European Polymer Journal 76 (2016) 53–62

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Entirely S-protected silicone oil as second generation mucoadhesive agent

Alexandra Partenhauser, Ožbej Zupančič, Snežana Podričnik, Julia Rohrer, Christina Leichner, Andreas Bernkop-Schnürch *

Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, Innsbruck, Austria

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 9 January 2016
Accepted 14 January 2016
Available online 16 January 2016

Keywords:
Silicone oil
S-protection
Thiomers
Mucoadhesion
Mucoprotection

ABSTRACT

Aim: Design of entirely S-protected silicone oils providing superior mucoadhesion and potential mucoprotection.
Methods: 2-Mercaptonicotinic acid (MNA) and its dimer (MNA–MNA) were coupled to amino-modified silicone oil. Thiolated and S-protected silicone oil were assessed regarding rheological mucus interaction, mucoadhesion and potential mucoprotection.
Results: For thiolated silicone–MNA and S-protected silicone–MNA–MNA almost quantitative coupling efficiencies could be achieved with total MNA amounts of 193 ± 15 μmol/g and 398 ± 44 μmol/g, respectively. Especially for S-protected silicone oil a firm mucus interaction was determined with an up to 28-fold increase in viscosity in comparison to non-thiolated control. Both silicone thiomers showed enhanced mucoadhesive features with remaining amounts of 18.1 ± 2.5% for silicone–MNA and 44.9 ± 4.8% for silicone–MNA–MNA after 8 h on intestinal mucosa, whereas non-thiolated control was washed away within 4 h. In view of a potential mucoprotective effect, methylene blue release was below 15% for silicone–MNA–MNA upon 8 h of incubation on intestinal and conjunctival mucosa in contrast to 100% leakage for the control.
Conclusion: Entirely S-protected silicone oil as second generation mucoadhesive can be ascribed with outstanding mucoadhesion and mucoprotective potential, which renders it a promising tool for mucosal targeting and inflammation treatment modalities.

1. Introduction

Silicone oils are optically clear, non-flammable and generally considered as inert as well as non-toxic polymeric organosilicon compounds. Mainly these features render them appealing, amongst others, for medical or pharmaceutical applications. For example, silicone oils are active ingredients in the treatment of flatulence [1], within pediculicides [2], for various dermatological indications [3,4] or vitreoretinal surgery [5]. In addition, thiolated silicone oils have recently been generated with enhanced affinity to small intestinal mucosa [6]. Thiomers in general as innovative sulfhydryl-containing class of biomaterials are already known for their outstanding degree of mucoadhesiveness. In order to obviate undesired disulphide crosslinking of thiolated polymers prior to attaching to the intended mucosal site of action, the concept of entire S-protection has been established [7]. Therefore, the respective thiol ligand is firstly coupled to MNA as leaving group via a disulphide bond. Then the S-protected thiol moiety is conjugated to the polymer via amide bond formation. It has been elu-

* Corresponding author.
E-mail address: Andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

http://dx.doi.org/10.1016/j.eurpolymj.2016.01.028
0014-3057/

Nomenclature

DIC	N,N′-diisopropylcarbodiimide
DTDP	4,4′-dithiodipyridine
G'	elastic modulus
G''	viscous modulus
HOBt	1-hydroxybenzotriazole hydrate
MNA	2-mercaptonicotinic acid
MNA–MNA	dimer of MNA
η^*	dynamic viscosity
Silicone oil	poly[dimethylsiloxane-co-(3-aminopropyl)methylsiloxane]
Silicone–MNA	silicone oil coupled with MNA
Silicone–MNA–MNA	silicone oil coupled with MNA–MNA; entirely preactivated silicone oil
TNBS	2,4,6-trinitrobenzenesulphonic acid solution 5% (w/v) in demineralized water
γ	shear deformation
δ	phase shift angle
τ	shear stress

cidated that S-protection of thiomers seems to facilitate mucosal interactions as enhanced mucoadhesive properties compared to their thiolated counterparts were evident [7,8]. With versatile mucosal surfaces in the human body gastrointestinal, pulmonary, vaginal, rectal or ocular targets are accessible for mucoadhesive devices. The reason for the paramount mucoadhesion of thiomers in general is their capability for thiol–disulphide exchange reactions with mucus glycoproteins [9]. So-called gel-forming mucins, such as MUC5AC in the conjunctiva [10] or MUC2 in the intestine [11], are able to crosslink via disulphide bonds due to cysteine-rich subdomains [12–14]. These gel-forming mucins are therefore responsible for rheological properties and thiomer interactions of physiological mucus.

As far as mucosal surfaces and silicone oils are concerned, dimethicone as well-known representative of silicone oils can be attributed with mucoprotective features as it has been evaluated beneficial in the treatment of reflux oesophagitis [15,16]. As mucosal surfaces in the human body create a protective physical barrier, they have to face all kinds of potentially harmful agents including extreme pH and temperature shifts of food or irritating medications [17]. With regard to mucoprotection, previous studies suggest that sulfhydryl-containing compounds mediate gastric cytoprotection and might have potential for preventing or treating hemorrhagic gastric erosions [18–20]. In addition, a beneficial effect of a small molecule thiol has been reported for intestinal ischemia/reperfusion injury, presumably due to reducing oxidative stress [21]. A potential mucoprotective effect of thiomers, however, has not been investigated yet.

The aim this study therefore was to create novel entirely S-protected silicone oil thiomers exhibiting both superior mucoadhesive and potential mucoprotective features. MNA for thiolation as well MNA dimer for entire S-protection were covalently coupled via amide bond formation to amino-modified silicone oil with a functional group equivalent weight of 4400 Da. Both thiomers were evaluated with regard to mucus interaction, mucoadhesion as well as potential-protection and cytotoxicity.

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), 1-hydroxybenzotriazole hydrate (HOBt), 4,4′-dithiodipyridine (DTDP), hydrogen peroxide, trimethylamine, 1-(2-methoxyphenylazo)-2-naphthol (Sudan red G), dichloromethane (DCM), dimethyl sulfoxide (DMSO), L-glutamine, 2,4,6-trinitrobenzenesulphonic acid solution 5% (w/v) in demineralized water (TNBS), Triton X 100, L-cysteine, minimum essential medium (MEM) and methylene blue were purchased from Sigma Aldrich (Steinheim, Germany). RPMI-1640 was purchased from Thermo Scientific (Wien, Austria). 2-Mercaptonicotinic acid 98% (MNA) was purchased from ABCR GmbH & Co KG (Karlsruhe, Germany). All other chemicals, reagents and solvents were received from commercial sources. Porcine intestine and conjunctiva were kindly donated from a local slaughterhouse.

2.2. Synthesis and purification of thiolated silicone oil

The amino-modified silicone oil was thiolated according to a procedure described previously by our research group [6]. In brief, 1 mmol of silicone oil and 2 mmol of triethylamine were dissolved in DCM. Then 2 mmol of MNA were dissolved in DMSO and 2 mmol of HOBt were added. The mixture was cooled to 0 °C and 2 mmol of DIC were dropwisely added. The solution was stirred for 1 h at 0 °C and for 24 h at room temperature. 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