INCREASING THE SOLUBILITY CHARACTERISTICS OF D-NORGESTREL WITH CYCLODEXTRINS

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ABSTRACT

Levonorgestrel dissolves only slightly in water. Attempts were made to increase the solubility properties of this drug by complexing with cyclodextrins. The products were investigated with a dissolution tester and a Sartorius resorption model. X-ray and NMR spectra of the inclusion complexes were analysed.

1. INTRODUCTION

Levonorgestrel (**Lev**) is one of the most popular contraceptives. It is used in combination with estrogen. It was discovered by *Hughes et al.* (1963). It is a white or nearly white powder, which dissolves only slightly in water.
Cyclodextrins (**CD**s) form inclusion complexes with numerous guest drug molecules and this complexation increases the solubility and rate of dissolution of these drugs.
Our aim was to increase the solubility of **Lev** by using **CD**s and to investigate these products (dissolution rate, in vitro diffusion properties, X-ray, NMR, etc.).

2. MATERIALS AND METHODS

2.1. Materials

Levonorgestel (D-norgestrel, **Lev**), 13-ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one (Chemical Works of G. Richter Ltd., Budapest, Hungary) [1].
α-, β-, γ-CD, methyl-β-CD, dimethyl-β-CD, hydroxyethyl-β-CD, hydroxypropyl-β-CD, RAMEB (Cyclolab Ltd., Budapest, Hungary) [2].

2.2. Apparatus

USP rotating-basket dissolution apparatus, type DT; kneading mixer, type LK5 (Erweka Apparatebau GmbH., Heusenstamm, Germany); Sartorius resorption model (Germany); Spektromom 195 (MOM, Budapest, Hungary); Specord UV-VIS (C. Zeiss, Jena, Germany); DRON UM-1 X-ray apparatus; BRUKER Avance Drx 400 NMR spectrometer.

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2.3. Preparation of products

Products were prepared in four different mole ratios (**Lev**:**CD** mole ratio = 2:1, 1:1, 1:2 and 1:3).
Physical mixtures: The ground components were mixed in a mortar and sieved through a DIN 0.315 mm sieve.
Kneaded products: Physical mixtures of **Lev** and **γ-CD** were mixed (Erweka LK5) in the same quantity of ethanol + water (1:1). They were kneaded until the bulk of the solvent mixture had evaporated. After this, they were dried at room temperature and then at 105 °C, and were next pulverised and sieved (DIN 0.315 mm).
Products were stored under normal conditions at room temperature in closed glass containers.

2.4. Dissolution of drug

In the USP rotating-basket dissolution apparatus, 20 mg of pure **Lev**, or product containing 20 mg of **Lev**, was examined in 900.0 g of distilled water. The basket was rotated at 100 rpm. Sampling was performed after 5, 10, 15, 30, 60 and 90 min. The sample volume was 5.0 mL. The **Lev** contents of the samples were determined spectrophotometrically.

2.5. Membrane diffusion

Measurements were performed on 100.0 mL of artificial gastric juice (pH = 1.1 ± 0.1) or artificial intestinal juice (pH = 7.0 ± 0.1) and artificial plasma (pH = 7.5 ± 0.1). 20 mg of active agent, or product containing 20 mg of active agent, was in the donor phase in all cases. The temperature was 37.5 ± 1.5 °C. During the examination, 5.0 mL samples were taken five times (after 30, 60, 90, 120 and 150 min) and their active agent contents were determined spectrophotometrically. The amount of diffused active agent was calculated.

2.6. NMR spectra

The high-resolution NMR spectra were measured on a BRUKER Avance DRX 400 Fourier transform NMR spectrometer at 400 MHz ^1H frequency. The samples were dissolved in $CDCl_3$ and the deuterium signal of the solvent was used to lock the spectrometer. The spectra were recorded at room temperature; 32 K data points were used with a digital resolution of 0.26 Hz/pt.

3. RESULTS AND DISCUSSION

3.1. Preliminary experiments

A mixture of 0.03 g of **Lev** and 0.50 g of **CD** derivative was diluted to 20.0 g with water and stirred for 15 min. Suspension systems were filtered and the UV spectra were recorded. A system without **CD** was used as a control. As **γ-CD** had the highest influence on the solubility (by a factor of 310), this derivative was used for the further examinations.
The absorption maximum was 243 nm. The absorption obeyed the Bouguer-Lambert-Beer law in the concentration interval 0-15 μg/mL.

3.2. Dissolution studies

The amount of **Lev** that dissolved in distilled water during 90 min was less than 2.64%.

The physical mixtures yielded a higher dissolution of active agent as compared to **Lev**. The highest value for physical mixtures was obtained for the 1:3 composition (10.85 mg/900 mL), which is more than a 20-fold solubility increase relative to the pure active agent. Maximum dissolution was attained at 30 min, and this value did not change significantly later (saturation).
On dissolution of the kneaded products, similarly as for the physical mixtures, the best results were obtained for the 1:3 composition (17.34 mg/900 mL). Dissolution was better and faster than for the physical mixtures. The maximum was reached at about 5-15 min.
Summarizing: increasing CD ratio increased the amount of dissolved material. The rate of dissolution and the amount of dissolved material depended on the preparation methods. There were significant differences in the amount of dissolved drug between analogous compound products made by different preparation methods.

3.3. Membrane diffusion examinations

The results of these examinations can be seen in **Tables 1 and 2**.

TABLE 1. Membrane diffusion examinations in artificial gastric juice (mg /100 mL)

		Physical mixtures				Kneaded products			
Time	**Lev**	2:1	1:1	1:2	1:3	2:1	1:1	1:2	1:3
30'	0.0664	0.0987	0.0483	0.0806	0.0894	0.0763	0.0922	0.0786	0.0998
60'	0.0971	0.1020	0.0521	0.0899	0.0993	0.0976	0.1041	0.0930	0.1190
90'	0.0878	0.1152	0.0818	0.1015	0.1059	0.1069	0.1226	0.1012	0.1349
120'	0.0747	0.1053	0.0796	0.1179	0.1020	0.1174	0.1259	0.1078	0.1503
150'	0.0894	0.1119	0.0916	0.1201	0.1097	0.1267	0.1346	0.1127	0.1487

TABLE 2. Membrane diffusion examinations in artificial intestinal juice (mg/100 mL)

		Physical mixtures				Kneaded products			
Time	**Lev**	2:1	1:1	1:2	1:3	2:1	1:1	1:2	1:3
30'	0.1168	0.0905	0.1300	0.1695	0.1185	0.1168	0.1284	0.1531	0.1432
60'	0.1234	0.1020	0.1201	0.1695	0.1331	0.1284	0.1333	0.1744	0.1794
90'	0.1152	0.1119	0.1366	0.1794	0.1421	0.1514	0.1580	0.1827	0.2041
120'	0.1168	0.1201	0.1333	0.1810	0.1399	0.1547	0.1563	0.1893	0.1991
150'	0.1366	0.1185	0.1300	0.1909	0.1564	0.1563	0.1432	0.1909	0.2041

Summarizing: most of the products showed a slight increase in diffusion as compared to **Lev**. There was no significant difference between the different compositions. The amount of **Lev** that diffused from the kneaded product was higher.

3.4. X-Ray investigations

Inclusion complex formation was investigated by X-ray techniques. As can be seen in **Fig. 1, Lev** and **γ-CD** have crystalline structures, while their 1:3 kneaded product is amorphous: the spectrum contains no characteristic peaks of **Lev** or **γ-CD**. The change in this spectrum during 1 month is not important.

3.5. ^{1}H NMR investigation

Comparison of the ^{1}H NMR spectra of norgestrel and its complex formed with CD revealed the following changes:

- the chemical shift of the olefinic proton at position 4 moved from 5.83 ppm to 5.97 ppm;

- the chemical shift of the ethinyl proton at position 17 showed a paramagnetic shift from 2.59 ppm to 3.11 ppm;
- the chemical shifts of the angular ethyl group at position 13 exhibited a paramagnetic shift.

From the paramagnetic shifts summarized above, it can be seen that, during the complexation, a H-bond is formed between the hydroxy groups of **CD** and the 3-oxo and 17-OH groups of **Lev**. The paramagnetic shielding effect of the oxygens of **CD** is best seen on the neighbouring protons, e.g. at positions 4 and 17 (**Fig. 2**).

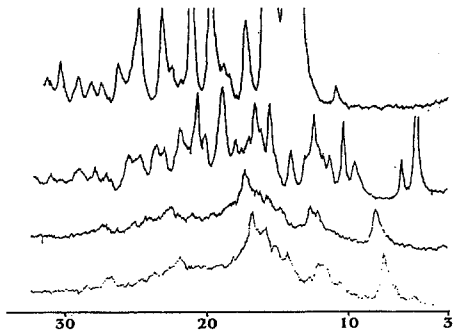

Fig. 1. X-ray spectra of **Lev**, **γ-CD**, 1:3 kneaded product

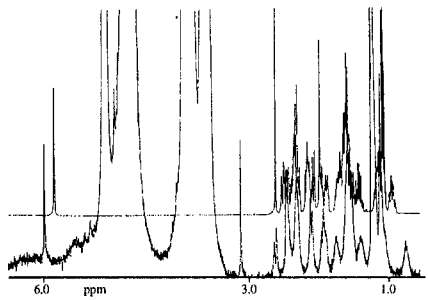

Fig. 2. ^{1}H NMR spectra of **Lev** and 1:3 kneaded product

4. CONCLUSIONS

- **CD** derivatives increase the solubility of **Lev**; the best solubility increase was found for γ-CD.
- Dissolution of the active agent increases on increase of the **CD** content; the best dissolution results were obtained for the 1:3 **Lev**:**γ-CD** composition.
- The dissolution rate is more than 20 times for the physical mixture, and more than 33 times for the kneaded product.
- Influence of the preparation method: kneading better increases the dissolution properties.
- The **CD**s slightly infuence the diffusion rate of **Lev**; the increase is not significant.
- The X-ray and NMR spectra of the products confirmed the solubility and diffusion results and showed the presence of complex formation.

The use of **CD**s affords possibilities of increasing the solubility characteristics of **Lev**, and by this means the quantity of drug in the dosage forms may be decreased.

References

[1] USP XXIII and NF XVIII. US Pharmacopeial Convention, Inc., 1994
[2] Szejtli, J., Cyclodextrin Technology, Kluwer Academic Publishers, Dordrecht, 1988