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Regulatory aspects in the pharmaceutical development of nanoparticle drug delivery systems designed to cross the intestinal epithelium and M-cells

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ABSTRACT

This article reviews the field of oral uptake of nanoparticles across the gastrointestinal epithelium for the period 2006–2016. Analysis is conducted from the viewpoint of i) M-cell genetics and model development, ii) drug targeting to Peyer's patches and M-cells, and iii) physicochemical interactions of nanoparticles in the intestinal milieu. In light of these recent developments, regulatory considerations in the development of orally-absorbable nanoparticle drug products are discussed and focused on Module 3.2.P sub-sections of the Common Technical Document. Particular attention is paid to novel excipients, ligands and the non-standard method of manufacture. The novelty of this drug delivery system demands not only a multi-disciplinary scientific and regulatory approach but also a risk-adjusted consideration for a system defined by both processes and specifications. Given the current state of scientific development in the field it is suggested (in the author's personal opinion) that the design of nanoparticulate drug delivery systems should be kept as simple as possible (from a regulatory and manufacturing perspective) and to target the entire gastrointestinal epithelium.

1. Introduction

Given the rapid pace of innovation, miniaturisation and convergence of technologies, it can be expected that over the next decade or so nanotechnologies administered via the oral route will be able to reliably deliver molecules that are currently prone to either poor absorption or acid-mediated enzymatic degradation.

This mini-review will summarise the scientific advances made in the area since 2006 in the area of M-cell biology, how its genetics affords possibility specific targeting and inducing intestinal transcytosis of the nanoparticulate system, and crucially how the entire system (carrier ± ligand plus other excipients in the formulation) needs to remain stable in the intestinal contents before reaching the epithelial surface. The attendant regulatory aspects[1] to be considered in the pharmaceutics development of such systems is then discussed. For such drug delivery systems to be considered meaningful and effective, the article concludes by bringing together the aforementioned interrelated and multi-disciplinary subjects and offers possible ways forward for realisation of this intriguing and beguiling area of research.

2. Basic scientific progress since 2006

Since the 1920s it has been known that small amount of particulate matter can be absorbed intact from the gastrointestinal tract (GIT), particularly Peyer's patches (for a review on the early investigations in this phenomena, see Owen, 1999). Specialised M-cells discovered in the 1960s were found to overlay the gut-associated lymphoid tissue (GALT) particularly on aggregates known as Peyer's patches (PP). M-cells have a unique physiology of reduced or absent microvilli, the ability to phagocytize nano- and microparticulates and show a marked reduction in the level of cellular lysosomal degradation of particulates. The reader is referred to reviews that document the early years of basic

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[1] Opinions expressed in this article are my own, are not necessarily shared by other assessors at the MHRA, and cannot be considered to be UK policy. In addition, the thoughts expressed herein are also not necessarily shared by other assessors from EU working parties and committees, and again cannot be considered to be EU policy. The ideas, therefore, expressed in this article is for information only. They are not to be relied on as the full explanation of any aspect of drug safety or regulation.

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scientific investigation into the phenomenon conducted by four principal set of investigators (Owen, 1999; Florence, 1997; LeFevre et al., 1989; Volkheimer, 1977).

In the 'classical' situation, two components need to be assessed: the nature of the nanoparticulates and its interaction with M-cells. Later in this article, it will be seen that this equation has morphed somewhat from true nanoparticulates (being absorbed systemically) to a range of nanotechnologies that behave in different ways e.g. attachment of delivery devices to the epithelia via microneedles, and use of non-lymphoid tissue to increase the available intestinal surface area for nanoparticle translocation into the systemic compartment.

A search on PubMed revealed 1494 references on Peyer's since 2006. It is accepted that this is not a comprehensive search strategy (given the multitude of bibliographic electronic databases available) but the aim here was simply to ascertain broad developments over the last decade on the feasibility of exploiting this route for drug delivery of gut-labile molecules. Eliminating for duplication, review articles and relevance, 44 articles were examined to elucidate findings and patterns. These articles fell into three broad areas: i) M-cell genetics and model development; ii) drug targeting to Peyer's patches and M-cells; and, iii) physiochemical interactions of nanoparticles in the GIT milieu.

2.1. M-cell genetics and model development

The last decade has seen several important concerning the genetic processes leading to M-cell development and expression of specific receptors. Umod was identified as a novel M-cell-specific gene, the translation products of which is thought to contribute to the uptake function of M-cells (Sato et al., 2013). One such product is the ETS-family transcription factor Spi-B specifically expressed in M cells. Spi-B-deficient mice show reduced expression of most, but not all, other M-cell-specific genes and M-cell surface markers. Further, uptake of *Salmonella typhimurium* via M cells is reduced in Spi-B-deficient mice but not completely absent suggesting that whilst Umod may be the overall master-regulator gene, further dissection of the molecular pathways of M-cell differentiation is still required. However, the importance of Spi-B transcription factor is not be underestimated. Kanaya (2014) has also shown similar results in their efforts to develop reliable cell cultures of M-cells. Absence of Spi-B silenced the expression of various M-cell markers and prevented the differentiation of M cells in mice severely reducing the immune response to *Salmonella typhimurium*. Further, activation of T cells via the oral route was substantially impaired in the intestine of Spi-B-deficient mice.

The scarcity of M-cells cells *in vitro* and *in vivo* has hampered investigation in the development of models to truly elucidate if this route has the feasibility for targeted drug delivery. In parallel with the above cited studies, Nakato et al. (2016) have very recently further delved into the molecular mechanisms of antigen transcytosis and differentiation of M-cells by investigating the role of epithelium-intrinsic microRNAs. By generating mice with an intestinal epithelial cell-specific deletion of Dicer1, mice displayed a marked decrease in M-cells numbers compared to control floxed Dicer mice, suggesting an essential role of microRNAs in maturation of these cells. Using such technology can aid the reliable and perhaps tuneable development of *in vitro* cell line models as previous attempts have yielded mixed results. However, such models in the short-run are likely to be expensive and technically demanding.

In contrast, Rouch et al. (2016) demonstrates a simpler approach can suffice by inducing excised human intestinal crypt containing Lgr5+ intestinal stem cells into M-cells. Activation of the Spi-B transcription factor using RANKL (Receptor Activator of Nuclear factor Kappa-B Ligand, also known as tumour necrosis factor ligand superfamily member 11 and osteoclast differentiation factor), dramatically induced M-cell specific markers including the glycoprotein-2 (GP2) cell surface receptor. Furthermore, confocal microscopy demonstrated GP2 positive M-cells preferentially took up microparticles. As the authors suggest, this study was the first to demonstrate that M- cells can be induced to form from primary human intestinal epithelium and that large numbers can be generated for further functional studies. *In vivo* confirmation has been shown by Maharjan et al. (2016) where intraperitoneal injection of RANKL resulted in significantly higher number of functional GP2 positive M-cells leading higher transcytosis of fluorescent beads through them. To corroborate the effect of RANKL in antigen delivery through M-cells, orally delivered microparticulate antigen to mice treated with RANKL induced strong protective IgA and systemic IgG antibody responses against orally delivered antigen in RANKL-treated mice. Knoop et al. (2009) previously showed that RANKL deficient mice had less than 2% of wild-type levels of Peyer's patch M-cells and markedly diminished uptake of 200 nm diameter fluorescent beads.

Following the plethora of genetic investigations cited above, identification of M-cell surface receptors has finally begun to emerge albeit in non-humanoid species. To bridge the gap, Nakato et al. (2009) performed comparative gene expression profiling of chicken and murine follicle associated epithelium (FAE) to identify commonly expressed genes by M-cells in both species. Transcriptome analysis revealed that 28 genes were commonly upregulated in FAE from both species. Several co-localization of experimental ligands known to target the Peyer's patches with receptors were shown such as Annexin A10 with *Ulex europeaus* lectin. Further, cellular prion protein (PrPC) was expressed on the luminal side of the apical plasma membrane of M-cells, which co-localised with GP2 that recognizes only M-cells in murine PP. Given the conservation of genes across mammalian species it is highly probable that the human M-cells also express similar receptors that can allow nanoparticulate targeting.

The disadvantage of *in vitro* methods is that they do not take account of the actual realties encountered in the gut e.g. stability of the nanoparticulate carrier and/or the surface-tethered ligand, the effect of mucus and food content. It was this reason that the group of Florence and Hussain (2001) preferred direct oral experiments in rats to accelerate feasibility testing of the PP route for the potential of oral delivery of nano-encapsulated gut-labile molecules. Another approach to further validate the feasibility of targeting M-cells is to use rodents that are deficient either in PP or M-cell functionality. The Yoshida rat strain, for example, is a naturally occurring laboratory species that has been long known for reduced or absent Peyer's patches; however procuring and maintaining this rate rat strain is difficult. Recently, a number of knockout mice models have been described that can allow for controlled experiments. For example, Westphal et al. (2008) have described the resistance of Chemokine Receptor 6-Deficient knockout mice (CCR6) to *Yersinia enterocolitica* infection. It was shown that following oral challenge with *Yersinia enterocolitica*, control mice suffered from lethal septic infection whereas CCR6-deficient mice showed very limited symptoms of infection. As the pathogenic microorganism *Yersinia enterocolitica* exploits M-cells for the purpose of mucosal tissue invasion exclusively through PPs, and M-cells are reduced in number in CCR6-deficient mice, this type of validated model could be most useful in further understanding the potential of this oral drug delivery targeting route. However, generating knockout mice is equally troublesome and expensive. To overcome the disadvantage of either procuring natural stock or genetically engineering a new one, Donaldson et al. (2012) transiently depleted M-cell numbers in the follicle-associated epithelium (FAE) of mice PP by RANKL neutralisation. Depletion was achieved using intraperitoneal injection of the IK22-