

Biopharmaceutical properties of chitosan-based thermogelling system for donepezil nasal delivery

Mirna Perkušić^{1*}, Laura Nižić Nodilo¹, Cvijeta Jakobušić Brala¹, Jasmina Lovrić¹, Ivan Pepić¹, Marie Trenkel², Regina Scherließ², Anita Hafner¹

¹University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

²Kiel University, Faculty of Mathematics and Natural Sciences, Kiel, Germany

Introduction

Donepezil oral delivery is related to several drawbacks such as gastrointestinal adverse effects and low brain delivery efficiency. As a viable alternative, nasal administration of donepezil may provide its efficient and direct delivery to the brain, thus avoiding donepezil systemic absorption and side-effects (Agrawal et al., 2018).

Innovative nasal platforms, such as *in situ* gelling thermoresponsive polymer/drug solutions stand out as formulations with great potential for effective brain-targeted delivery (Agrawal et al., 2020). These systems can incorporate gel-forming constituents and mucoadhesive polymers that contribute to a sustained drug release, prolong nasal retention time and increased bioavailability.

In this work we prepared chitosan-based thermogelling system for donepezil nasal delivery characterised by harmonised biopharmaceutical properties. Studies on *in vitro* release, *in vitro* biocompatibility and permeability, *ex vivo* mucoadhesion and *in vivo* irritability (using slug mucosal irritation assay) were performed to evaluate the potential of the developed formulation in donepezil nose-to-brain (N2B) delivery.

Materials and methods

Thermosensitive chitosan-based formulation for donepezil N2B delivery was prepared using low molecular weight chitosan (C; Sigma-Aldrich, Germany) and β -glycerophosphate (B; Biosynth Ltd., Slovakia) as the gelling agent. The C solution (1.5%, w/w) was added dropwise to the B solution (49%, w/w) at 4°C in 1.6:1 volume ratio. The proper amount of donepezil (D;

Carbosynth Ltd., UK) was added to the prepared C-B solution. Concentrations of D, C and B in the final formulation (DCB) were 0.30, 9.23 and 188.00 mg mL⁻¹, respectively.

Donepezil *in vitro* release profile was determined using the automated Franz diffusion cell testing system PhoenixTM RDS (Teledyne Hanson, USA). *Ex vivo* mucoadhesiveness was assessed using porcine nasal mucosa, on texture analyser TA.XT Plus (Stable Micro Systems, UK) equipped with mucoadhesion rig. Mucoadhesive properties were expressed as the maximum detachment force (F_{max}) and the work of adhesion (W_{adh}). For *in vitro* release profile and mucoadhesiveness assessment two control samples were used: the sample with a chitosan concentration different from the DCB sample (control formulation (CF) – C concentration 6.15 mg mL⁻¹) and corresponding aqueous D solution (donepezil solution (DS) – D concentration 0.3 mg mL⁻¹).

Biocompatibility and permeability were assessed using Calu-3 cells. Corresponding aqueous D solution was used as control, while Hank's balanced salt solution (HBSS) served as a negative control.

The slug mucosal irritation assay (SMI), quick and accurate screening method for prediction of nasal discomfort early in the formulation development, was performed to screen the formulation potential to cause irritation at the sensitive nasal mucosa. SMI was performed according to Trenkel and Scherließ (2021). Phosphate buffer saline (PBS) was used as a negative control (no irritation) and 1% (w/v) benzalkonium chloride (BAC) solution was used as positive control (maximum irritation).

Results and discussion

DCB thermosensitive nasal formulation provided prolonged D release in comparison to CF and DS ($32.5 \pm 4.0\%$, $62.9 \pm 1.0\%$, $90.6 \pm 3.5\%$ of D released in 45 min for the DCB, CF and DS, respectively).

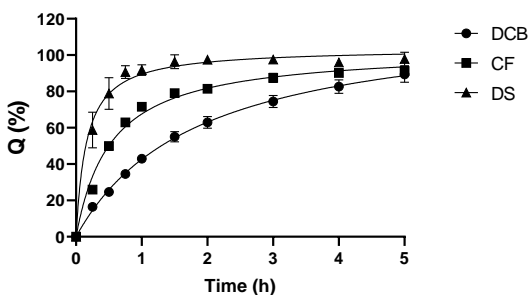


Fig. 1. *In vitro* release profiles of donepezil from the DCB, CF and DS. Data are expressed as the mean \pm SD, $n = 3$.

DCB formulation showed prominent mucoadhesive properties: a five-fold higher F_{\max} and a 20-fold higher W_{adh} in relation to the DS. Also, DCB presented a 2.4-fold higher F_{\max} and 2.0-fold higher W_{adh} in relation to the CF formulation.

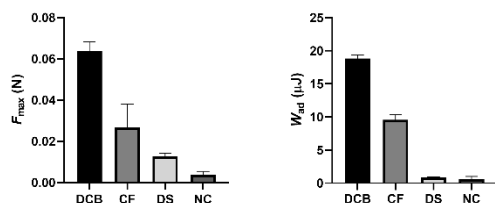


Fig. 2. F_{\max} (left) and W_{adh} (right) of DCB, CF, DS and filter paper as the negative control (NC). Data are expressed as the mean \pm SD, $n = 3$.

Table 1. Results of the SMI assay: total mucus production after exposure of the slug to the DCB formulation, in relation to positive and negative control. Data are expressed as the mean \pm SD, $n = 3$.

Sample	Total mucus production (%)
DCB	6.64 ± 1.04
Negative control	0.48 ± 1.50
Positive control	17.64 ± 4.33

Calu-3 cells exposed to DCB formulation retained viability above 80% in relation to negative control. The P_{app} values of DCB formulation showed 1.5-fold higher D permeation in relation to DS.

SMI assay of DCB showed 2.7 times lower mucus production compared to the positive control. Results of the SMI assay are presented in Table 1. The nasal DCB formulation showed acceptable irritability profile, paving the way for its safe nasal delivery.

Conclusion

Formulating D as thermosensitive chitosan-based formulation for N2B delivery resulted in prolonged drug release, increased mucoadhesiveness and enhanced drug permeation across Calu-3 monolayer. Results on *in vitro* biocompatibility confirmed the formulation potential for safe and efficient D nasal delivery. In addition, SMI assay showed that DCB is a formulation of tolerable irritability, confirming the appropriate selection of excipients in formulation development.

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