European Journal of Pharmaceutical Sciences 106 (2017) 1–9

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Diabetes development increased concentrations of the conjugated bile acid, taurocholic acid in serum, while treatment with microencapsulated-taurocholic acid exerted no hypoglycaemic effects

Sangeetha Mathavan[a], Momir Mikov[b], Svetlana Golocorbin-Kon[c], Hani Al-Salami[a,*]

[a] Biotechnology and Drug Development Research Laboratory, School of Pharmacy, Curtin Health Innovation Research Institute, Curtin University, Perth, WA, Australia
[b] Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[c] Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

ARTICLE INFO

Keywords:
Taurocholic acid
Bile acids
Microencapsulation
Diabetes mellitus
Inflammation
Type 1 diabetes

ABSTRACT

Context: The bile acid taurocholic acid (TCA) is endogenously produced, and has shown formulation-stabilising effects when incorporated into microcapsules containing potential antidiabetic drugs. This study aimed to develop and characterise TCA-microcapsules, and test their antidiabetic effects, in an animal model of Type 1 diabetes (T1D).

Methods: Using the polymer sodium alginate (SA), SA-microcapsules (control) and TCA-microcapsules (test) were prepared, and assessed for morphology, surface composition, chemical and thermal stability, swelling, buoyancy, mechanical, release and rheological properties. TCA-microcapsules were gavaged as a single dose (1.2 mg/300 g) to alloxan-induced diabetic rats, and blood glucose and TCA concentrations in serum, tissues (ileum, liver and pancreas) and faeces, were measured. One healthy and one diabetic group were used as control and gavaged SA-microcapsules.

Results: TCA-microcapsules showed consistent size, TCA presence on surface and all layers of microcapsules, chemical and thermal stability, enhanced swelling, buoyancy and targeted-release properties and rheological analysis showed Non-Newtonian flow properties. TCA serum concentrations were lower in the healthy group, compared with the diabetic and diabetic-treated groups, but there was no significant difference between diabetic control and diabetic treated groups, in terms of TCA levels, and blood glucose concentrations.

Conclusions: The developed TCA-microcapsules showed good stability and release properties, but did not lower blood glucose levels in T1D, which suggests absence of insulin-mimetic effects, when using a single 1.2 mg/rat oral dose.

1. Introduction

Taurocholic acid (TCA) is a dominant conjugate form of bile acids in humans. It is chemically known as 2-(3α,5β,7α,12α)-3,7,12-trihydroxy-24-oxocholan-24-yl,amino,ethanesulfonic acid, and has a molecular formula of $C_{26}H_{45}NO_7S$ and molecular weight of 515.70 g/mol. TCA is a tri-hydroxy bile acid that has an OH group at the C-3, C-7 and C-12 positions. TCA is also known as cholaic acid, and it is a taurine conjugate of cholic acid, and constitutes > 30% of bile acid pool and extensively metabolised in the small intestine by gut microflora. It is hydrophilic in nature and has a melting point of 125 °C (Budavari, 1996). Being an acid, TCA has a pKa value of 1.4 and is partially soluble in water but is soluble in alcohol and ether (Hisadome et al., 1980).

Although TCA is not a globally registered drug with clinical indications to treat a disease, it has shown potential applications in inflammatory disease models. It has shown substantial antiinflammatory and immunoregulatory effects in healthy mice treated with proinflammatory endotoxins (Wang et al., 2013a). It has also shown permeation-enhancing capabilities, when combined with other drugs. In recent studies, TCA enhanced the permeation of heparin-docetaxel

Abbreviations: TCA, Taurocholic acid; T1D, Type 1 diabetes; SA, Sodium alginate; TCA-SA, Taurocholic acid-sodium alginate; $CaCl_2$, Calcium chloride; OM, Optical microscopy; SEM, Scanning electron microscopy; EDXR, Energy dispersive X-ray spectroscopy; DSC, Differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; USP, United States Pharmacopoeia; UV–Vis, Ultraviolet-Visible spectroscopy; LC-MS, Liquid chromatography-mass spectroscopy; C18 column, Octadecyl carbon chain (C18) bonded silica; ABC-protein, ATP-binding cassette transporter protein; ANOVA, Analysis of variance

* Corresponding author at: School of Pharmacy, Curtin University, GPO Box U1987, Perth, Western Australia 6845, Australia.
E-mail address: hani.al-salami@curtin.edu.au (H. Al-Salami).
URL: http://healthsciences.curtin.edu.au/teaching/pharmacy_people.cfm/Hani.Al-Salami (H. Al-Salami).

http://dx.doi.org/10.1016/j.ejps.2017.05.041
Received 3 March 2017; Received in revised form 11 May 2017; Accepted 17 May 2017
Available online 19 May 2017
0928-0987/

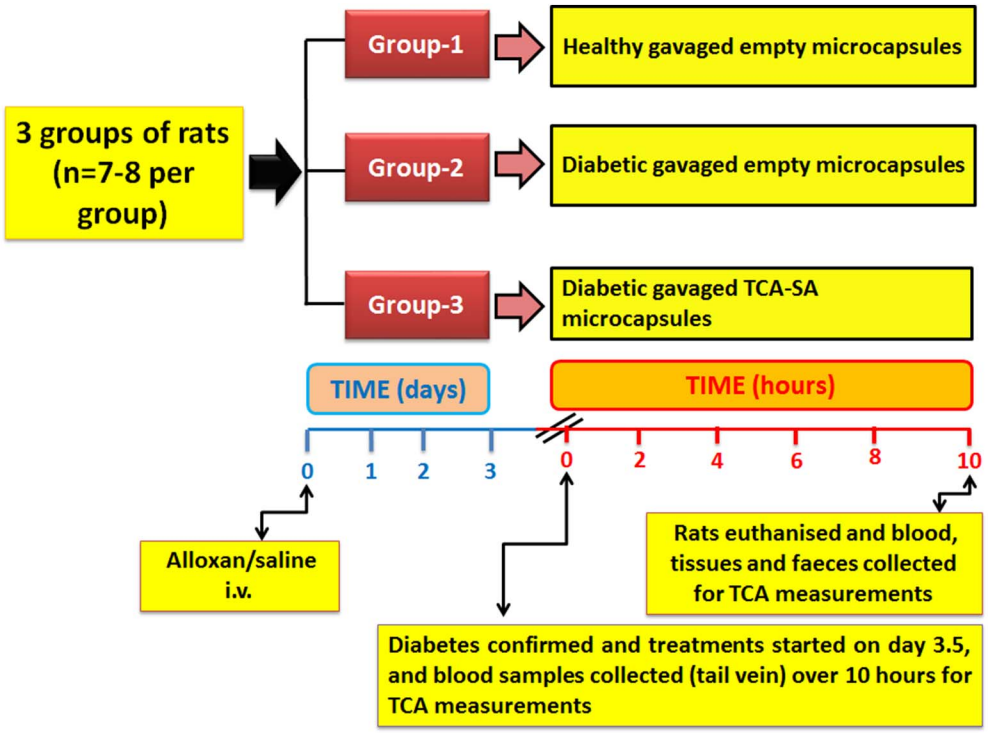

Fig. 1. Study design detailing animal work.

conjugates, in the lower intestine *via* transcellular and/or paracellular pathways (Lee et al., 2009; Khatun et al., 2013). Moreover, in one study, TCA exerted protective and desirable effects on muscle and pancreatic β-cells, which suggests potential antidiabetic effects (Mooranian et al., 2016a). Since TCA metabolism starts in the duodenum, it needs to be encapsulated in targeted-delivery matrices, which target the lower part of the intestine, and maximise its absorption (Moghimipour et al., 2015).

Accordingly, this study aimed to develop, characterise and test antidiabetic effects of new microcapsules with oral targeted-delivery properties, which can deliver TCA to the lower part of the ileum for maximum absorption. The study also aimed at investigating changes of TCA concentrations in serum, tissues and faeces, associated with diabetes development and TCA-microcapsules treatment. Thus, in this study, sodium alginate (SA) was used as a polymer, to form microcapsules without (control) and with TCA (test). The microcapsules were characterised *in-vitro*, then gavaged as a single dose to one diabetic group. Two other groups (one healthy and one diabetic) were gavaged SA-microcapsules and used as control. Blood, tissues and faeces were collected 10-hour postdose for blood glucose and TCA measurements. The *in-vitro* studies include imaging and morphological studies, surface elemental-analysis, stability, rheological properties, swelling, mechanical strength, and buoyancy analysis and release profiles at various temperature and pH values. The *in-vivo* studies include blood and tissues concentrations measurements of glucose and the bile acid, TCA.

2. Materials and methods

2.1. Materials

Taurocholic acid (96.0%) and alloxan (> 98%) were purchased from Sigma Chemical Co, USA. Sodium alginate was obtained as low viscosity, from Acros Organics, USA. Anhydrous calcium chloride ($CaCl_2$) was purchased from Scharlab S.L., Australia. Ultrasonic gel was obtained from Australian Medical Association, Australia. All other solvents and reagents used in this study were supplied by Merck, NSW, Australia and were of analytical grade.

2.2. Drug stock preparation

The stock suspension of TCA (4 mg/ml) was prepared by solubilising the crystalline powder of TCA with the ultra water-soluble gel (10%). The calcium chloride stock solution (10% w/v) was prepared by dissolving the salt to deionized water. Alloxan was mixed with saline prior to injection. All preparations were mixed for 3 h at room temperature to ensure uniform mixing and were used within 24 h of preparation.

2.3. Formation of microcapsules

The microencapsulation of TCA was carried out by combining a mixture of SA and TCA (1.8% and 4 mg/ml) and dispersing in deionized water (prior to use by microencapsulation process) using our well-established microencapsulating methodologies (Mooranian et al., 2016a; Mathavan et al., 2016a; Negrulj et al., 2016).

2.4. Microencapsulation efficiency, analyte content, production yield and microcapsules characterisations

0.1 g of microcapsules were weighed, and dissolved in 200 ml of phosphate buffer (pH 7.8) and the suspension was stirred and aliquots of the dissolution medium (2 ml) were withdrawn and TCA measured using established Liquid Chromatography Mass Spectroscopic methods (Mikov et al., 2007). The analyte contents, production yield and microencapsulation efficiency were calculated using our established methods, as described elsewhere (Mooranian et al., 2014a, 2014b, 2014c, 2015a).

The characterisation studies were done using Optical microscopy (OM)- Nikon YS2-H, Japan; Scanning electron microscopy (SEM)- Zeiss Neon 40EsB FIBSEM (Cambridge, MA) and Energy dispersive X-ray spectra (EDXR)- Oxford Instruments, INCA X-Act, Concord, MA), as per our established methods. Briefly, OM was used to evaluate the shape of the formulated microcapsules, while SEM and EDXR were used to investigate the surface morphological features of the microcapsules and elemental distribution of the atoms distributed across the surface of the