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Preformulation experiences and *in vitro* model studies with spironolactone-containing suppositories

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Dedicated to Prof. em. Dr. B. Selmeczi, Szeged (Hungary), on the occasion of his 70th birthday

The optimal suppository base for the formulation of rectal suppositories containing diuretic spironolactone was selected experimentally. Model studies were carried out about the effect of solubility-increasing additives on the release of the drug from the suppositories. During the *in vitro* examinations acceptor phases of different pH values were used, and both diffusion time and the number of samplings were changed. Among the lipophilic and hydrophilic suppository bases studied the hydrophilic Macrogolum 1540 was found to be optimal. The release and diffusion of spironolactone was the most favourable from these suppositories. During storage these suppositories remained stable and the values of release did not decrease significantly ($p < 0.05$).

1. Introduction

Dosage form research is part of drug research as the same dose of a given drug can result in a different intensity of effect depending on whether it is administered orally or rectally [1, 2]. Recent observations have revealed that bioavailability, stability and drug safety can differ even with the same route of drug administration depending on the vehicles and additives used in drug formulation [3–9].

Rectal suppositories are widely used [10–12]. The pharmacon chosen as a model for our experiments was spironolactone, which is well-known in therapy but has not been used rectally to date; our aim was to formulate a new diuretic preparation. The new product increases the choice of therapeutic possibilities [12–15]. There is clinical need for rectally used diuretics, which were earlier examined by the authors in the case of furosemide and theophylline [16–18].

In modern pharmaceutical technology the biopharmaceutical view has to be considered, this is best described by the abbreviation LADMER [19]. A fundamental effect is exerted by the first phase, that is liberation, on all the kinetic processes. The last letter of the abberation refers to R = response, which shows the therapeutic effect. *In vitro* pharmacon liberation studies are very useful with respect to subsequent *in vivo* trials, as the optimal suppository base can be screened well. The authors and Kedvessy have studied factors influencing drug liberation from suppositories for decades [20–21]. Other authors share the view that it is important to get to know the bioavailability of various drug [22–28]. Therefore the optimal suppository base and the necessary additives have to be chosen with scientific accuracy [29–31]. The biopharmaceutical importance of the use of additives was shown by Regdon et al., too [32–34].

2. Investigations, results and discussion

The experimental suppository compositions and conditions are presented in the Table for a better comparison of the results. The results considered to be the most characteristic and the most important are discussed in the following.

Fig. 1 shows drug liberation and diffusion from suppositories prepared with three hydrophilic and four lipophilic vehicles. The acceptor phase was water. Less than 2% of the standard powder diffused through the membrane in 240 min. Liberation was even worse from lipophilic vehicles, while approximately 5 times as much drug, that is about 10% was liberated and entered the acceptor phase with the use of hydrophilic Macrogolum 1540.

The tensides added to Macrogolum 1540 as additives did not increase liberation. The same percentage was measured in the case of Tween® and diffusion was slightly hindered by Span® (Fig. 2).

Then diffusion time was increased from 4 to 8 h, and samples were taken more frequently. Instead of the previous 4 samples 6 and 10 were now taken for spectrophotometric analysis and cumulative values were determined from the obtained values (Fig. 3). Drug liberation from one lipophi-

Table: Experimental suppository compositions and examination conditions

Base	Form of the drug	Medium	Number of samples
Massa Estarinum 299	powder	water/buffer	4*, 6** and 10***
Witepsol H 15	powder, physical mix, kneaded product	water	4 and 6
Witepsol W 35	powder, kneaded product	water/buffer	4 and 6
Witepsol S 55	powder	water	4 and 6
Macrogolum 1540	powder, physical mix, kneaded product	water/buffer	4, 6 and 10
Macrogolum 1540 + 10% Macrogolum 400	powder	water	4
Macrogolum 1540 + 5% Span 20	powder, kneaded product	water/buffer	4
Macrogolum 1540 + 5% Tween 20	powder	water	4

* 30, 60, 120, 240 min
** 30, 60, 90, 120, 180, 240 min
*** 30, 60, 90, 120, 180, 240, 300, 360, 420, 480 min

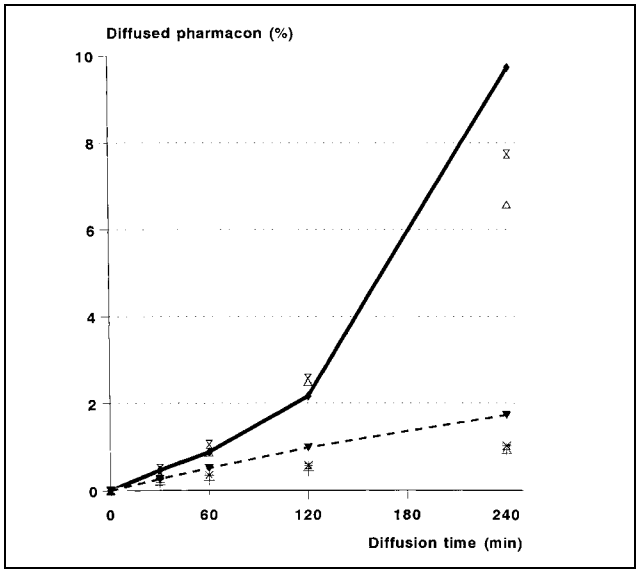

Fig. 1: Membrane diffusion of spironolactone from suppositories prepared with lipophilic and hydrophilic vehicles, acceptor phase: water. ▼ Powder, + Witepsol W 35, ✳ Massa Estarinum 299, □ Witepsol H 15, × Witepsol S 55, ◆ Macrogolum 1540, △ Massa macrogoli, ⧖ 90% Macrogolum 1540/10% Macrogolum 400

lic and one hydrophilic suppository base was studied in these experiments. It was found that the quantity of diffused spironolactone did not increase with the greater number of samples, and in the case of lipophilic vehicles liberation was not enhanced by the longer diffusion time. The increase observed in the diffusion value after 240 min could be explained by the high melting point of Macrogolum 1540. The reason for this is that the hydrophilic base does not melt at 37 °C, while it slowly dissolves in water, that is why diffusion shows a considerable increase in the second 4 h, too. The small extent of liberation is probably due to the poor water-solubility of the pharmacon.

The solubility-increasing effect of cyclodextrin derivatives in the case of spironolactone was already studied both in the forms of physical mix and kneaded product (abbreviated in the figure as 'ph' and 'kn', respectively) and was

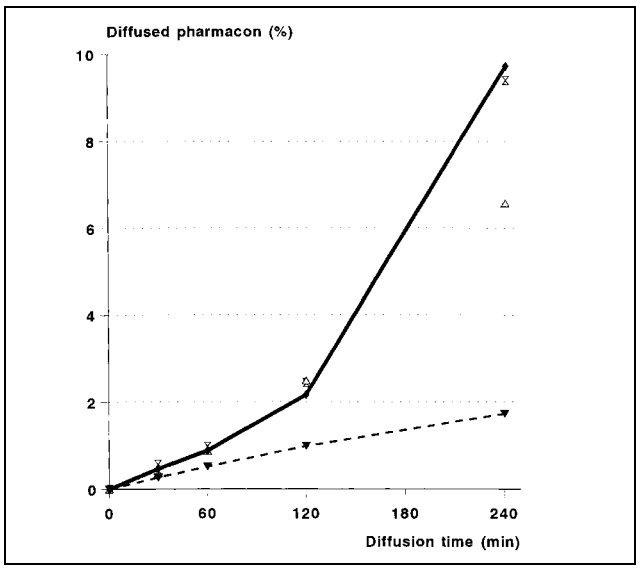

Fig. 2: The effect of Span 20 and Tween 20 on the membrane diffusion of spironolactone from suppositories prepared with the Macrogolum 1540 base acceptor phase: water. ▼ Powder, ◆ Macrogolum 1540, △ +5% Span 20, ⧖ +5% Twen 20

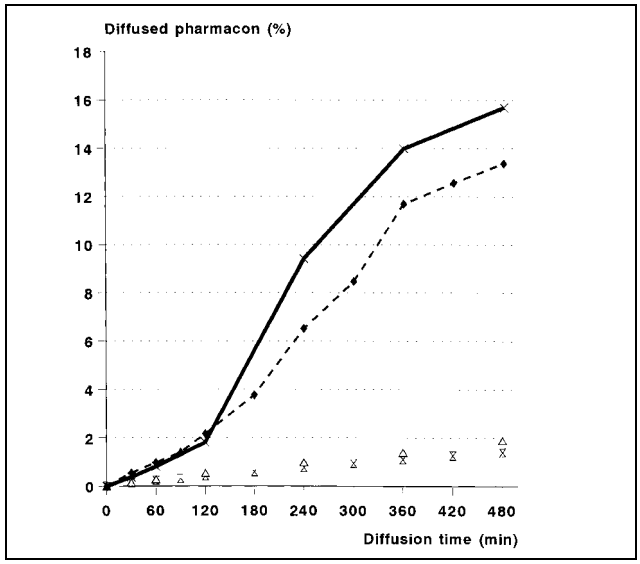

Fig. 3: Membrane diffusion of spironolactone measured at 6 and 10 different times diffusion time: 8 hours, acceptor phase: water. ◆ Macrogolum 1540 (6), ◆ Macrogolum 1540 (10), △ Massa Estar. 299 (6), ⧖ Massa Estar. 299 (10)

found to be favourable [34–35]. As far as we know they had not been tested in suppositories, therefore several experiments were carried out in this respect. Some results are shown in Fig. 4. The proportion of spironolactone/beta-cyclodextrin was 1 : 3 both in the physical mix and in the kneaded product. Diffusion of spironolactone from suppositories prepared with Macrogolum 1540 was increased significantly ($p < 0.05$) by cyclodextrin complexes. The kneaded product proved to be the best.

Fig. 5 shows the comparison of the *in vitro* relative availability values compared to the standard (100%), with the use of two acceptor phases of different pH values. Several conclusions can be drawn from the column diagrams: the lipophilic suppository bases were below the standard, therefore they are not recommended for use. On the other hand, excellent results were obtained with Macrogolum

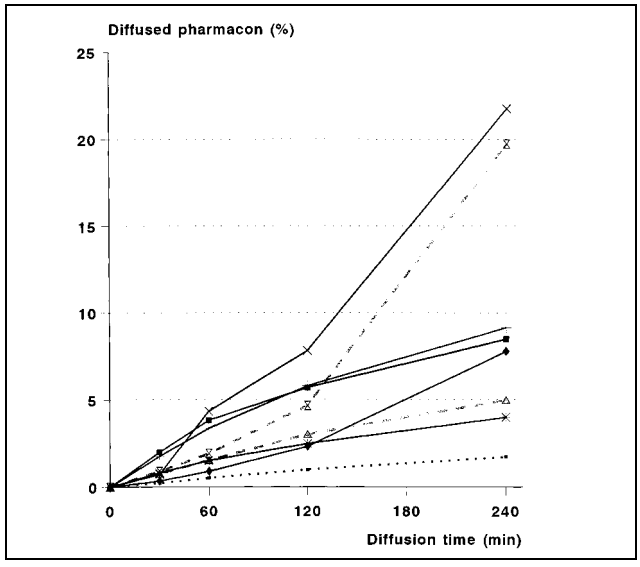

Fig. 4: Membrane diffusion of spironolactone + β-CD physical mix and kneaded product from lipophilic and hydrophilic vehicles, acceptor phase: water. ▼ Powder, + Kneaded product, ✳ Witepsol W 35 (kn), ■ Witepsol H 15 (kn), × Macrogolum 1540 (kn), ◆ Massa macrogoli (kn), △ Witepsol H 15 (ph), ⧖ Macrogolum 1540 (ph)

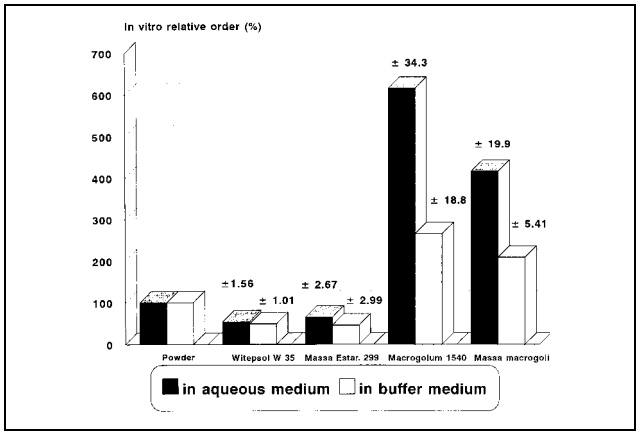

Fig. 5: In vitro relative order of spironolactone-containing suppositories

1540, the drug liberation from suppositories increased significantly ($p < 0.01$). Finally, it can be stated that a smaller extent of diffusion took place whenever phosphate buffer solution was used as the acceptor phase. The pH value of 7.5 did not have a good effect on spironolactone liberation and diffusion. Indifferent distilled water is more suitable for this purpose as an *in vitro* availability value of 600 relative % could be achieved with Macrogolum 1540 as a vehicle.

Furthermore, the effect of storage time on spironolactone liberation is presented with a few examples (Fig. 6). The column diagrams clearly show that Witepsol W 35, containing partial glycerides, had liberation properties practically as poor as the triglyceride Massa Estarinum 299; these did not change significantly ($p < 0.05$) during storage. *In vitro* liberation was by far the best from the hydrophilic Macrogolum 1540 vehicle, the quantity of spironolactone diffusing through the membrane was significantly higher ($p < 0.01$). This value did not decrease significantly ($p < 0.05$) after 6-months of storage. Suppositories were qualified as "acceptable" even after storage for 12 months.

Finally, it should be noted that the highest of spironolactone liberation was measured from Macrogolum 1540. The beta-cyclodextrin additive had a favourable effect on drug liberation and diffusion. During the *in vitro* model examinations distilled water was found to be a more suitable acceptor phase than buffer solution. Neither the number of sampling, nor diffusion time needs to be doubled. Suppositories prepared with the hydrophilic Macrogolum 1540 remained stable during 6 months of storage, drug liberation did not decrease significantly.

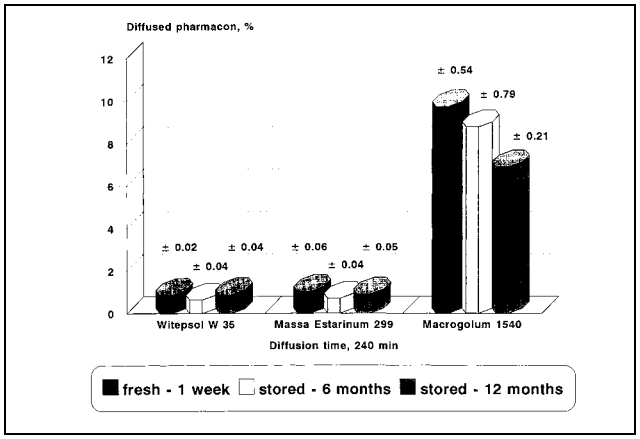

Fig. 6: The change of the membrane diffusion of spironolactone after storage for 6 and 12 months, acceptor phase: water

3. Experimental

3.1. Materials

Spironolactone, a diuretic drug with antihypertensive action (EGIS Pharmaceutical Factory, Budapest, Hungary), was used as model drug. The yellowish-white crystalline powder is practically insoluble in water. The 8 different lipophilic and hydrophilic suppository bases included Witepsol, Estarinum bases and Macrogolum derivatives (Hüls AG, Troisdorf/Germany), and Span, Tween and beta-cyclodextrin additives were used to increase solubility (Atlas Chemie/Germany, Chinoin/Hungary).

3.2. Methods

3.2.1. Formulation of spironolactone suppositories

Spironolactone was used in therapeutic doses, that is in the quantity of 50 mg/2.0 g suppository. During suppository formulation the drug was suspended in the melted suppository bases in the form of fine powder (particle size < 160 µm), and then it was homogenized. The suppositories were wrapped in aluminium foil and stored at room temperature for 12 months.

3.2.2. In vitro release studies

The Vibrotherm apparatus based on the principle of dynamic diffusion was used (Hungarian Academy of Sciences, Kutesz, Budapest/H). Five samples from each experimental composition were placed into a Visking-dialyzing membrane (Serva Feinbiochemia GmbH & Co. Heidelberg, Germany), which had a diffusion surface of about 12 cm^2. The apparatus performed 50 slight horizontal shakes per minute. The acceptor phase on the other side of the membrane was changed in order to find out how liberation and diffusion was influenced by the pH of the medium, that is by distilled water (pH = 5.8) and by phosphate buffer solution (pH = 7.5). The temperature was 37 ± 0.5 °C during the examinations. Drug liberation was monitored by taking samples at 4, 6 and 10 different times. The quantity of the diffused spironolactone was measured in each period spectrophotometrically at $\lambda = 241.5$ nm (Spektromom 195-D/Hungary). It was found that the vehicles did not influence the absorbance values. The absolute quantity of the released drug was measured spectrophotometrically and the relative order was determined *in vitro*. The calculation was based on the diffusion of 50 mg of spironolactone powder without a vehicle, which served as a standard. The following equation was used to calculate relative order:

$$\text{In vitro relative order (\%)} = \frac{m_k \cdot D_p}{m_p \cdot D_k}$$

where

m_k = the quantity of the pharmacon diffused from the studied suppository in mg
m_p = the quantity diffused from the standard preparation in mg
D_k = the spironolactone content in the studied suppository in mg
D_p = the quantity measured in the standard preparation in mg

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