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Buccal absorption of diazepam is improved when administered in bioadhesive tablets—An *in vivo* study in conscious Göttingen mini-pigs

Emil Meng-Lund[a], Jette Jacobsen[a,*], Anette Müllertz[a], Erling B. Jørgensen[b], René Holm[a,c,1]

[a] Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark
[b] Analytical Development Substance, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark
[c] Pharmaceutical Science and CMC Biologics, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark

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ABSTRACT

Buccal delivery may be clinically beneficial for compounds with a high gastrointestinal and hepatic first pass metabolism or in situations where a fast systemic absorption is desired. The delivery of a crystalline low soluble compounds, *e.g.* diazepam, may be limited due to the low volume of saliva available to facilitate solvation in order to drive the permeation of drug through the buccal mucosa. Therefore, the present study investigated the potential benefits of administering diazepam either as an amorphous or as a crystalline form in mucoadhesive tablets to conscious Göttingen mini-pigs. Presentation of the compound in the amorphous form lead to a very fast absorption, however, the obtained bioavailability was at the same level observed following buccal administration of a commercially immediate release tablet. Addition of chitosan, as a mucoadhesive excipient, resulted in a higher absolute bioavailability compared to tablets without chitosan. The absorption rate for the chitosan-based tablets was significant slower, probably due to the slower diffusion of the compound out of the tablet. *In vitro* release data was able to predict the variations in t_{max}, but otherwise no correlation could be found between *in vitro* and *in vivo* data.

1. Introduction

Diazepam is a widely used benzodiazepine for the treatment of various disorders in the central nervous system (Muzyk et al., 2013; Calcaterra and Barrow, 2014). In general, benzodiazepines have been used in the treatment of disorders requiring rapid onset of action such as seizures and status epilepticus, but also as daily dosing in chronic treatment of diseases as multiple sclerosis (Wolf, 2011; Tullman, 2013; McKee and Abou-Khalil, 2015). Oral administration of diazepam is normally the preferred route; however, alternative routes are sometimes necessary. This can be in situations where the treated disease requires a special therapeutic profile, as in the treatment of acute convulsive seizures in children where rectal administration is often chosen (Khan et al., 2014). For the treatment of convulsive seizures buccal and nasal administered midazolam has emerged as an effective and more convenient route of administration compared to rectal administration of diazepam (Ülgey et al., 2012). Accordingly, buccal administration of diazepam could also be considered. Beside the avoidance of gastro-intestinal and hepatic first pass metabolism, buccal administration offers fast absorption owing to the high blood flow and readily permeable mucosa compared to *e.g.* the skin (Johnson et al., 1987).

Predicting the performance of new buccal formulations is mainly conducted *in vitro* or in animal studies using anaesthetised mini-pigs, rats, dogs, or hamsters. Very few studies have been conducted in conscious animals (Tsagogiorgas et al., 2013; Meng-Lund et al., 2014a). These studies have demonstrated that anaesthesia have a profound effect on the *in vivo* performance after buccal administration. This may be due to an effect on salivary glands and blood vessels causing reduction in saliva and blood flow in the oral cavity, but other factors may also be involved. Porcine buccal mucosa has been used as a gold standard for predicting human buccal permeability due to a high level of similarities in terms of composition, epithelial thickness, and histology (Nielsen and Rassing, 2000; Lesch et al., 1989). This, together with the fact

* Corresponding author.
E-mail address: jette.jacobsen@sund.ku.dk (J. Jacobsen).
[1] Pressent address: Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium.

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that very few *in vivo* studies have been performed in mini-pigs, highlights the needs of further empirical data.

The use of bioadhesive polymers is a vital formulation variable when addressing buccal drug delivery (Chinna Reddy et al., 2011; Salamat-Miller et al., 2005; Laffleur, 2014). Many polymers have been used to facilitate bioadhesion with various mechanisms of actions. Chitosan is a widely studied polymer and has received excessive interest as a bioadhesive excipient in pharmaceutical formulations (Patel et al., 2012). Chitosan is a naturally derived non-toxic polysaccharide comprising of alternating glucosamine and *N*-acetyl-glycosamine units with a pK_a of 6.5 (Liu et al., 2005). By interacting with epithelial surface-bound mucins in the mucus layer, chitosan has shown to be an effective agent to facilitate bioadhesion using hydrophobic, hydrophilic, electrostatic, and hydrogen bonding (Sogias et al., 2008). The advantages of bioadhesion are extended retention time and higher drug concentration at the site of absorption, which results in increased drug absorption due to a steeper concentration gradient across the tissue (Haas and Lehr, 2002).

Previous studies, using the complex coacervation model and the tensile detachment model, have shown a pH dependent mucoadhesion of chitosan (Meng-Lund et al., 2014b). Based on this, and the fact that bioadhesion may result in increased drug absorption due to steeper concentration gradient, it could be suggested that an increased strength of mucoadhesion could have a positive effect on the *in vivo* absorption of drug compounds administered following buccal administration. In relation to the present study, this could be relevant for diazepam also given the clinical potential of a buccal formulation for the compound. Further, diazepam is an amine with a pK_a value of ~3.4, why diazepam is unionised at the pH range relevant for the oral cavity (Manallack, 2007; Aframian et al., 2006). The compound will therefore not affect the bioadhesive properties of chitosan at the pH range relevant for the oral cavity making it a suited model compound for investigation of the mucoadhesive effect *in vivo*.

For compounds, like diazepam, with a low aqueous solubility it may be challenging to obtain a good absorption when administered bucally in the crystalline form. The small volume of saliva available in the mouth could limit the amount of diazepam solvated in the oral cavity and thereby the fraction absorbed. One potential way to circumvent this could be to increase the solubility and dissolution rate of the compound, *e.g.* by dosing the compound in an amorphous state. Amorphous systems are often stabilised by polymers (Bhugra and Pikal, 2008; Serajuddin, 1999; Taylor and Zografi, 1997), and in mucoadhesive systems with chitosan, the polymer for this purpose could be chitosan, *i.e.* the excipient could potentially have a dual function in buccal formulations. Buccal administration of an amorphous compound has, to our knowledge not been evaluated before. Therefore, the aim of the present study was to study the impact on buccal absorption of diazepam in chitosan based buccal tablets in mini-pigs applying (i) crystalline diazepam in tablets with high and low bioadhesion and (ii) amorphous diazepam.

2. Materials and methods

2.1. Materials

Diazepam (DIAZ) and clozapam were kindly provided from Takeda (Osaka, Japan) and H. Lundbeck A/S (Valby, Denmark), respectively. Isolute® C2 (50 mg/L ml) solid-phase extraction columns were purchased from Biotage (Uppsala, Sweden). Methanol, 2-(*N*-morpholino)ethanesulfonic acid (MES) hydrate, 85% phosphoric acid, dibutyl phthalate and hydrogen chloride were purchased from Sigma-Aldrich (St Louis, MO, USA). Chitopharm® L (MW 500–5000 kDa) was kindly provided by Cognis GmbH (Monheim, Germany). Sodium hydroxide and triethylamine were obtained from Merck (Darmstadt, Germany). Diazepam "DAK" 5 mg tablets ("DAK" tablet) were purchased from Takeda (Osaka, Japan). Solution for injection Stesolid® 5 mg/mL was purchased from Actavis (Dublin, Ireland). Deionized water was obtained from a Millipore Milli-Q Ultrapure Water purification system (Billeria, MA).

Table 1
Composition of liquid feeds for spray-drying.

Excipients	Neat chitosan feed	SD feed	MES feed
Chitosan (g)	3	0.5	–
MES (g)	–	–	10
Diazepam (g)	–	1	–
HCl 2.5 M (mL)	15	22.5	–
NaOH 10 M (mL)	–	–	17
Deionised water	ad 600 mL	ad 600 mL	ad 50 mL

2.2. Preparation of spray-dried particles

Amorphous solid dispersions (SD) of diazepam were prepared by spray-drying diazepam together with chitosan. The compositions of liquid feeds for spray-drying are shown in Table 1. Liquid feeds of neat chitosan or chitosan and diazepam were prepared by dissolving chitosan in a diluted solution of HCl overnight in order to allow complete solvation. Spray-dried particles were prepared using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). The system was heated to an inlet temperature of 200 °C, resulting in an outlet temperature of 81–86 °C. The pressure of the atomizing air was 35 mm Hg. An automatic vacuum pump was used for cleaning the nozzle every second min. The feed was supplied by a pump at a flow rate of 3 mL/min and an aspirator rate of 32 m^3/h.

2.3. Bioadhesive buccal tablets

Buccal tablets with unidirectional release of diazepam were prepared as previously described (Meng-Lund et al., 2014a,b). Briefly, the excipients (Table 2) were mixed manually and core tablets (gross mass 80 mg) were compressed (Diaf TM 20, Diaf, Vibe Mølle, Denmark) using a circular tablet punch with a diameter of 6 mm.

The core tablets of formulation 1, 2, and 3 (see Table 2), as well as the commercial oral diazepam tablet, used for buccal administration in this study, were coated with a backing layer consisting of ethyl cellulose (5%, w/v) in acetone with dibutyl phthalate (30%, w/w dry weight of polymer) as plasticizer and riboflavin as colouring agent (in excess as a suspension). The coating layer was manually sprayed in 3–4 individual layers on the core tablets, leaving only one surface uncoated, which was placed towards the buccal tissue and thereby promoting unidirectional release of diazepam.

2.4. Chemical and physical stability studies of diazepam formulations

Buccal tablets formulation 1, 2, and 3 (see Table 2) were stored at 25 °C and 60% relative humidity (RH). Stability was tested at day 0, 30, and 90. Chemical stability was tested by dissolving a tablet in 200 mL of 0.1 M HCl (n = 3) followed by filtering and dilution to an appropriate concentration of diazepam to be within the linear range of the HPLC calibration curve (see the analytical section).

Physical stability of diazepam in the buccal tablets was tested by X-ray powder diffraction (XRPD), where measurements were performed on an X'Pert PRO MRD diffractometer from PANalytical (Almelo, The Netherlands) equipped with a TCU 100 temperature control unit and an X'Celerator detector using nickel-filtered CuK_α