are liposome-like structures that have extremely low toxicity, can act as adjuvants[2]. If the spheres are >250 nm in diameter they can induce a predominantly Th1 (cell-mediated) immune response[3]. NISVs can also induce cytotoxic T-cell responses against protein antigens[4].

'We decided to follow up several years of research in this area by looking at the ability of NISV to act as mucosal vaccine carriers following oral administration,' says Brewer. However, the group had experience of the disastrous effects of detergents on NISVs and had observed that the greatest obstacle to the stability of NISVs in the gut was the detergent effects of bile salts. To circumvent this problem, the group made bile salts an integral part of the NISV membrane 'because it has been recognized for some time that this stabilizes the lipid vesicles against the action of bile salts in the gut,' explains Brewer[5] (Fig. 1).

Efficacy of bilosomes

In the initial phase of the study, the ability of bilosomes to withstand disruption by bile salts was confirmed by comparing the amount of entrapped bovine serum albumin (BSA) retained in NISVs with that in bilosomes after the addition of bile salt solutions of various concentrations. Both types of vesicle remained stable when incubated with a 5 mM bile salt solution, but when the concentration was increased to 20 mM, 60% of the BSA was lost from the NISVs, compared with only 15% from the bilosomes. Bilosomes, into which a synthetic measles peptide or influenza subunit vaccine had been incorporated, were then used in various immunization protocols in mice. 'The most effective of these was to give two doses of vaccine, three days apart, and then repeat this protocol two weeks later,' reports Brewer.

After immunization, the mice mounted a specific cell-mediated immune response, as measured by splenocyte proliferation and interleukin-2 (IL-2) production. Furthermore, the observed antibody response to the influenza subunit vaccine was equal to that induced by a parenterally administered vaccine containing the same amount of antigen. The Th1:Th2 balance of the response was also similar, irrespective of whether the animals were immunized orally with bilosomes, or parenterally with the traditional vaccine form[1].

The study appears to confirm that the effectiveness of bilosomes is a result of the preparation of the vesicles with bile salts. 'Bile salts have been used as pharmaceutical penetration enhancers for drugs given orally, and this ability is thought to be because of the membrane destabilizing activity of bile salts,' says Brewer. Although bile salts act initially to destabilize liposome membranes, they also subsequently stabilize lipid vesicles against the further effects of bile acids. 'So, whereas NISVs tend to be broken down in the gut, bilosomes are protected,' he adds. This also seems to hold true *in vivo*; Brewer reports that greater amounts of bilosome-entrapped radiolabelled antigens are retained in the mouse gut for longer periods compared with free antigens (unpublished observation).

Future studies

Brewer and colleagues have done some follow-up experiments examining the distribution of antigens prepared in bilosomes after oral delivery, and have been working with bilosome-based vaccines in an infectious disease model. Recently, the international technology commercialization company, BTG (London, UK), acquired the rights to bilosome technology and is currently in discussion with several biotechnology companies interested in vaccine development or drug delivery. Lorraine Jones, a Licencing Executive at BTG, says, 'At this stage, it is not possible to speculate what projects might arise, but the first licence agreements could be signed in the next 4–9 months.'

Brewer is hopeful that bilosomes will be developed as an oral vaccine delivery system for clinical use in the relatively near future. 'The only orally administered vaccine at present is the attenuated polio vaccine, which has the problem that it can revert to virulence,' he says, 'Bilosomes are much safer than attenuated vaccines because they have no potential for reversion and have extremely low toxicity; we hope they will go far!'

References

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