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Insulin availability from mucoadhesive tablets

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The widespread implementation of peptides as drugs encounters numerous obstacles, the main being invasive and inconvenient parenteral administration. Oral transmucosal administration is one of the possible alternatives, valuable for its noninvasiveness and easy accessibility. The aim of our study was to determine the implementation possibilities of mucoadhesive tablets prepared on a methylcellulose and sodium alginate basis with an addition of absorption-modifying hyaluronic acid, as carriers for peptides destined for oral transmucosal administration. Two series of 50 mg tablets containing 5mg of insulin were prepared for the study. The first series contained methylcellulose, hyaluronic acid and mannitol, while the second series' formulation included sodium alginate, hyaluronic acid and mannitol. Carried out study confirmed that insulin administration in the form of mucoadhesive tablets lowers blood glucose levels in rabbits. Better effects were reached *in vivo* in the case of MC-based tablets, for which stronger and longer glycemia lowering was achieved.

1. Introduction

The widespread implementation of peptides as drugs encounters numerous obstacles, the main being invasive and inconvenient parenteral administration. Oral transmucosal administration is one of the possible alternatives, valuable for its noninvasiveness and easy accessibility.

Bioadhesive tablets used as peptide carriers, enable constant contact with the absorbing mucous membrane and elongate drug retention at the administration site, maintaining a high concentration gradient and thus increasing peptide bioavailability (Müller and Hildebrand 1997). Furthermore, mucous permeability modulation as well as protection from proteolytic enzymes are rendered possible through the use of appropriate adhesive polymers (Müller and Hildebrand 1997; Bernkop-Schnürch et al. 1998; Senel and Hincal 2001).

Alginic acid is a linear polysaccharide formed by (1–4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues. Alginic acid salts are widely used in pharmaceutical technology and their mucoadhesive properties are important for buccal adhesive tablet technology, increasing the bioavailability of some therapeutic agents. Moreover, the use of sodium alginate enables the application of the direct tabletting method for tablet production (Miyazaki et al. 1995; Han-Gon Choi and Chong-Kook Kim 2000; Holte et al. 2003).

Other polymers frequently used in pharmaceutical technology include cellulose ethers, of which the most common are methylcellulose (MC), hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC). Selecting the right derivatives permits modulating the rate of drug release (Jian-Hwa Guoet et al. 1998; Samani et al. 2003; Cherng-ju Kim 1999).

Hyaluronic acid is a natural, highly adhesive mucopolysaccharide composed of disaccharides, themselves composed of D-glucuronic acid and D-*N*-acetylglucosamine, linked together via alternating β-1,4 and β-1,3 glycosidic bonds. In mammals, hyaluronic acid naturally occurs in all extracellular structures, filling intercellular space in tissues and answering for water balance between inter- and intracellular fluids. Good biocompatibility, low immunogenicity and good mucoadhesiveness – comparable to acrylic acid derivatives, characterize hyaluronate. It is deemed that mucoadhesive and mucotropic properties can increase drug permeability, this also concerning peptid drugs (Prestwich and Vereruysse 1998; Merck 1996b).

The aim of our study was to determine the implementation possibilities of mucoadhesive tablets prepared on a methylcellulose and sodium alginate basis with an addition of absorption-modifying hyaluronic acid, as carriers for peptides destined for oral transmucosal administration. Insulin, a 5760Da peptide with an isoelectric point of 5.3 was used as model peptide.

2. Investigations and results

2.1 Tablet properties

Tablets containing 70% of mucoadhesive polymer – methylcellulose or sodium alginate, were tested in terms of disintegration time in water, absorption capacity and adhesion strength according to Pharm. Eur. 6th edition. The adhesions strengths measuring device is shown in Fig. 1; results are given in Table 1; differences in absorption capacity and adhesion strength reaches statistical significance (student's T-test, $p = 0.05$).

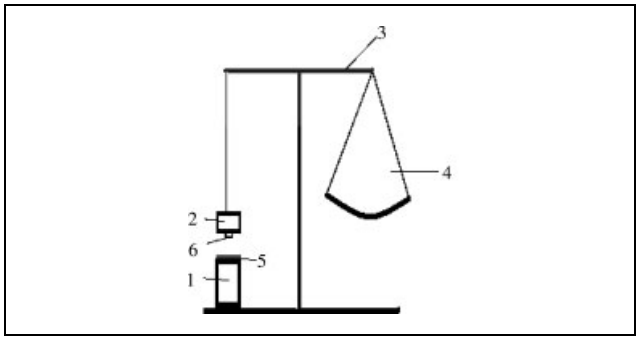

Fig. 1: Adhesion strength measuring device
1 – lower platform, 2 – upper platform, 3 – beam, 4 – opposite pan, 5 – gut, 6 – tablet

Table 1: Mean disintegration times, absorbed water quantity and mean adhesion strength of mucoadhesive tablets

Tablet series	Disintegration time ± SD [min]	Mean amount of water absorbed per 1 g of tablet ± SD	Mean adhesion strength ± SD [N/mm^2]
t-MC	60.44 ± 1.47	3.069 ± 0.107	0.0038 ± 0.0008
t-ANa	63.87 ± 4.27	9.701 ± 0.539	0.0110 ± 0.0014

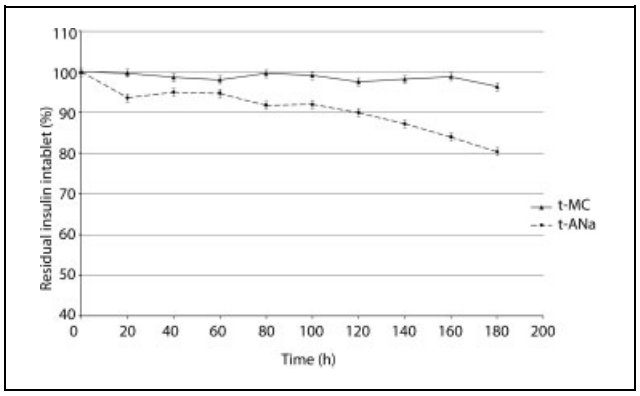

Fig. 2: Insulin release kinetics from t-MC and t-ANa tablets on USP Dissolution Apparatus 2

2.2 Insulin release from mucoadhesive tablets

2.2.1 In vitro release tests

In testing insulin release kinetics from tablets on a dissolution apparatus 2, considerable impact of matrix formulation on amounts of released substance was found. Results are shown in Fig. 2, while process kinetics parameters are given in Table 2. Higher amounts of insulin were released from sodium alginate-based tablets at a mean of 19.63%, while MC-based tablets released a mean of only 3.62%. During the whole process, differences in amounts of released drug remained statistically significant (ANOVA, $p = 0.05$, NIR post-hoc test). In both series of tablets, variations of drug release rate in time were observed, which may indicate the role played by matrix hydration in the drug release process.

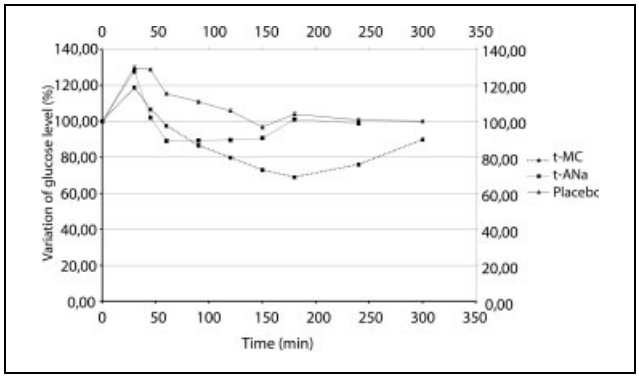

Fig. 3: Variation kinetics of rabbit blood glucose levels following t-MC or t-ANa tablet administration and after placebo administration. (Glucose concentrations were calculated as percentage of variation in blood glucose profile, using glucose levels at time t = 0 (100%) as reference)

2.2.2 In vivo release tests

As shown in Fig. 3, insulin administration in the form of mucoadhesive tablets lowers blood glucose levels in rabbits. Better effects were reached *in vivo* in the case of MC-based tablets, for which stronger and longer glycemia lowering was achieved. Lowest glucose levels in the case of MC-based tablets amounted to 69.04% of the initial value, and to only 89.69% of the initial value for alginate-based tablets.
Statistical analysis with ANOVA/MANOVA ($p = 0.05$) and NIR post-hoc test showed statistically significant differences in glucose level decrease between MC-based tablets and placebo at 60 min to 240 min timepoints from tablet administration.
For alginate-based tablets, statistically significant differences were noted only at 60 to 150 min timepoints. Significant differences were also found between MC- and alginate-based tablets at 60 to 240 min timepoints. An initial transient increase in blood glucose concentrations was noted in all 3 animal groups, and was connected with rabbit sedation induction.

3. Discussion

Mucoadhesive tablet disintegration time has significant impact on contact duration between tablet and mucous membrane. However, the test conditions – especially regarding quantity of water and mechanical forces – widely differ from those found in the oral cavity – that is the target administration site. For those reasons, obtained tablet disintegration times may only serve as reference points, and differences between selected series may vary widely.
The amount of water absorbed by the tablet as well as absorption rate strongly influence the tablet's mechanical

Table 2: Linear trend equations for insulin release kinetics on USP Dissolution Apparatus 2

Series	R-Pearson coefficient	Linear trend equation for dependence of % residue on time	R^2	Linear trend equation for dependence of ln % residue on time	R^2
t-MC	−0.712	y = −0.012x + 99.661	0.3957	y = −0.0001x + 4.6018	0.396
t-ANa	−0.949	y = −0.0858x + 98.433	0.8643	y = −0.001x + 4.5939	0.8553

resistance, and may also affect mucoadhesiveness and drug release rate (Bernkop-Schnürch et al. 1998; Tamburic and Craig 1997; Colombo et al. 1999a, b). Colombo et al. demonstrated that drug release from hydrophilic matrices is connected with their hydration, leading to matrix structure relaxation and its accelerated erosion (Colombo et al. 1999a, b). Tamburic and Craig discovered that the first 10 min of the hydration process are most significant – following this time, stabilisation of the formed bonds occurs (Tamburic and Craig 1997).

Buccal tablet adhesion determines contact between tablet and mucous membrane, through which drug absorption occurs. Thus, the distance between drug releasing and absorbing surfaces is minimized and, additionally, gel formation protects the released substance, limiting i.e. enzyme activity. Matrix hydration, connected with the number of hydrogen bonds between polymer and mucous membrane, is also an important factor in mucoadhesion.

Numerous simultaneous processes account for drug release from hydratable matrices i.e.: hydrogel formation, dissolution-matrix erosion. Thus, available water quantity and matrix absorption rate are essential elements, determining further phenomena, such as polymer matrix relaxation connected with polymer properties, and final hydrogel dissolution. Considering the above, it should be stressed that pharmaceutical availability test conditions recommended by Ph. Eur. considerably differ from those found in the oral cavity.

The amount of available solution seems to be a critical parameter. High water amounts lead to quick matrix hydration, gel formation and eventual dissolution, which loosens tablet structure and may lead to accelerated insulin release. In our study, alginate-based tablets showed higher absorption capacity and released greater insulin amounts in pharmaceutical availability tests. Yet, *in vivo* tests showed lower insulin release in comparison to MC-based tablets. Thus, it seems that low conformity between *in vitro* and actual natural conditions leads to low correlation between obtained *in vivo/in vitro* results.

The conducted study demonstrated that MC-based or sodium alginate-based mucoadhesive tablets with an addition of hyaluronic acid may be used as peptide carriers. Moreover, it was proven that peptides administered this way permeate the mucous membrane, thus therapeutic effects can be reached through with the use of this drug form. It should also be stated that further investigations are needed in order to establish test conditions closely matching those naturally found at the drug administration site.

4. Experimental

4.1. Material

Methocel 65HG – Fluka Chemie GmbH, Switzerland (MC), Alginic acid sodium salt – Sigma-Aldrich Chemie GmbH, Steinheim, Germany (Ana), d-(-)-Mannitol – Riedel-de-Haen, Germany (Man), Hyaluronic acid – Croma Pharma GmbH (HA), Insulin WOS– Polfa Tarchomin, Poland (Ins)

4.2. Mucoadhesive tablet production technology

Two series of 50 mg tablets containing 5 mg of insulin were prepared for the study. The first series contained methylcellulose, hyaluronic acid and mannitol, while the second series' formulation included sodium alginate, hyaluronic acid and mannitol. Methylcellulose or sodium alginate, hyaluronic acid and mannitol were carefully mixed (63 : 10 : 27 ratio), appropriate amounts of insulin were subsequently added, the whole was re-mixed and tabletted. Direct compression tabletting was applied, with the use of a tabletting machine and 3-punch matrix of 6 mm diameter. In order to monitor tabletting correctness, the following parameters were tested in accordance with Ph. Eur. 6th Edition: mean tablet mass, mean tablet thickness and mechanical resistance.

4.3. Tablet disintegration time testing

Tablet disintegration time in water was determined for 6 tablets from each series on an device meeting Ph.Eur. requirements (Erweka). Tests were conducted at a temperature of $37 \pm 0.5\,^{\circ}C$, an oscillation amplitude of 5.5 ± 0.5 cm and a frequency of 30 ± 2/min. Total tablet disintegration or dissolution was considered the test end-point. Obtained results are given in Table 1.

4.4. Determining water absorption capacities

Water absorption capacity was evaluated in 5 randomly chosen tablets from each series. Tablets were weighed with an accuracy of ± 0.001 g on an analytical scale (Sartorius), subsequently placed in copper mesh handles, re-weighed and immersed in purified water for 30 min. After this time, the tablets were taken out, drained on blotting paper and weighed. The amount of adsorbed water was calculated from the equation:

$$S_w = \frac{M_2 - M_1}{m_t} \quad (1)$$

where S_w – quantity of absorbed water, M_1 – pre-test mass of the tablet in clasp, M_2 – post-test mass of the tablet in clasp, m_t – tablet mass. Mean test results are presented in Table 1.

4.5. Evaluation of mucoadhesive performance

Adhesion strength was evaluated in 6 randomly selected tablets from each series. Tests were conducted on a balance-based device, described by Han-Gon Choi et al. (2000, Fig. 1). A tablet was fixed to the upper mobile platform using a special tape. The static lower platform was covered with pig gut – moistened with ca. 0.5 ml of purified water. The upper platform, together with the tablet, was lowered onto to lower platform and left loadless for 5 min. Subsequently, the opposite balance pan was weighed down every minute according to two schemes. For methylcellulose tablets, a 2 g mass was added every minute to a total of 10 g, followed by 1 g masses. For sodium alginate tablets, the pan was weighed down with 10 g masses every minute.

Adhesion strength was calculated from the equation:

$$F = \frac{W \cdot G}{A} \quad (2)$$

where F – adhesion strength $N \cdot m^{-2}$, W – maximum load at which tablet detachment occurred (kg), G – earth gravity ($m \cdot s^{-2}$), A – contact area (mm^2). Mean adhesion strength values are shown in Table 1.

4.6. Evaluation of insulin availability from mucoadhesive tablets

4.6.1. Testing drug release from tablets on USP dissolution apparatus 2

Insulin release from mucoadhesive tablets was tested on a dissolution apparatus 2 according to the method described in USP XXVI for drug release from tablets. The test was conducted at $37 \pm 1\,^{\circ}C$, with 750 ml of purified water used as acceptor fluid. The acceptor fluid was mixed at 50 rotations/min, and 4-ml samples were collected every 20 min during a total of 3 h. Spectrophotometry was used in order to determine insulin amounts in each sample. The pharmaceutical availability profiles obtained are shown on Fig. 2.

4.6.2. Determination of bioavailability

Bioavailability was evaluated in 4–4.5 kg rabbits. Animals were sedated through intravenous administration of xylazin and thiopenthal. Tablets were placed on rabbits' buccal mucous membrane. Blood samples were drawn from the auricular marginal vein at 0, 30, 45, 60, 90, 120, 150, 180, 240 min after tablet administration. Samples were tested for glucose using the Glucose GODFS assay (Diasys). Obtained glucose concentrations were calculated as percentage of variation in blood glucose profile, using glucose levels at time $t = 0$ (100%) as reference. Variations in rabbit blood glucose levels are presented in Fig. 3.

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