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Thiolated silicone oil: Synthesis, gelling and mucoadhesive properties

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ABSTRACT

The aim of this study was the development of novel thiolated silicone oils and their evaluation with regard to gelling and mucoadhesive properties. A thiol coupling of 220 ± 14 and 127 ± 33 μmol/g polymer for 3-mercaptopropionic acid (MPA)- and cysteine-coupled silicone oil was determined, respectively. The dynamic viscosity of MPA–silicone raised significantly ($p < 0.000001$) after oxidation with iodine to a maximum of 523-fold within 1 h. During tensile studies, MPA–silicone showed both the highest results for total work of adhesion (TWA) and maximum detachment force (MDF) with a 3.8- and 3.4-fold increase, respectively, compared to the control. As far as the residence time on small intestinal mucosa is concerned, both silicone conjugates were detectable in almost the same quantities for up to 8 h with 56.9 ± 3.3 and 47.8 ± 8.9% of the initially applied conjugated silicone oil. Thiolated silicone oils can be regarded superior in comparison to commonly used silicone oils due to a prolonged retention time in the small intestine as site of action. Gelling and mucoadhesive features are advantageous for antiflatulent as well as mucoprotective biomaterials. Thus, these novel thiomers seem promising for an upgrade of currently available products for the treatment of dyspepsia, reflux oesophagitis and even inflammatory bowel diseases such as ulcerative colitis or Crohn's disease.

1. Introduction

Silicone oils are mixtures of polydimethylsiloxanes with dimethicone and simethicone as their most commonly used representatives. The difference between them is, that simethicone additionally contains small amounts of silicone dioxide. Those silicone oils, alone or in combination with other drugs, have been evaluated as efficient and well-tolerated agents for versatile gastrointestinal indications. For instance in the treatment of acute diarrhoea [1,2], premedication for upper endoscopy [3] and anorectal ultrasonography [4], functional dyspepsia [5,6] and reflux oesophagitis [7,8]. As dimethicone and simethicone bare antifoaming properties [9–11], they are supposed to disintegrate excess gastrointestinal gas, thus relieving flatulence and abdominal discomfort. Furthermore, simethicone revealed visceral antinociceptive effects on stress-induced colonic hypersensitivity in rats [12] as well as an inhibition of Helicobacter pylori [13].

One major drawback of the current formulations is, that they have to be applied three or four times a day [6,8], which is not fostering patient's compliance. With a prolonged retention time in the gastrointestinal tract, the dosing frequency can be reduced. This can be achieved with thiolated and thus mucoadhesive polymers, which strongly attach to mucosal membranes [14–16]. So-called thiomers have great potential for versatile pharmaceutical applications with the first product containing thiolated chitosan (Lacrimera® eye drops, Croma-Pharma) already entering the European market this year [17]. This class of biomaterials can be obtained by the covalent attachment of a thiol ligand to the polymeric backbone. As the thiolated derivatives feature free thiol groups on their surface, they can interact with the mucus layer and attach due to the formation of disulphide bonds [18]. Furthermore, thiomers bare in situ gelling properties as they can form inter- and/or intramolecular crosslinked networks via oxidative disulphide bond formation between polymer chains [19–21].

It was therefore the aim of this study to develop thiolated silicone oils and evaluate them regarding gelling and mucoadhesive features. The goal was to achieve a prolonged residence time on mucosal membranes as the gastrointestinal tract is the intended site of action. Such novel biomaterials are supposed to be advantageous in comparison to currently available products as the dosing frequency can be reduced. As outlined above, silicone oils can be attributed with antiflatulent and mucosaprotective features. As a consequence, the treatment of excessive gastrointestinal gas accumulation, reflux oesophagitis and even inflammatory bowel diseases such as ulcerative colitis or Crohn's disease can be regarded as pharmaceutical targets for thiolated silicone oils. For thiomer synthesis, an amino-modified silicone oil with a functional

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group equivalent weight of 4400 and a viscosity of 100 cSt was chosen. The thiolation was achieved via amide bond formation between the primary amino groups of the silicone oil side chain and the carboxylic acid group of two thiol ligands, namely MPA and cysteine. Iodine as oxidizing agent was chosen to enhance the viscoelastic properties of the silicone conjugates to give evidence for a successful thiolation. Furthermore the affinity for small intestinal mucosa was evaluated with regard to a prolonged residence time for thiolated silicone oils.

2. Materials and methods

2.1. Materials

Poly[dimethylsiloxane-co-(3-aminopropyl)methylsiloxane] with a functional group equivalent weight of 4400 Da (silicone oil), N,N′-diisopropylcarbodiimide (DIC), N,N′-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole hydrate (HOBt), 1,1′-carbonyldiimidazole (CDI), 3-mercaptopropionic acid (MPA), L-cysteine hydrochloride monohydrate (cysteine), 4,4′-dithiodipyridine (DTDP), 2,4,6-trinitrobenzenesulphonic acid solution 5% (w/v) in demineralized water (TNBS), iodine, pyridine, triethylamine and 1-(2-methoxyphenylazo)-2-naphthol (sudan red G) were purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals, reagents and solvents were received from commercial sources.

2.2. Synthesis of thiolated silicone oil

2.2.1. Carbodiimides

The synthesis with carbodiimides was based on commonly applied methods [22,23], but modified for silicone oils. A solution of 1 mmol (1 equivalent) of silicone oil, 2 equivalents of pyridine and 2 equivalents of DCC or DIC, respectively, in 40 mL DCM was cooled to 0 °C. Afterwards 2 equivalents of acid (MPA or cysteine, respectively) dissolved in 2 mL DMSO was dropwisely added. The solution was stirred for 1 h at 0 °C and for 24 h at room temperature.

2.2.2. Carbodiimides and HOBt

The usage of the active ester HOBt in combination with carbodiimides was based on previously described procedures [24–26] and modified for silicone oils. First, 1 mmol (1 equivalent) of silicone oil and 2 equivalents of triethylamine were dissolved in 40 mL DCM. Then 2 equivalents of acid (MPA or cysteine, respectively) dissolved in 2 mL DMSO and 2 equivalents of HOBt were added. The mixture was cooled to 0 °C and 2 equivalents of DCC or DIC, respectively, were dropwisely added. The solution was stirred for 1 h at 0 °C and for 24 h at room temperature.

2.2.3. CDI

The synthesis was based on a previously described one-pot-method [27], but adapted strongly. In brief, a mixture of 2 mmol of acid (MPA or cysteine, respectively) dissolved in 2 mL DMSO and 2 mmol of CDI in 40 mL DCM was stirred for 3 h at room temperature. Then 1 mmol of silicone oil was dropwisely added and the solution was stirred for 24 h at room temperature.

2.3. Purification of thiolated silicone oils

The modified silicone oil solution was purified via filtration and five washing steps with demineralized water until the pH of the aqueous phase was neutral. The residual solvent was removed under vacuum. The thiolated silicone oil was finally centrifuged two times for 10 min at 13,400 rpm and stored at 4 °C until further use.

2.4. Determination of thiol groups with DTDP

Another method to determine the total amount of thiol groups attached to the silicone oil was based on a procedure with DTDP [28,29] and modified for silicone oil. The reaction medium was prepared by addition of triethylamine (0.1%, v/v) to DCM and stirring for 10 min in ambient air. 1.0 mg of the polymer conjugate was dissolved in 500 μL of the reaction medium. Subsequently, 500 μL of DTDP reagent (0.9 mg in 10 mL of reaction medium) was added. After 5 min the reaction was terminated with 30 μL of neat acetic acid. Aliquots of 200 μL were transferred to a microtitration plate and the absorbance at 360 nm was read against DCM with a microtitration-plate reader (Tecan infinite M200 spectrophotometer, Grödig, Austria). The quantity of bound thiol ligand was calculated using a standard curve obtained by the thiol group determination of a series of solutions containing increasing concentrations of cysteine.

2.5. Degree of modification

The degree of modification was determined by measuring the free amino groups of unmodified and modified silicone oil, using TNBS. The unmodified silicone oil served as 100% control. For the assay, 1.0 mg of each conjugate was dispersed in 500 μL of 0.5% w/v sodium chloride and 1% w/v Tween 80 solution. Then 500 μL of 0.1% TNBS solution was added and the mixture was incubated for 2 h at 37 °C [30]. Aliquots of 200 μL were transferred to a microtitration plate and the absorbance was measured at 450 nm with a microtitration-plate reader (Tecan infinite M200 spectrophotometer, Grödig, Austria). The amount of remaining free amino groups was calculated using a standard curve obtained by a series of solutions containing increasing concentrations of cysteine hydrochloride.

2.6. Rheological measurements

2.6.1. With Iodine as oxidizing agent

The gelling properties of the thiolated silicon oils were determined as previously described by our research group [21]. In this study, iodine was chosen as oxidizing agent. In brief, a plate–plate combination rheometer (Haake Mars Rheometer, 379-0200, Thermo Electron GmBH, Karlsruhe, Germany; Rotor: C35/1°, D = 35 mm) was used to determine the viscoelastic characteristics of the modified and unmodified silicone oils. Firstly, 800 μL of each polymer sample was admixed with 400 μL of 10% w/v iodine solution in ethanol. Following that, the samples were incubated at room temperature for 1, 8, 16 and 24 h. Dynamic oscillatory tests within the linear viscoelasticity range were performed with 1 mL aliquots of the samples in triplicate. Unmodified silicone oil served as control. From oscillating measurements, the parameters obtained thereby were the phase shift angle (δ), the shear stress (τ) and the shear deformation (γ). The elastic modulus (G'), the viscous modulus (G'') and the dynamic viscosity (η^*) were calculated by the equations below (1)–(3):

$$G' = \left(\frac{\tau_{max}}{\gamma_{max}}\right)\cos\delta \quad (1)$$

$$G'' = \left(\frac{\tau_{max}}{\gamma_{max}}\right)\sin\delta \quad (2)$$

$$\eta^* = \left(\frac{G''}{\omega}\right) \quad (3)$$

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