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(54) Title: NOVEL COMPOSITIONS

(57) Abstract: A pharmaceutical composition which comprises an antibody together with one or more excipients selected from (a) carnitine, an acyl carnitine, or a salt of carnitine or an acyl carnitine; (b) a salt or an ester of a bile acid; and (c) an alkylsaccharide; said composition being in solid or semi-solid form adapted for release of the active ingredient in the ileum and/or the colon. The invention also provides methods for stabilising an antibody in the presence of intestinal fluid and methods for preventing a disease or condition in a subject.



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Novel Compositions

Field of the Invention

- 5 This invention relates to novel compositions and their use.

Background

Antibodies and antibody-derived molecules are an important class of pharmaceuticals, demonstrating high specificity for particular targets. Many therapeutic uses have been
10 proposed and implemented, and monoclonal antibodies (mAbs) are the highest selling class of biotherapeutics. However, delivery of the antibody to the desired target continues to be a major challenge in pharmaceutical industry as well as an unmet medical need for several diseases. Specifically, oral or rectal administration is generally not feasible for antibodies, because the antibody is highly unstable in the presence of gastric and intestinal fluids, and for
15 this reason, intravenous or subcutaneous delivery remains the most feasible option for delivery of antibodies. Such delivery is not, usually, however, the most convenient method of administration of a drug to a patient, and issues include pain at the site of injection, poor patient compliance, and frequent hospital visits for intravenous infusions. There can also be other major disadvantages of intravenous or subcutaneous delivery, and for some
20 applications, high systemic doses of mAbs have been reported to be associated with serious systemic adverse effects. High systemic exposure can also lead to a decline in efficacy with repeated administration due to formation of anti-drug antibodies.

For some drugs, the targeting of the drug to the colon has been utilised as a means of
25 achieving local therapy or systemic treatment. For example, WO 2007/122374 describes compositions having a delayed release coating which can be used to target release of a drug from a core to the intestine, particularly the colon. The colon is susceptible to a number of disease states, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), constipation, diarrhoea, infection and carcinoma. IBD is a chronic, medically incurable
30 condition involving inflammation of the gut. There are two main forms of IBD: ulcerative colitis (UC), which typically begins in the descending colon and rectum and may extend continuously to involve the entire colon (pancolitis), and Crohn's disease (CD), which most commonly involves the terminal small intestine and ascending colon. UC usually affects only the epithelial layer of the bowel wall, while CD may affect all layers of the intestinal

wall. A number of mAb therapies are currently available for the treatment of IBD, but all involve either intravenous or subcutaneous administration. Because of the stability problems discussed above, none is currently available as an oral treatment.

- 5 Many strategies have been proposed for the stabilisation of antibodies in the presence of body fluids. For example, PEGylation and glycosylation are established techniques in which the antibody is modified in order to increase the circulation time of the antibody in the bloodstream. However, modification of the antibody clearly has disadvantages, and even for modified antibodies, the route of administration remains predominantly intravenous or
10 subcutaneous.

There remains an unmet need for a method of stabilising antibodies in the presence of body fluids, specifically intestinal fluids. More specifically, there remains a need for a method of stabilising antibodies sufficiently for them to be delivered to the body, and particularly to the
15 intestine including the ileum and/or the colon, by rectal or, especially, oral administration.

Summary of the invention

Accordingly, the invention provides a pharmaceutical composition which comprises an antibody together with one or more excipients selected from (a) carnitine, an acyl carnitine,
20 or a salt of carnitine or an acyl carnitine; (b) a salt or an ester of a bile acid; and (c) an alkylsaccharide; said composition being in solid or semi-solid form adapted for release of the active ingredient in the ileum and/or the colon. Surprisingly, the presence of such excipients, which are not believed to have been used as stabilisers previously, has proved effective in stabilising the antibody on contact with intestinal fluids. The composition of the invention is
25 in solid or semi-solid form, preferably in a form suitable for rectal or, especially, oral administration. Most preferably it is in a solid or semi-solid form suitable for oral administration, and adapted for selective release of the antibody in the ileum and/or the colon, especially the colon.

- 30 The excipients (a), (b) and (c) are well-known as enhancers of penetration (“penetration enhancers”), and have been proposed for use in a number of pharmaceutical formulations. However, they have not been proposed for use together with antibodies. Further, nothing in the prior art known to the inventors suggests that they might have any effect on the stabilising of any active ingredient administered as a pharmaceutical composition, and particularly an

antibody so administered. The present inventors have found that, surprisingly, they stabilise antibodies in the presence of intestinal fluids. Accordingly, the present invention further provides a method of stabilising an antibody in the presence of intestinal fluid, which comprises delivering the antibody in a pharmaceutical composition to the intestine together with an excipient selected from (a), (b) and (c) above. Further, the invention provides the use of an excipient selected from (a), (b) and (c) above for the stabilisation of an antibody which has been administered as a pharmaceutical composition and delivered to the intestine, especially to the ileum and/or the colon.

It is an advantage of the present invention that not only do said excipients stabilise the antibody, they also continue to act as penetration enhancers, facilitating penetration/absorption of the antibody into tissue. The result is the ability to formulate a composition which, in a preferred embodiment, is capable of delivering an antibody to the ileum and/or the colon, following oral or rectal administration, and in which penetration of the antibody into the intestinal tissue can occur without significant degradation of the antibody.

Detailed description of the invention

The antibody

As used herein, “antibody” means an immunoglobulin molecule that recognizes and specifically binds to a target antigen, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, bacteria or virus, or combination thereof through at least one antigen recognition site within the variable region of the immunoglobulin molecule. The term “antibody” encompasses polyclonal antibodies, monoclonal antibodies, multispecific antibodies such as bispecific antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. An antibody can include any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations. In one embodiment, the antibody is IgG1 or IgG4. The

term “antibody” is also intended to include conjugates of the antibody, for example conjugates with polyethylene glycol, PEG.

Further, except where the context requires otherwise, the term “antibody” should be understood to encompass complete antibodies and antibody fragments comprising an antigen-binding region of the complete antibody. Antibody fragments may for example be single domain antibodies (e.g. V_HH domain antibodies), monovalent or divalent Fab, Fab', F(ab')₂, scFv, Fc, bispecific antibodies, diabodies, minibodies or multispecific antibodies formed from antibody fragments, for example minibodies composed of different permutations of scFv fragments or diabodies, and optionally Fc fragments or CH domains, such as scFv-Fc, scFv-Fc-scFv, Fab-scFv, (Fab'FcFv)₂, scDiabodies, scDiabody-Fc, scDiabody-CH₃, scFv-CH₃, and scFv-CH₂-CH₃ fusion proteins. An antibody fragment can be produced by enzymatic cleavage of a complete antibody, or by synthetic means such as recombinant DNA techniques, phage display or yeast display technologies or using transgenic mice, or liquid or solid phase peptide synthesis.

Where an antibody is used in a composition according to the invention, it may be any one whose therapeutic effect is advantageously realised by administration via the colon. Specific antibodies of particular interest in the context of the present invention include existing commercial IBD therapeutic antibodies such as adalimumab, infliximab, cetrolizumab pegol, golimumab, natalizumab, vedolizumab, ustekinumab, and additional antibodies in development for IBD treatment which target pathways and molecules (agonists or antagonists) implicated in pathogenesis of IBD, such as, for example CD40. Targeting to the colon additionally affords the possibility to improve treatment of colorectal cancer by targeting and localization of anti-cancer therapeutic antibodies, or of different possible formats as mentioned above, to the tumour.

Further, it is recognised that stabilisation of biomolecules in the gastrointestinal (GI) tract and penetration into GI tissues additionally may offer the potential for transmission of therapeutics through GI tissue and into the systemic circulation, thus affording the opportunity to target a much larger range of diseases.

The excipient

(a) carnitine, an acyl carnitine, or a salt of carnitine or an acyl carnitine

Carnitine and certain acyl carnitines, or salts thereof are known as excipients for pharmaceutical compositions, and any of these may be used in the compositions of the invention.

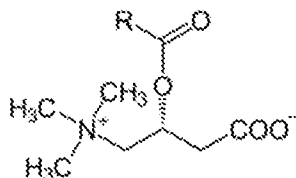
For example, compositions of the invention may comprise carnitine, or a salt thereof.

Alternatively, compositions of the invention may comprise an acyl carnitine, or a salt thereof.

Alternatively, compositions of the invention may comprise carnitine and an acyl carnitine, or a salt thereof.

Carnitine and acyl carnitines are enantiomeric compounds. Compositions of the invention may comprise L- or D-forms of carnitine or an acyl carnitine. Preferably, compositions of the invention comprise the L-form of carnitine, herein referred to as L-carnitine, or the L-form of an acyl carnitine, herein referred to as an acyl-L-carnitine.

Suitably the acyl carnitine has the general formula:



in which R represents an alkyl or alkenyl group having from 1 to 20 carbon atoms, for example at least 2, at least 8, at least 10 or at least 12 carbon atoms. R may for example have from 6 to 16 carbon atoms. Specific acyl carnitines of interest include, acetyl carnitine, lauroyl carnitine, myristoyl carnitine, stearoyl carnitine, propionyl carnitine, palmitoylcarnitine, valeryl carnitine, hexanoyl carnitine and octanoyl carnitine, especially hexanoyl carnitine, octanoyl carnitine and lauroyl carnitine. Most preferred acyl carnitines are valeryl carnitine, hexanoyl carnitine, octanoyl carnitine, lauroyl carnitine, myristoyl carnitine and stearoyl carnitine.

Salts of carnitine or an acyl carnitine which are suitable for use in the present invention are those wherein a counterion is pharmaceutically acceptable. Suitable salts include those

formed with organic or inorganic acids or bases. In particular, suitable salts formed with acids according to the invention include those formed with mineral acids, strong organic carboxylic acids, such as alkanecarboxylic acids of 1 to 4 carbon atoms which are unsubstituted or substituted, for example, by halogen, such as saturated or unsaturated dicarboxylic acids, such as hydroxycarboxylic acids, such as amino acids, or with organic sulfonic acids, such as (C₁-C₄) alkyl or aryl sulfonic acids which are unsubstituted or substituted, for example by halogen. Pharmaceutically acceptable acid addition salts include those formed from hydrochloric, hydrobromic, sulphuric, nitric, citric, tartaric, acetic, phosphoric, lactic, pyruvic, acetic, trifluoroacetic, succinic, perchloric, fumaric, maleic, glycolic, lactic, salicylic, oxalic, oxaloacetic, methanesulfonic, ethanesulfonic, p-toluenesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic, isethionic, ascorbic, malic, phthalic, aspartic, and glutamic acids, lysine and arginine. Suitable salts formed with bases include those formed with base salts include ammonium salts, alkali metal salts, for example those of potassium and sodium, alkaline earth metal salts, for example those of calcium and magnesium, and salts with organic bases, for example dicyclohexylamine, N-methyl-D-glucamine, morpholine, thiomorpholine, piperidine, pyrrolidine, a mono-, di- or tri-lower alkylamine, for example ethyl-, tert-butyl-, diethyl-, diisopropyl-, triethyl-, tributyl or dimethyl-propylamine, or a mono-, di- or trihydroxy lower alkylamine, for example mono-, di- or triethanolamine. Corresponding internal salts may furthermore be formed.

(b) a salt or ester of a bile acid

Certain salts and esters of bile acids are known as excipients for pharmaceutical compositions, and any of these may be used in the compositions of the invention. Salts and esters of bile acids such as taurocholic, turodexycholeic, deoxycholeic, cholic, lithocholate, chenodeoxycholeic, ursodeoxycholeic,ursocholeic, dehydrocholeic and fusidic acids, and their salts and/or esters, are examples. Suitable cations which may be present in salts include alkali metal cations, especially sodium, or ammonium or amino cations. Suitable esters are those with alkylcarboxylic acids having from 1 to 20, especially having at least 8, preferably at least 10, for example from 6 to 16, carbon atoms in the alkyl group, as for example in lauroylcholine chloride. Preferably a salt is used. Most preferred is the use of sodium taurocholate.

(c) alkylsaccharide

Suitably the saccharide is a mono- or, especially, di-saccharide, for example a maltose or sucrose. The alkyl saccharide may for example be an ether-linked alkyl saccharide. The alkyl group suitably has from 1 to 20, especially having at least 8, preferably at least 10, carbon atoms in the alkyl group. The alkyl group may for example be a lauryl, myristyl, stearyl or palmityl group. Suitable alkylsaccharides include lauryl- β -D-maltoside (also known as dodecyl- β -D-maltoside), lauryl sucrose, myristyl sucrose, and palmityl sucrose.

Pharmaceutical formulations

The pharmaceutical compositions according to the invention may be in any suitable form, but as stated above, it is preferred that they are in solid or semi-solid form, and preferably they are suitable for oral or rectal administration. They may be prepared by any known method. For example, the antibody and the required excipient may be admixed together, optionally together with other excipients required in the dosage form.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, tablets, mini-tablets, or pellets, or as powders, granules or crystals. In a solid composition, the minimum diameter of each particle is typically at least 10^{-4} m, usually at least 5×10^{-4} m and, preferably at least 10^{-3} m. The maximum diameter is usually no more than 30 mm, typically no more than 20 mm and, preferably, no more than 10 mm. In preferred embodiments, the particle has a diameter from about 0.2 mm to about 15 mm, preferably from about 1 mm to about 4 mm (e.g. for pellets or mini-tablets) or from about 6 mm to about 12 mm (e.g. for certain tablets or capsules). The term "diameter" refers to the largest linear dimension through the particle.

As well as the required excipients (a), (b) and (c), compositions according to the invention may of course contain any further conventional excipients as required. Excipients used in solid forms include for example, microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate, calcium sulfate, sorbitol, glucose and/or lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art. Suitable binders include starch, gelatine, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Disintegrators include without limitation starch, methylcellulose, agar, bentonite, xanthan gum and the like. Fast

dissolving diluents include mannitol, lactose, sucrose and/or cyclodextrins. Lubricants, glidants, flavours, colouring agents and stabilizers may also be added for ease of fabrication and use. Lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride.

5

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded
10 tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the antibody. Preferred examples of coatings are given below.

15 Capsules may have solid, semi-solid or non-solid contents. Exemplary contents for capsules may include suspensions which can contain, for example, microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, and methylcellulose as a viscosity enhancer, as well as any of the solid or semi-solid forms above.

20 Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter, synthetic glyceride esters or polyethylene glycol. Such carriers are typically solid at ordinary temperatures, but liquefy and/or dissolve in the rectal cavity to release the drug.

25 Preferred unit dosage formulations are those containing an effective dose, or an appropriate fraction thereof, of the active ingredient. Release from certain formulations may be sustained, if the composition contains suitable controlled-release excipients. However, in preferred formulations, release is pulsatile.

30 The compositions according to the invention will typically comprise a therapeutically effective amount of the antibody which may be from 0.01 wt % to 99 wt %, based on the total weight of the composition. The actual dosage would be determined by the skilled person using common general knowledge. However, by way of example, "low" dose formulations typically comprise no more than 20 wt % of the antibody, and preferably comprise from 1 wt

% to 10 wt %, e.g. 5 wt %, of the antibody. "High" dose formulations typically comprise at least 40 wt % of the antibody, and preferably from 45 wt % to about 85 wt %, e.g. 50 wt % or 80 wt %.

- 5 Whilst the antibody may be used as the sole active ingredient in a composition according to the invention, it is also possible for the antibody to be used in combination with one or more further therapeutic agents. Thus, the invention also provides a composition according to the invention containing a further therapeutic agent in addition to the antibody. If desired, the composition according to the invention may be administered together with a further
10 composition, by simultaneous, sequential or separate administration.

Except where the context requires otherwise, throughout this Specification and claims, any reference to a pharmaceutical composition in solid or semi-solid form should be understood to include individual solid or semi-solid particles or unit forms which are solid or semi-solid
15 throughout, as well as those having a solid or semi-solid exterior and a non-solid, for example liquid or gel, interior. For example, a capsule may have liquid or gel contents.

Enteric coatings

In a particularly preferred embodiment of the invention, the composition as adapted for
20 selective release of the antibody in the intestine, especially the ileum and/or the colon, suitably following rectal or, especially, oral administration. This may be accomplished by the use of particular coatings.

The compositions according to the invention may have an enteric coating. Enteric coatings
25 which protect the active ingredients in a composition from attack and degradation in the stomach, and permit release within the intestines, are well known. The optimal coating for any particular formulation depends on the exact intended use, and coatings may be tailored to release the active ingredient in a particular region of the intestines, or at a particular time following ingestion. In one embodiment, the composition of the present invention is in a
30 solid or semi-solid form which comprises an enteric coating. Such a formulation may if desired contain one or more intermediate layers between the active ingredient and the outer enteric coating. In this case, it is possible for a composition of the invention to release a portion of its contents at one particular region of the intestines, and a further portion of its contents at a lower region of the intestines.

Delivery to the ileum and/or colon

Of particular interest in the context of the present invention are formulations which provide release only within a specific part of the GI tract, particularly the ileum or, especially, the colon. WO 2007/122374 (the contents of which are incorporated herein by reference) describes such formulations, and these form one preferred embodiment of the invention.

Accordingly, the invention further provides a composition comprising a particle with a core and a coating for the core, the core comprising an antibody together with one or more excipients selected from (a) carnitine, an acyl carnitine, or a salt of carnitine or an acyl carnitine ; (b) a bile acid or a salt or an ester thereof; and (c) an alkylsaccharide; the coating comprising a mixture of a first material which is susceptible to attack by intestinal bacteria and a second material which has a solubility threshold at pH 5 or above, preferably pH 7 or above, wherein the first material comprises a polysaccharide selected from the group consisting of starch; amylose; amylopectin; chitosan; chondroitin sulfate; cyclodextrin; dextran; pullulan; carrageenan; scleroglucan; chitin; curdulan and levan.

In the above formulations, preferably the polysaccharide is selected from the group consisting of starch; amylose; amylopectin; chitosan; chondroitin sulfate; cyclodextrin; dextran; and carrageenan. For example the polysaccharide may be starch, amylose or amylopectin. The second material is an enteric material which has a pH threshold which is the pH below which it is insoluble and at or above which it is soluble. The pH of the surrounding medium triggers dissolution of the second material. The normal pH of gastric juice is usually in the range of 1 to 3, while the pH of intestinal juice gradually increases from about 5.5 in the duodenum to about 7 to 8 in the colon. The second material preferably has a pH threshold of 6.5 or greater, especially 7 or greater.

The second material is typically a film-forming polymeric material such as an acrylate polymer, a cellulose polymer or a polyvinyl-based polymer. Examples of suitable cellulose polymers include cellulose acetate phthalate ("CAP"); cellulose acetate trimellitate ("CAT"); and hydropropylmethylcellulose acetate succinate. Examples of suitable polyvinyl-based polymers include polyvinyl acetate phthalate ("PVAP"). The second material is preferably a co-polymer of a (meth)acrylic acid and a (meth)acrylic acid C₁₋₄ alkyl ester, for instance, a copolymer of methacrylic acid and methacrylic acid methyl ester. Such polymers include

those available under the Trade Marks Eudragit L, Eudragit S and Eudragit FS. The use of Eudragit S as the second material is particularly preferred.

In such compositions, multi-unit dosage forms comprising particles having a diameter of less than 3 mm are preferred. The "core" is usually a single solid body. The core may consist of the antibody together with one or more of excipients (a), (b) and (c). More usually, however, the core will comprise a mixture of the antibody and said excipient together with one or more additional excipients. The core may for example include a filler or diluent material, e.g.

lactose or cellulose material such as microcrystalline cellulose; a binder, e.g.

polyvinylpyrrolidone (PVP); a disintegrant, e.g. croscarmellose sodium; and/or a lubricant, e.g. magnesium stearate. The core may be a compressed granulate comprising at least some of these materials.

Release from such compositions is delayed until the intestines and preferably the ileum

and/or the colon. Such compositions have application in a multi-phasic release composition comprising at least two pluralities of particles, e.g. coated pellets, in the same dosage form, e.g. a capsule, in which the particles of one plurality are differentiated from the particles of the or each other plurality by the coating. The coatings may differ from one plurality to the next in terms of coating thickness or composition, e.g. the ratio and/or identity of

components. Multi-phasic release formulations would be particularly suitable for sufferers of Crohn's disease affecting different regions along the intestine, including the ileum and/or colon.

Medical applications

The present invention provides a pharmaceutical composition according to the invention for use in therapy. It also provides a method of treating or preventing a disease or condition in a subject, especially a human subject, which comprises administering to the subject a pharmaceutical composition according to the invention in which the antibody in the composition is medically indicated for treatment or prevention of said disease or condition.

In a preferred embodiment of the invention, the compositions are adapted for administration via the oral or rectal route, and although in one embodiment the invention finds utility in the treatment of diseases of the intestine and particularly in the treatment of diseases of the ileum and/or the colon, it also has application as a portal for entry of the antibody into the systemic circulation by absorption from the intestine, and particularly the ileum and/or the colon, and

hence finds utility in the treatment of a wide range of diseases and conditions. It may for example find utility in the treatment or prevention of autoimmune diseases.

The invention finds particular utility in the treatment or prevention, including maintenance of remission or prevention of relapse, of a disease or condition of the ileum and/or the colon, especially the colon, for example inflammatory bowel disease (including ulcerative colitis and Crohn's disease), constipation, diarrhoea, infection, or cancer, and the invention therefore further provides the use of at least one excipient (a), (b) and/or (c) in a method of manufacture of a medicament comprising an antibody for the treatment and/or prevention of one or more of these conditions. The treatment and/or prevention of IBD is of particular importance.

The following Examples illustrate the invention.

Materials and methods

Rat Colon Model

A rat colonic model based on a mixed fecal inoculum was used to mimic the luminal environment of the rat large intestine. An anaerobic workstation (Electrotek 500TG™ workstation, Electrotek, West Yorkshire, UK) maintained at 37 °C and 70% relative air humidity was used to set up the model. Three healthy male wistar rats were sacrificed and the fecal contents were collected. The fecal material was transferred in the anaerobic workstation and diluted with freshly prepared basal medium to obtain 20% w/w slurry by homogenization. The basal media provides nutrients and growth factors to the microbiota allowing viability for upto 24 hours. The homogenized bacterial media was sieved through an open mesh fabric (SefarNitex™, pore size 350 µm) to remove any nonhomogeneous fibrous material. The pH was maintained at approximately 6.5 to mimic the colonic luminal pH of the rat.

Human Colon Model

A human colonic model based on a mixed fecal inoculum was used to mimic the luminal environment of the human large intestine. An anaerobic workstation (Electrotek 500TG™ workstation, Electrotek, West Yorkshire, UK) maintained at 37°C and 70% relative air humidity was used to set up the model. The fecal material was transferred in the anaerobic workstation and diluted with freshly prepared basal medium to obtain 20% w/w slurry by

homogenization. The basal media provides nutrients and growth factors to the microbiota allowing viability for up to 24 hours. The homogenized bacterial media was sieved through an open mesh fabric (SefarNitex™, pore size 350 µm) to remove any nonhomogeneous fibrous material. The pH was maintained at approximately 7 to mimic the colonic luminal pH of the human.

Antibody incubation studies

Antibody stock solution (infliximab, anti-CD40, vedolizumab) was prepared in PBS at 2 mg/ml and added to 20% human or rat faecal slurry to obtain an incubation concentration of 1 mg/ml and 10% w/w faecal slurry. Samples were withdrawn at appropriate time points and added to a protease inhibitor cocktail (Sigma, P2714) in a ratio of 1:3. The samples were centrifuged at 9.6 g for 10 mins and the supernatant was analysed by size exclusion-HPLC (SE-HPLC).

SE-HPLC

Sample analysis was performed using a high performance liquid chromatography (HPLC) system (Agilent Technologies, 1260 Infinity II Series™) equipped with a pump (model G1311C), autosampler (model G1329B) and a diode-array UV detector (model G1314B). A 600 × 7.8-mm Biosep™ 5 µm SEC-s3000 290 Å (Phenomenex, Torrance, CA) size exclusion (SE) chromatography column was used for sample separation using phosphate buffer saline (pH 7.3) prepared in sterile HPLC grade water as the mobile phase for elution, at a flow rate of 1 ml/min. The analysis was operated at room temperature and UV detection wavelength was set at 280 nm. Each sample was run for 40 minutes to allow complete elution of the sample proteins and reduce run-over. The retention time for IgG1 antibody, F(ab')₂ and Fab/Fc fragments was 17, 18.2 and 20.3 minutes, respectively.

Ussing chamber system

A NaviCyte vertical ussing system (Harvard Apparatus, Cambridge, UK) was used to measure transport across epithelial membranes which are polar structures possessing an apical (mucosal) and basolateral (serosal) side. The chambers are made of solid acrylic and supports the tissue membrane in such a way that each side of the membrane is isolated and faces a different chamber representing the apical and basolateral chamber. The working system consists of a unit to fit a maximum of six vertical chambers, a gas manifold for carbogen purging (95% O₂, 5% CO₂) and a heater block to maintain the temperature of the chambers at 37°C during the experiments with the use of a circulating water bath. The

chambers are two-piece assemblies held together by a high spring-tension retaining ring to ensure leak-free operation during the experiments.

The EVOM™ voltohmmeter (World Precision Instruments, Inc., Hertfordshire, UK) and Ag/AgCl electrodes (Harvard Apparatus, Cambridge, UK) were used to measure the trans-epithelial electrical resistance (TEER) of the tissue samples. TEER monitors the presence of functional tight junctions, which are responsible for the barrier function and which limit Paracellular permeation of water and solutes. TEER value of 200 Ω/cm^2 was set as the lower limit to confirm the tissue viability and tight junction integrity.

For the tissue penetration studies, the freshly excised colon of male Wistar rats was collected and transferred to an ice-cold solution of Krebs-Bicarbonate Ringer solution (KBr) of pH 7.4. The tissue was cut open transversally and was washed with KBr solution to remove the luminal contents and was then mounted in the Ussing chambers. The mucosal surface of the colon tissue was facing the apical chamber, and the endothelial surface of the tissue was facing the basolateral chamber. The exposed tissue area on each side of the chamber was 0.29 cm^2 and the tissue mounting region was 4 x 8 mm (Figure 3.6). The volume of KBr in apical and basolateral chamber was 5 ml and the pH was maintained at 7.4. The tissue was allowed to incubate with KBr for 20 minutes before addition of the drug. Antibody concentrations tested during the penetration experiments was 0.5 mg/ml. The penetration of drugs into the tissue was tested for 3 hours and in a minimum of 3 rats. The tissue without drug was incubated in parallel for the same time which acted as the negative control. The chambers were purged with carbogen and kept at 37°C by water jackets during incubation. The TEER was continuously monitored during the experiment to confirm the viability and integrity of the tissue. Tissues with TEER value below 200 were not used for the experiments.

Cryosectioning and staining

The tissue section exposed to the drug was precisely cut at the end of the experiment and immediately transferred to a cryostat (Leica CM3050, Leica Microsystems, Milton Keynes, UK) at -30°C. The tissue was allowed to freeze for 15-20 minutes. After the tissues were frozen, thin sections of the tissue (10 μm) were sliced and mounted on adherent microscope slides (SuperFrost® Plus, VWR International, Leuven, Belgium). Up to 10 sections from the

entire length of the tissue exposed to the drug were sliced. Similarly, sections were cut from the tissue not exposed to drug that served as negative controls.

The slides were kept at room temperature for 15 minutes before the initiation of the staining procedure. The tissue sections were fixed in 4% paraformaldehyde (Sigma-Aldrich, UK) for 10 minutes followed by incubation with 0.1% Triton X-100 (Sigma-Aldrich, UK) surfactant for 5 minutes to open up the tight junctions. The sections were then incubated with 1% bovine serum albumin (BSA) (Sigma-Aldrich, UK) for 30 minutes to avoid any protein-protein non-specific binding. Washing steps were included at each stage using PBS pH 7.4.

The sections were then stained with secondary antibody, 10µg/ml (Red) (anti-human IgG from goat, Alexa Flour® 633, Molecular Probes, UK) for 1 hour. This was followed by staining with CellMask™ green plasma membrane stain (Green) (Molecular Probes, UK) (0.5 X solution in PBS) at 37°C for 1 minute. This step is to stain the cell components including cell membrane and cytoplasm. The sections were then stained with vectashield Hard Set™ mounting medium with DAPI (Blue) (Vector Laboratories, Inc., Burlingame, CA, USA) to stain the cell nuclei. The slides were stored at 2-8°C in the dark until analysis by confocal laser-scanning microscopy (LSM 710, Zeiss, Cambridge, UK). The images were processed and analysed by Zen 2012 imaging software (Carl Zeiss Ltd., Cambridge, United Kingdom).

Tissue homogenization and antibody extraction for quantification by ELISA

Freshly excised colon tissues following completion of the ussing chamber permeation study for 3 hrs were weighed and the appropriate amount of extraction buffer was added in the ratio 20 mg: 1 ml. The tissue was homogenized, and the homogenate was incubated for 2 hrs at 4°C. After centrifugation, the supernatant was tested by ELISA (protocol described below) to determine the UV signal observed due to any non-specific binding of colon tissue proteins secondary antibody. The UV readings of blank colon tissue homogenate were directly compared with the UV readings obtained with dilution buffer. The readings obtained with colon tissue homogenate were similar to the blank readings observed with dilution buffer, confirming that there is no interaction of the colon tissue proteins with secondary antibody that could lead to high background signal.

For quantification of infliximab, an anti-TNF α antibody detection ELISA kit (Alpha Diagnostic International, Texas, USA) was used. The kit comprised of TNF- α coated 96 well plate with 12 removal strips of 8 microwells each, an anti-human Fc domain specific IgG labelled with HRP, wash solution, dilution buffer and TMB substrate. The protocol below

5 describes the details of all the variables optimized and implemented at each step:

1. Prior to testing, all wells and reagents were brought to room temperature for at least 30 mins. The wells were filled with wash buffer and incubated for 5 mins after which the wash buffer was poured off. The wells were pat dried on a paper towel after each washing step.

10 2. Primary antibody incubation

The extraction buffer supernatant obtained following tissue homogenization and extraction of infliximab was diluted using dilution buffer. 100 μ l of the diluted test sample, positive control (infliximab in PBS), negative control (homogenate of colon tissue without drug) or calibration curve samples (1000, 500, 100, 50, 25, 10 ng/ml
15 infliximab) were added into the wells of the 96 well plate in triplicates and incubated at 50 rpm shaking for 1 hr at room temperature. Post incubation, the wells were washed with wash buffer 4 times for 5 mins each.

3. Secondary antibody incubation

Anti-human IgG-HRP conjugated (100x) solution was diluted to 1x using dilution
20 buffer and 100 μ l of the solution was added to each well. The plate was incubated for 30 mins at 50 rpm shaking at room temperature, followed by 5 washes for 5 mins each with wash buffer.

4. Substrate incubation

To each well, 100 μ l of TMB substrate was added and incubated in the dark for 15
25 mins. The wells began to turn blue due to the reaction between HRP and TMB.

5. Stop step

The enzyme-substrate reaction was stopped by addition of 100 μ l of stop solution (1% sulfuric acid).

6. Absorbance reading

The plate was read within 30 mins of addition of stop solution at 450 nm using a SpectraMax M2^e multi-mode microplate reader (Molecular Devices, Sunnyvale, USA).

7. Data analysis

The optical density (O.D) readings of the calibration curve samples were plotted against log concentration (3, 2.699, 2, 1.699, 1.398, 1) in a 4-parameter logistic regression curve fit (4-PL) using software GraphPad Prism 7 (GraphPad Software Inc., San Diego, USA). The equation obtained from the calibration curve is as follows:

$$x = \text{LogIC}_{50} - \frac{\text{Log}(T - Y)/(Y - B)}{\text{Hillslope}}$$

x=log concentration, Y=O.D (450 nm). LogIC₅₀, Top (T), Bottom (B), hillslope values and the goodness of fit (R²) were provided by the software. By fitting the O.D values and other given values, the log concentration was determined. The log concentration values were then converted to concentration in ng/ml (10^{log conc}) to calculate the recovery of the calibration curve.

Example 1. Stabilisation of monoclonal antibody infliximab in the Rat Colon Model

Colon stability was assessed using the Rat Colon Model with the amount of intact antibody remaining at each time point assessed by SE-HPLC as described in the Methods section. The experiment was carried out in the absence of any excipient, and then in the presence of 20mM lauryl-L-carnitine, sodium taurocholate or dodecyl-D-maltopyranooside. For comparison, a further excipient known as a penetration enhancer, sodium caprate, was tested under the same conditions.

The results are shown in the following Table.

Excipient	None (control)	Lauryl-L-carnitine	Sodium taurocholate	Dodecyl-β-D-maltopyranoside	Sodium caprate (comparative)
% Infliximab remaining after 5 hours	<15	98	75	62	<15

The results show that infliximab was degraded by the colonic microbiota in the absence of test stabilizer molecules. However, in the presence of lauryl-L-carnitine, sodium taurocholate or dodecyl- β -D-maltopyranoside, a significant stabilising effect was obtained. In the presence of 20mM sodium caprate, no stabilization effect was observed.

5

Example 2. Stabilisation of anti-CD40 antibody.

Stability of anti-CD40 monoclonal antibody was assessed using the Rat Colonic Model with stabilizers at 20 mM with detection performed using SE-HPLC. Lauryl-L-carnitine and dodecyl- β -D-maltopyranoside stabilized the anti-CD40 antibody against colonic microbiota mediated degradation with approximately 93% and 83% drug remaining after 6 hrs respectively, compared to 35% remaining without excipient.

10

Example 3. Tissue uptake of infliximab in rat ascending colon tissue and translocation of infliximab across the tissue into the basolateral compartment.

The amount of intact IgG remaining in the tissue was detected by ELISA post homogenization and extraction of the antibody using extraction and quantification methods as described in the Methods section. Without any excipient, approximately 20 μ g and 4 μ g of antibody was detected at apical pH 6 and 7.4 respectively. In the presence of lauryl-L-carnitine, sodium taurocholate and dodecyl- β -D-maltopyranoside, approximately 113, 134 and 5 μ g of infliximab was detected. Approximately 85 and 130 ng of antibody was detected in the basolateral compartment of the Ussing chamber at apical pH 6 (without excipient) and dodecyl- β -D-maltopyranoside (20 mM). This shows that the excipients used were effective in enhancing tissue penetration by the antibody.

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Example 4. Stabilisation of monoclonal antibody infliximab in the Human Colon Model

Colon stability was assessed using the Human Colon Model with the amount of intact antibody remaining at each time point assessed by SE-HPLC as described in the Methods section. The experiment was carried out in the absence of any excipient, and then in the presence of 50 mM L-carnitine, valeryl-L-carnitine, hexanoyl-L-carnitine, octanoyl-L-carnitine, lauroyl-L-carnitine, myristoyl-L-carnitine or stearoyl-L-carnitine.

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The results are shown in the following Table.

Excipient	Concentration	% Infiximab remaining after 0 hours (T= 0 h)	% Infiximab remaining after 3 hours (T= 3 h)
Infiximab alone (control)	1 mg/ml	100	18.9
L-carnitine	50 mM	100	47.2
valeryl-L-carnitine	50 mM	100	52.3
hexanoyl-L-carnitine	50 mM	100	55.3
octanoyl-L-carnitine	50 mM	100	60.1
lauroyl-L-carnitine	50 mM	100	46.6
myristoyl-L-carnitine	50 mM	100	40.7
stearoyl-L-carnitine	50 mM	100	46.4

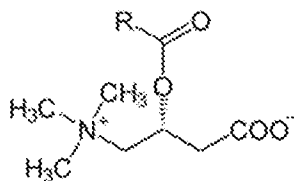
The results show that infiximab was degraded by the human colonic microbiota in the absence of test stabilizer molecules. However, in the presence of L-carnitine, valeryl-L-carnitine, hexanoyl-L-carnitine, octanoyl-L-carnitine, lauroyl-L-carnitine, myristoyl-L-carnitine or stearoyl-L-carnitine, a significant stabilising effect was obtained.

5

Claims

1. A pharmaceutical composition which comprises an antibody together with one or more excipients selected from (a) carnitine, an acyl carnitine, or a salt of carnitine or an acyl carnitine; (b) a salt or an ester of a bile acid; and (c) an alkylsaccharide; said composition being in solid or semi-solid form adapted for release of the active ingredient in the ileum and/or the colon.

2. A composition as claimed in claim 1, comprising L-carnitine or an acyl carnitine having the general formula:



in which R represents an alkyl or alkenyl group having from up to 20 carbon atoms.

3. A composition as claimed in claim 2, comprising L-carnitine, lauroyl carnitine, myristoyl carnitine, stearoyl carnitine, propionyl carnitine, palmitoyl carnitine, valeryl carnitine, hexanoyl carnitine and/or octanoyl carnitine.

4. A composition as claimed in claim 3, comprising lauroyl carnitine.

5. A composition as claimed in any one of the previous claims, comprising a salt or an ester of a bile acid selected from taurocholic, turodexycholic, deoxycholic, cholic, lithocholate, chenodeoxycholic, ursodeoxycholic,ursocholic, dehydrocholic and fusidic acids.

6. A composition as claimed in claim 5, comprising sodium taurocholate.

7. A composition as claimed in any one of the previous claims, which comprises an alkyl mono- or di-saccharide having from 1 to 20 carbon atoms in the alkyl group.

8. A composition as claimed in claim 7, which comprises lauryl maltoside, lauryl sucrose, myristyl sucrose, and/or palmityl sucrose.

9. A composition as claimed in claim 8, which comprises lauryl maltoside.

5

10. A composition as claimed in any one of the preceding claims, in which the antibody is selected from adalimumab, infliximab, cetrolizumab, golimumab, natalizumab, vedolizumab, ustekinumab, and anti-CD antibody.

10 11. A composition as claimed in any one of the preceding claims in a solid or semi-solid form suitable for oral or rectal administration.

12. A composition as claimed in claim 11, in a solid form suitable for oral administration, said composition having an enteric coating.

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13. A composition as claimed in any one of the preceding claims comprising a particle with a core and a coating for the core, the core comprising an antibody together with one or more excipients selected from (a) carnitine, an acyl carnitine, or a salt of carnitine or an acyl carnitine ; (b) a salt or an ester of a bile acid; and (c) an alkylsaccharide, and the coating
20 comprising a mixture of a first material which is susceptible to attack by intestinal bacteria and a second material which has a solubility threshold at pH 6.5 or above, preferably pH 7 or above, wherein the first material comprises a polysaccharide selected from the group consisting of starch; amylose; amylopectin; chitosan; chondroitin sulfate; cyclodextrin; dextran; pullulan; carrageenan; scleroglucan; chitin; curdulan and levan.

25

14. A composition as claimed in claim 13, in which the polysaccharide is starch, amylose or amylopectin.

15. A composition as claimed in either claim 13 or claim 14, in which the second material is
30 an acrylate polymer, a cellulose polymer or a polyvinyl-based polymer.

16. A composition as claimed in claim 15, in which the second material is selected from cellulose acetate phthalate; cellulose acetate trimellitate; hydropropylmethylcellulose acetate succinate; and polyvinyl acetate phthalate.

17. A composition as claimed in claim 16, in which the second material is a co-polymer of a (meth)acrylic acid and a (meth)acrylic acid C₁₋₄ alkyl ester.

5 18. A method of treating or preventing a disease or condition in a subject which comprises administering to the subject a pharmaceutical composition according to any one of the preceding claims.

19. A method as claimed in claim 18, wherein the disease or condition is inflammatory bowel disease; irritable bowel syndrome; constipation; diarrhoea; infection; or cancer

10

20. A composition as claimed in any one of claims 1 to 17 for use in therapy.

21. A method of stabilising an antibody in the presence of intestinal fluid, which comprises delivering the antibody to the intestine together with an excipient selected from (a) carnitine, 15 an acyl carnitine, or a salt of carnitine or an acyl carnitine ; (b) a salt or an ester of a bile acid; and (c) an alkylsaccharide.

22. The use of an excipient selected from (a) carnitine, an acyl carnitine, or a salt of carnitine or an acyl carnitine; (b) a salt or an ester of a bile acid; and (c) an alkylsaccharide; for the 20 stabilisation of an antibody which has been administered as a pharmaceutical composition and delivered to the intestine.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/053332

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K47/14 A61K39/395 A61K9/50 A61P1/00 A61P1/12 ADD. A61P35/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K C07K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/173068 A1 (UNIV MARYLAND [US]; FENG HANPING [US]; YU HUA [US]; HOAG STEPHEN [US];) 5 October 2017 (2017-10-05) paragraph [0006] - paragraph [0016] examples 2,4	1-22
Y	US 5 780 028 A (GRAHAM CONOR JOHN [AU]) 14 July 1998 (1998-07-14) column 2, line 22 - line 28 column 3, line 21 - column 4, line 67 examples 1-2	1-22
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 19 February 2019		Date of mailing of the international search report 27/02/2019
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Giró, Annalisa

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2018/053332

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CAON THIAGO ET AL: "Enhancing the Buccal Mucosal Delivery of Peptide and Protein Therapeutics", PHARMACEUTICAL RESEARCH, SPRINGER NEW YORK LLC, US, vol. 32, no. 1, 29 August 2014 (2014-08-29), pages 1-21, XP035420365, ISSN: 0724-8741, DOI: 10.1007/S11095-014-1485-1 [retrieved on 2014-08-29] table I	1-22
Y	----- SHAJI JESSY ET AL: "Protein and Peptide drug delivery: oral approaches", INDIAN JOURNAL OF PHARMACEUTICAL SCIENCESI,, vol. 70, no. 3, 1 May 2008 (2008-05-01), pages 269-277, XP009174425, ISSN: 1998-3743, DOI: 10.4103/0250-474X.42967 page 270, column 1, line 8 - line 20 page 274, column 2, line 7 - line 28 -----	1-22
X,P	WO 2018/071655 A1 (CURIRX INC [US]) 19 April 2018 (2018-04-19) example 14; table 8 -----	1,5,6, 11,18-22

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