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Formation, bioavailability and organoleptic properties of an inclusion complex of femoxetine with β-cyclodextrin

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Summary

Femoxetine, a new selective serotonin uptake inhibitor with antidepressant properties, possesses a very bitter taste which hinders the development of oral liquid formulations. The present study has shown that it is possible to improve the organoleptic properties by inclusion complexation of the drug with β-cyclodextrin. Phase solubility diagrams and UV-spectroscopic studies revealed the formation of 1 : 1 and 1 : 2 inclusion complexes, the strongest complex being obtained for femoxetine in free-base form. A microcrystalline inclusion complex was isolated and shown to have the stoichiometric composition of 1 : 2 (femoxetine : β-cyclodextrin). In a single dose cross-over study in 5 volunteers, the bioavailability of the solid complex formulated as an aqueous suspension was found to be similar to that observed for a sugar-coated tablet of femoxetine hydrochloride.

Introduction

Femoxetine, trans-(+)-3-(4-methoxyphenoxy)methyl-1-methyl-4-phenylpiperidine (Fig. 1), is a selective serotonin uptake inhibitor with antidepressant properties (Ghose et al., 1977; Børup et al., 1979; Reebye et al., 1982; Tamminen et al., 1982). The compound is used as the water-soluble hydrochloride salt and the recommended therapeutic dosage in treatment of endogenous depression is 600 mg per day. The drug is almost completely absorbed upon oral administration of capsules, plain tablets or enteric coated tablets (Lund et al., 1979; Mengel et al., 1983). A desire to

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develop an oral liquid formulation of the compound was, however, seriously hindered by its very bitter taste.

The present study was undertaken to obtain a liquid formulation of femoxetine with acceptable organoleptic properties through inclusion complexation with β-cyclodextrin. In recent years, cyclodextrin complexations have been widely studied and shown to be useful in the pharmaceutical formulation to improve e.g. the aqueous solubility, chemical and physical stability or bioavailability (for reviews, see Saenger, 1980; Uekama, 1981; Szejtli, 1982). However, no previous study has apparently been devoted to improve the organoleptic properties of drugs through inclusion complexation. Since femoxetine was found to form a rather strong complex with β-cyclodextrin it may, therefore, be a good candidate drug for investigating this potential application of inclusion complexation.

Materials and Methods

Materials

Femoxetine hydrochloride and femoxetine free-base were synthesized by Ferrosan Research Division, Copenhagen, while β-cyclodextrin (β-CyD) was obtained from Dumex, Copenhagen. All other chemicals used were of analytical grade.

Apparatus

UV-spectra were recorded using a Shimadzu UV-190 spectrophotometer and 1-cm cuvettes. Measurements of pH were done at the temperature of study using a Radiometer Type PHM 26 instrument. Differential scanning calorimetry (DSC) was done with a Perkin-Elmer DSC-1 instrument using a low temperature cell and acetone-dry ice as coolant. The sample size was 5–10 mg and the scanning rate $8° \cdot min^{-1}$

Solubility studies

Phase-solubility measurements were carried out according to the method described by Higuchi and Connors (1965). Excess amounts of femoxetine hydrochloride (13 mg) were accurately weighed into each of several screw-capped vials and 10 ml of aqueous 0.05 M carbonate buffer (pH 10.5) solutions containing various amounts of β-CyD were added. The vials were rotated on a mechanical spindle at room temperature (22°C) for 6 days, which was sufficient to ensure solubility

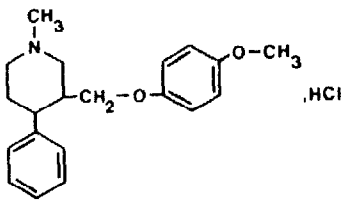

Fig. 1. Structural formula of femoxetine hydrochloride.

equilibrium. Following equilibration the contents of the vials were filtered. Aliquot portions of the filtrates were properly diluted with ethanol–water (1 : 1 v/v) and analyzed spectrophotometrically at 287 nm for total content of femoxetine. It was confirmed that Beer's law was obeyed and that no interference was produced from the cyclodextrin. A similar study was carried out using an isolated complex instead of the drug.

Spectroscopic studies

Complex formation between femoxetine hydrochloride and β-CyD was studied by UV-spectroscopy. Aqueous solutions containing about 3×10^{-4} M femoxetine hydrochloride and β-CyD in various concentrations up to 10^{-2} M were made and the UV-spectra were recorded using water as reference.

Preparation of a solid complex

A solid complex of femoxetine and β-CyD was prepared using conditions derived from Fig. 2: a mixture of 1 g of femoxetine hydrochloride and 1 litre of a 1.3×10^{-2} M β-CyD solution in 0.05 M carbonate buffer (pH 10.5) was stirred for one week at room temperature. The complex, which precipitated as a microcrystalline powder, was filtered, washed with water and dried in vacuo over phosphorous pentoxide for 24 h. The yield was 87%.

Composition of femoxetine suspension

The suspension used in the bioavailability study was formulated as follows: an amount of the complex of femoxetine with β-CyD corresponding to 1.75 g of femoxetine hydrochloride was suspended in 100 ml of an aqueous solution of 0.45 g sodium chloride, 0.12 saccharin sodium, 40 g sorbitol and 1 g β-CyD.

Bioavailability study

Volunteers

This study, in which 5 subjects were given a tablet and a liquid formulation, was part of another bioavailability study including comparison of different tablet formulations in 12 volunteers. After giving informed consent, 5 volunteers (Table 1) participated in the study, which was carried out in accordance with the Helsinki declaration II (1976). None of the volunteers were under regular treatment with or had taken any other drug (except oral anticonception) during the study.

Before entering the study it was checked by routine methods that the following variables were within the normal ranges: ECG, S-aspartate aminotransferase, P-bilirubin, P-creatinine, B-haemoglobin, B-leucocytes, and erythrocyte counts, U-albumin and U-glucose.

Dosing

The study was a single dose, cross-over study. Each volunteer was given the doses with an interval of at least 2 weeks between each administration. After having fasted overnight the volunteers were given the dose with 200 ml of water and a light,

standardized breakfast (one roll and a cup of tea or coffee). No other food was allowed within the next 2 h.

Blood sampling

Blood samples were drawn before and 1, 2, 4, 6, 8, 10, 14, 24, 48 and 72 h after intake of dose. Plasma was prepared within an hour and stored frozen until determination of femoxetine and norfemoxetine was performed as described by Bechgaard et al. (1977) and Bechgaard et al. (1983), respectively.

Formulations

One dose of each of the following formulations was given: (a) 30.0 g of a suspension of the femoxetine-β-cyclodextrin complex (see above) corresponding to 525 mg femoxetine hydrochloride; and (b) 6 sugar-coated tablets of 100 mg femoxetine hydrochloride.

Side-effects

After each administration the volunteers were asked to report side-effects, if any, together with the time of occurrence, the duration and degree.

Calculations

The area under the plasma concentration–time curves (AUC) was calculated using the trapezoidal rule. The elimination half-life, $t_{1/2(\beta)}$, was estimated graphically from the terminal phase of the plasma concentration curve.

Comparison was done using Wilcoxon test for paired samples.

Results and Discussion

Inclusion complexation in solution

The phase-solubility diagram obtained for femoxetine hydrochloride with β-CyD in 0.05 M aqueous carbonate buffer solution at pH 10.5 is shown in Fig. 2. According to Higuchi and Connors (1965), the diagram can be classified as type B_S with a microcrystalline complex precipitating from the solutions at the higher β-CyD

TABLE 1

VOLUNTEERS PARTICIPATING IN THE STUDY

Subject no.	Sex	Weight (kg)	Age (years)	Smoker
1	♀ *	63	25	yes
2	♂	82	22	no
5	♀	56	23	no
6	♂	75	25	no
11	♂	70	24	yes
Mean (± s.d.)		69 (± 10)	24 (± 1.3)	

* Using oral anticonception.

concentrations. The ascending linear part of a solubility diagram is generally ascribed to the formation of a 1 : 1 complex when the slope is less than 1. Assuming such a complex to be formed, the apparent formation constant can be determined from the initial straight line portion according to the following equation (Higuchi and Connors, 1965):

$$K = \frac{\text{slope}}{S_0(1 - \text{slope})} \tag{1}$$

where S_0 is the equilibrium solubility of the substrate in the absence of cyclodextrin ligand and thus equal to the intercept of the plot in Fig. 2. The value obtained for K was $5.0 \times 10^3\ M^{-1}$.

The diagram shows a plateau region before the descending part of the curve, making it possible, on the basis of the length of the plateau, to estimate the stoichiometry of the complexes precipitating from the solutions. In this case about 2.5×10^{-5} mole of the femoxetine base apparently complexed with 4.5×10^{-5} mole of β-CyD, indicating a 1 : 2 complex formation.

According to the phase rule of Gibbs, only one complex can precipitate at any given point of the descending part of the solubility curve though different complexes may appear at different points. If the reasonable assumption is made that the stoichiometry of the precipitating complex is 1 : 2 throughout the descending part of the curve the apparent formation constant $K_{1:2}$ for the complex can be calculated by Eqn. 2 (Higuchi and Connors, 1965):

$$K_{1:2} = \frac{S_B}{(S_X - S_B)(L_X - 2S_B)^2} \tag{2}$$

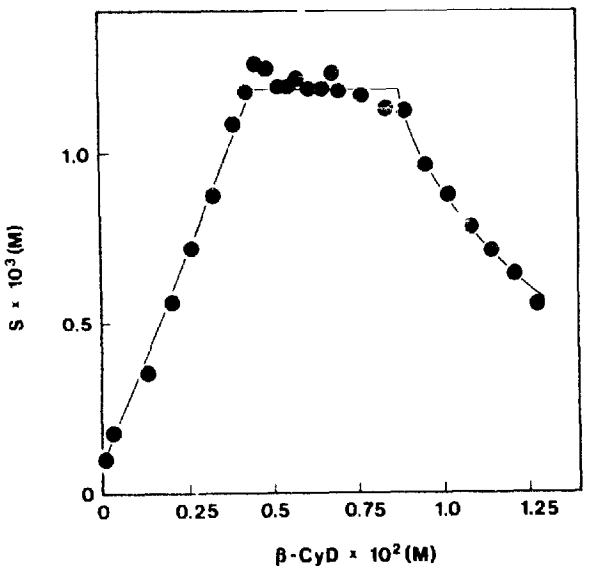

Fig. 2. Solubility (S) of femoxetine as a function of β-cyclodextrin concentration at 22°C in 0.05 M carbonate buffer solution of pH 10.5.

where S_B is the solubility of the 1:2 complex and S_X and L_X are the total concentrations of substrate and ligand, respectively, at any given point on the descending part. S_B was estimated to be about 2×10^{-4} M from a solubility study of the complex (Fig. 3). Using this value for S_B, $K_{1:2}$ was calculated to be 3.2×10^3 M^{-2}. The value can only be taken as an order of magnitude because of its small size.

The complexation of femoxetine cation was studied in aqueous solution by UV-spectroscopy. By addition of β-CyD to a solution of femoxetine hydrochloride the UV-spectrum showed a red shift. The absorbance changes observed were analyzed by Eqn. 3 (Benesi and Hildebrand, 1949) to determine the apparent $K_{1:1}$ stability constant:

$$\frac{1}{\Delta E} = \frac{1}{K_{1:1} \times S_t \times \Delta\varepsilon \times L} + \frac{1}{S_t \times \Delta\varepsilon} \tag{3}$$

where S_t is the total concentration of femoxetine hydrochloride, ΔE is the observed change in absorbance at 286 nm, $\Delta\varepsilon$ is the difference in molar absorptivity between the uncomplexed and complexed drug, and L is the concentration of free β-CyD. At sufficiently high concentrations of β-CyD L can be approximated to the total concentration, L_t, of β-CyD. As shown in Fig. 4, a plot of $1/\Delta E$ against $1/L_t$ is linear. From the ratio intercept/slope the stability constant was calculated to be 840 M^{-1}. It is interesting to note that although the complex constant for the femoxetine free-base is, as expected, larger than that for the ionized species, the value of the latter is quite impressive.

β-Cyclodextrin inclusion complexes are generally considered to be less soluble in water than β-CyD itself (Szejtli and Budai, 1979). However, the complex apparently formed with femoxetine hydrochloride showed an increased solubility. Thus, whereas β-CyD is soluble in water at room temperature to the extent of about 1.85% w/v, at least a 10% w/v solution could be obtained by adding femoxetine hydrochloride in equimolar amounts. It cannot be excluded, however, that this solubilizing effect is only due to complex formation.

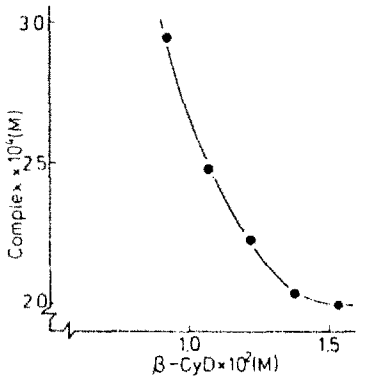

Fig. 3. Solubility (S) of the inclusion complex of femoxetine with β-cyclodextrin as a function of β-cyclodextrin concentration at 22°C in 0.05 M carbonate buffer solution of pH 10.5.

Inclusion complexation in the solid state

UV-spectrophotometric analysis of the solid complex isolated as described in the experimental section revealed a stoichiometry of exactly 1 mole of the drug to 2 moles of β-CyD. This stoichiometric composition is in agreement with that calculated above from the solubility-phase diagram. Differential scanning calorimetry further supported the existence of a true inclusion complex. As shown in Fig. 5 the thermograms of femoxetine and its physical mixture with β-CyD (the composition being 1 : 2 on a molar basis) show an endothermic peak at about 30 °C corresponding to the melting point of the femoxetine base. In contrast, this endothermic peak was not observable in the case of the assumed complex.

If 4.45 g of the solid complex corresponding to 600 mg femoxetine hydrochloride are suspended in 100 ml of diluted acid (i.e. conditions simulating those in the stomach) the complex will dissociate and transform to a 1 : 1 complex of protonated femoxetine, which is highly soluble as described above. Some of the β-CyD released through this complex transformation will precipitate. Knowing the $K_{1:1}$ value (840 M^{-1}) and the solubility of β-CyD (1.6×10^{-2} M^{-1}) it can be calculated that 93% of the drug will be dissolved in the complexed form and the rest as free drug. If the same amount were to be suspended in 200 ml instead of 100 ml 89% of the drug would be dissolved in complexed form. These calculations thus indicate that most of the drug given orally and in the form of a β-CyD complex will be present in the stomach as a soluble 1 : 1 complex of the protonated species.

Organoleptic properties

The bitter taste of femoxetine hydrochloride was greatly suppressed by inclusion complexation with β-CyD as revealed by testing in human volunteers. The taste of a suspension was further improved by including sorbitol and saccharin although the bitterness was not fully eliminated. Further improvement was achieved by addition of β-CyD to the suspension. The bitter taste is due to free femoxetine arising from dissociation of the complex in the suspension, and as seen from Fig. 3 the solubility of the complex can be depressed by adding free β-CyD.

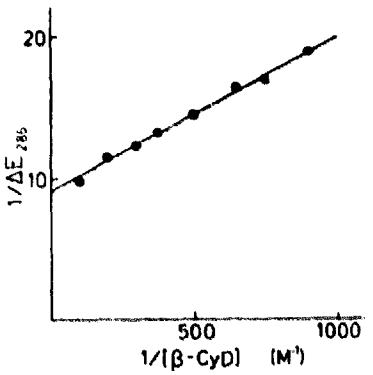

Fig. 4. Double-reciprocal plot of the spectral changes (at 286 nm) of femoxetine hydrochloride induced by complexation with β-cyclodextrin according to Eqn. 3.

Bioavailability

Two subjects (Nos. 1 and 11) vomited approximately 1 h after intake of the suspension. It was, however, assumed that the whole dose had been absorbed, since previous studies have shown, that the AUC was not diminished by vomiting 1 h or more after the administration of a solution of femoxetine hydrochloride. The results from the absorption study are given in Table 2. The AUC for the cyclodextrin complex varied from 23 to 96 $ng \cdot h \cdot kg \cdot ml^{-1} \cdot mg^{-1}$, while the corresponding values were 30 to 120 $ng \cdot h \cdot kg \cdot ml^{-1} \cdot mg^{-1}$ for the sugar-coated tablet. There was no significant difference ($P > 0.05$) between the AUCs for the two formulations. The rather large inter-individual variation was not diminished when the 'availability' of the active metabolite, norfemoxetine, was taken into consideration (cf. Table 2).

For both formulations the maximal plasma concentration (C_{max}) was reached within 1–4 h, and the variations in C_{max} were similar to those in AUCs. In several cases $t_{1/2(\beta)}$ could only be roughly estimated, but in all cases it seemed to be within the range (7–27 h) previously reported (Lund et al., 1979).

The plasma concentrations determined 2 h after administration were significantly correlated ($P < 0.001$) to the AUCs (Fig. 6). A similar correlation was found between C_{max} and AUC. This indicates that the magnitude of AUC, and hence the clearance of femoxetine, can be predicted after one (or few) blood samples taken after a single dose of the suspension or the sugar-coated tablets. Confirmation of this finding will, however, require more data.

In addition to the vomiting after the suspension, one subject (No. 5) reported

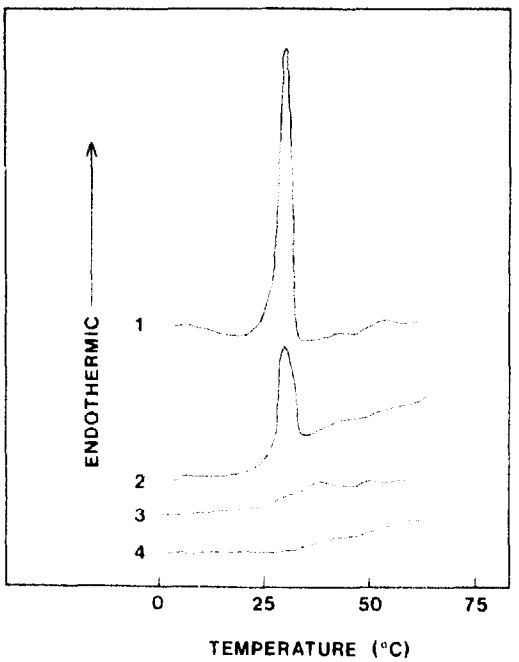

Fig. 5. Differential scanning calorimetry of femoxetine (1), a physical mixture of femoxetine and β-cyclodextrin (2), the inclusion complex of femoxetine with β-cyclodextrin (3) and β-cyclodextrin (4).

TABLE 2

RESULTS AFTER SINGLE ORAL DOSES OF FEMOXETINE HYDROCHLORIDE

Administration form		Subject no. 1	2	5	6	11	Mean (±S.D.)
Cyclodextrin complex (dose: 525 mg)	C_{max} [a] ($ng \cdot ml^{-1}$)	97	18	49	36	93	59 (±35)
	t_{max} (h)	1	2	4	4	2	2.6 –
	$\frac{AUC_{fem}}{dose}$ [b] ($ng \cdot h \cdot kg \cdot ml^{-1} \cdot mg^{-1}$)	96	23	60	35	92	61 (±33)
	$\frac{AUC_{norf}}{dose_{fem}}$ [c] ($ng \cdot h \cdot kg \cdot ml^{-1} \cdot mg^{-1}$)	19	0	17	11	38	17 (±14)
Sugar-coated tablet (dose: 600 mg)	C_{max} [a] ($ng \cdot ml^{-1}$)	130	30	112	42	27	68 (±49)
	t_{max} (h)	2	2	4	4	4	3 –
	$\frac{AUC_{fem}}{dose}$ [b] ($ng \cdot h \cdot kg \cdot ml^{-1} \cdot mg^{-1}$)	129	40	80	51	30	66 (±40)
	$\frac{AUC_{norf}}{dose_{fem}}$ [c] ($ng \cdot h \cdot kg \cdot ml^{-1} \cdot mg^{-1}$)	35	1	53	19	1	22 (±22)

[a] Not significantly different, $P > 0.05$.
[b] Not significantly different, $P > 0.05$.
[c] Not significantly different, $P > 0.05$.

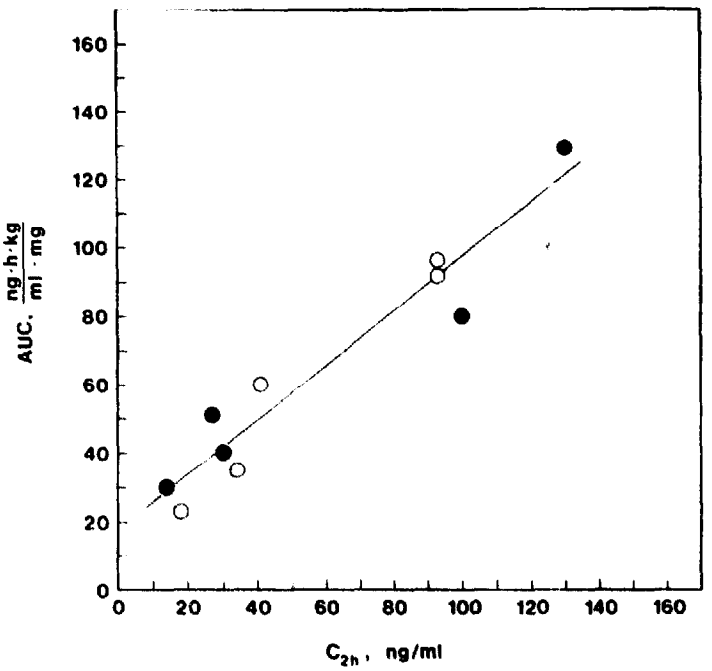

Fig. 6. Relation between the area under the plasma concentration curve (corrected for dose) and the plasma concentration 2 h (C_{2h}) after administration of 525 mg femoxetine as β-cyclodextrin complex (○) and 600 mg femoxetine hydrochloride as tablets (●) to 5 subjects.

nausea and was feeling uncomfortable 1–4 h after the administrations of both preparations.

In conclusion, the bioavailability of the β-CyD inclusion complex of femoxetine given as a suspension appears to be similar to that observed for the drug given as sugar-coated tablets.

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