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Inclusion complexation of furosemide in cyclodextrins

Part 2: Implication on bioavailability

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The effect of inclusion complexation of furosemide in cyclodextrins (CyD) on the bioavailability of the drug was studied on normal human volunteers. The excretion rate of the drug was determined by HPLC for a period of 24 h. post dosing. Several pharmacokinetic parameters were calculated and statistically analyzed. viz., peak excretion rate, peak excretion time, half peak time, elimination rate constant, area under the excretion rate time-curve as well as the total amount of drug excreted. The obtained results show that on administration of furosemide in the form of an inclusion complex in CyDs particularly β-CyD or its dimethyl derivative may improve its biological performance, taking into consideration that furosemide is characterized by a rapid onset and a short duration of action. Inclusion complexation of furosemide in CyDs leads to a more or less delay in its onset of action, a significant increase in its duration of action as well as significant augmentation in its overall biological availability.

1. Introduction

Inclusion of drug molecules in cyclodextrins (CyDs) affects many of their physicochemical properties and can result in increased aqueous solubility and stability [1, 2]. Such pharmaceutical formulations enhance dissolution rate [3–5], membrane permeability [6, 7] and, on the other hand, improve bioavailability [8–15] of slightly soluble drugs.

In the preceding communication [16], furosemide was shown to form more or less stable inclusion complexes with five cyclodextrins (CyDs). Complex formation improved both the solubility and dissolution characteristics of the drug. The objective of the present study was to investigate the implication of furosemide complexation with CyDs on the bioavailability of the drug. Because the drug induces diuresis, urinary furosemide excretion is the best correlate of activity [17, 18]. Hence, development of a new accurate and extremely sensitive HPLC method for determination of the drug in urine was necessary.

2. Investigations, results and discussion

The Fig. represents the change in excretion rate of furosemide after its oral administration as a plain drug or as an inclusion complex in CyDs as a function of time. It is evident that there exists a difference between the excretion profiles of the drug and those of the prepared complexes. The maximum excretion rate of the drug per se is 2 h post dosing; afterwards a sharp and marked decrease in the excretion rate takes place. The time to maximum values of excretion rate for the investigated complexes is also 2 h with the exception of the α- and γ-CyD complexes, whereby the time increases to 3 h. Furosemide γ-CyD complexes show a marked increase in the excretion rate compared to the drug; however, a distinct drop in the excretion rate was noticed after 3 h. The excretion rate profile with time for the β-CyD complexes shows a gradual and more or less uniform decrease in the excretion rate during the time period beyond 4 h post dosing up to 24 h. However, the excretion rate profile for the furosemide-dimethyl-β-cyclodextrin (DM-β-CyD) complex shows a re-

Table 1: Pharmacokinetic parameters of furosemide-cyclodextrin complexes in normal human subjects receiving a single oral dose

System	Peak excretion rate (mg h^{-1})	Peak excretion time (h)	Half peak time $t_{1/2p}$ (h)	Elimination rate constant k_{el} (h^{-1})	Half-life $t_{1/2}$ (h)	AUC_{0-24} (mg h^{-1} h)	Cumulative amount excreted over 24 h (mg)
Furosemide	1.68 ± 0.323 (0.9–2.68)	2.20 ± 0.20 (2–3)	2.42 ± 0.407 (1.1–3.5)	0.315 ± 0.065 (0.13–0.44)	2.92 ± 0.833 (1.57–5.46)	6.57 ± 0.908 (3.88–9.87)	5.76 ± 0.738 (3.73–7.80)
Furosemide-α-CyD complex	1.85 ± 0.316 (1.13–2.83)	2.0 ± 0.49 (1–3)	3.10 ± 0.565 (1.0–4.3)	0.325 ± 0.096 (0.15–0.74)	2.73 ± 0.554 (0.94–4.50)	8.19 ± 1.277 (4.07–12.02)	7.23 ± 1.132 (3.95–11.53)
Furosemide-β-CyD complex	2.27 ± 0.687 (0.93–4.86)	2.67 ± 0.365 (2–4)	4.3** ± 0.729 (1.6–6.0)	0.225 ± 0.044 (0.09–0.32)	3.93 ± 1.03 (2.17–7.62)	11.24* ± 2.814 (4.8–21.27)	9.94* ± 2.051 (4.42–19.45)
Furosemide-γ-CyD complex	2.76 ± 0.450 (1.33–4.25)	2.50 ± 0.245 (2–3)	2.47 ± 0.284 (2.0–3.7)	0.298 ± 0.062 (0.1–0.48)	2.98 ± 0.861 (1.45–6.66)	9.40 ± 1.172 (6.09–13.48)	8.52 ± 1.086 (5.57–9.81)
Furosemide-DM-β-CyD complex	2.95* ± 0.830 (0.81–5.10)	3.0** ± 0.490 (2–4)	3.03 ± 0.561 (2.0–4.4)	0.112** ± 0.023 (0.04–0.18)	7.78** ± 2.373 (3.77–17.77)	11.62* ± 2.938 (4.83–14.00)	10.27* ± 2.44 (4.62–19.06)
Furosemide-TM-β-CyD complex	1.53 ± 0.227 (0.91–2.43)	2.17 ± 0.337 (1–3)	3.23 ± 0.474 (1.8–4.3)	0.153* ± 0.013 (0.14–0.21)	4.61 ± 0.361 (3.27–5.78)	6.32 ± 0.716 (4.29–9.09)	5.87 ± 0.627 (3.87–8.19)

All data are the mean ±S.E of 6 experiments; ranges are shown in parentheses
* statistically significant ($P < 0.05$)
** statistically highly significant ($P < 0.01$)

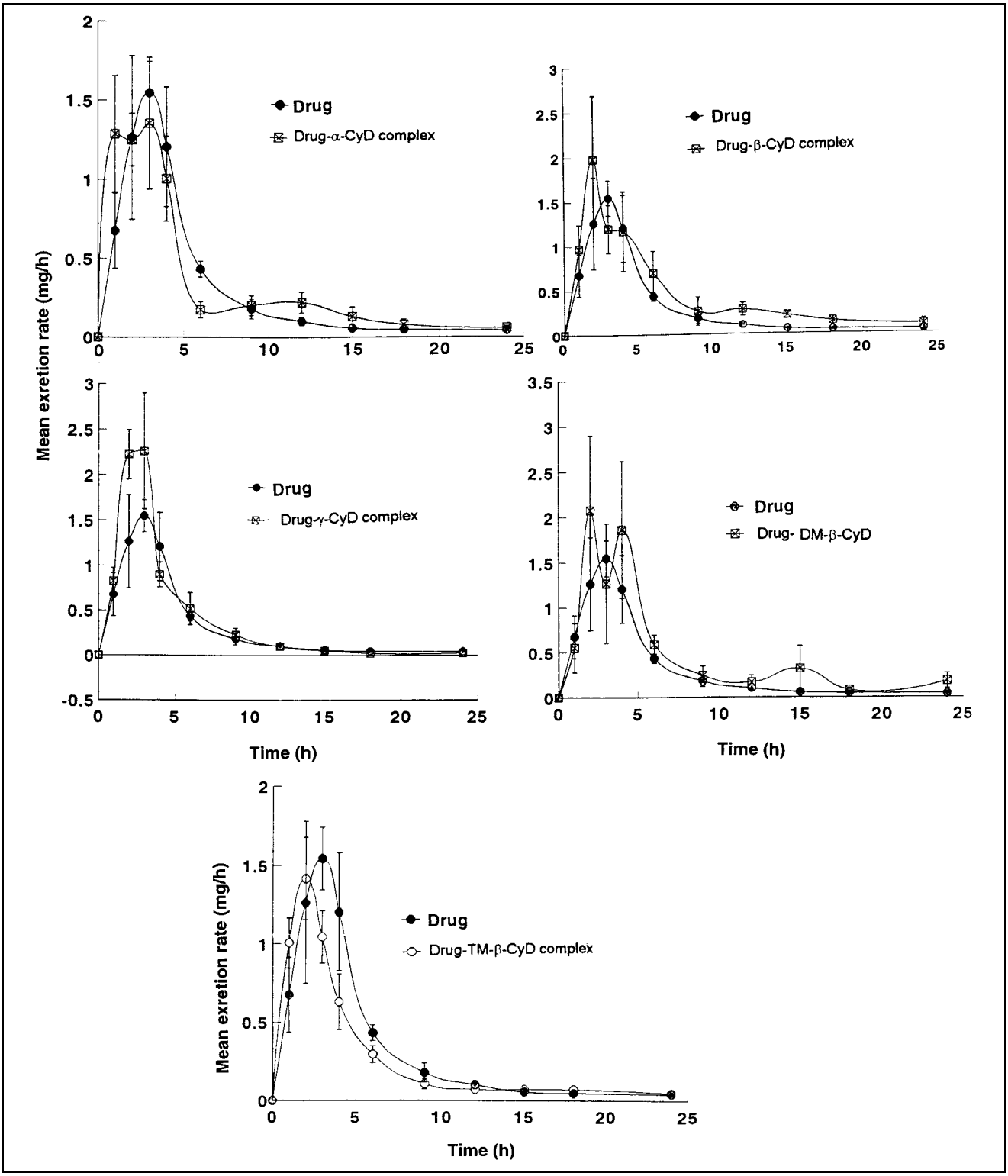

Fig.: Effect of cyclodextrins on the excretion rate of furosemide in human subjects (mean ± SE, n = 6)

newed increase in the excretion rate of the drug between 3 and 4 h as well as between 12 and 15 h after administration of the complex. This might be a result of further absorption of the drug in the distal parts of the small and the large intestine.

The pharmacokinetic parameters of the drug and its complexes appear in Table 1. Peak excretion rate of the drug increases with complexation in CyDs with the exception of trimethyl-β-cyclodextrin (TM-β-CyD). However, peak excretion time of the drug is more or less slightly affected by complexation, with the exception of DM-β-CyD, whereby the t_{max} increases. The $t_{1/2p}$-values for the investigated complexes, with the exception of furosemide-γ-CyD complexes, are generally higher than that of the drug per se. On the other hand, a marked increase in the $t_{1/2p}$-values is noticed for the furosemide-β-CyD complex, pointing to prolongation of drug action. The quotient R (R_Δ) [19] was calculated for all the investigated systems according to the equation

$$R_\Delta = \frac{t_{1/2p} \text{ of drug} - \text{CyD complex}}{t_{1/2p} \text{ of drug}}$$

Table 2: Effect of cyclodextrins on the duration of action of furosemide in normal human subjects receiving a single oral dose

system	R_Δ
Furosemide-α-CyD complex	1.28
Furosemide-β-CyD complex	1.88
Furosemide-γ-CyD complex	1.02
Furosemide-DM-β-CyD complex	1.25
Furosemide-TM-β-CyD complex	1.33

The data presented in Table 2 depict an increase in the duration of action of the drug via inclusion complexation in CyDs as revealed from the values of R_Δ, being higher than unity. This increase in the duration of action is more prominent for the furosemide-β-CyD complex whereby the R_Δ-value approaches 2.

The elimination rate constant (k_{el}) of the drug and its complexes was calculated from the slope of the straight line portion of the semilogarithmic graphical representation of the amount of drug remaining to be excreted ($X_U^\infty - X_U$) as a function of time and appear in Table 1. k_{el} of the drug decreases by complexation with CyDs. This is very evident for furosemide-DM-β-CyD complex, whereby its value decreases by more than 60%. This is reflected on a marked increase in the half-life time of the drug ($t_{1/2}$) for this system amounting more than 166%.

The values of AUC_{0-24h} (Table 1), which might be taken as a measure of bioavailability of the investigated systems would clearly depict augmentation of biological availability of the drug through inclusion complexation in CyDs. This augmentation is more pronounced for DM-β-CyD and β-CyD complexes; the increase amounts to about 77 and 71%, respectively. The cumulative amount of drug excreted during 24 h (Table 1) is generally higher for the investigated complexes compared to the drug itself. The highest cumulative amount excreted is associated with administration of complexes of the drug with DM-β-CyD and β-CyD. The percentage amounts of drug excreted relative to the administered amount are 49 and 48% for drug complexes with DM-β-CyD and β-CyD, respectively, compared to about 28% for the drug per se.

Statistical analysis of the above-mentioned data [20] reveals that the effect of DM-β-CyD on the biological performance of furosemide is generally significant. This effect is statistically highly significant on onset of action (peak excretion time), elimination rate constant (k_{el}) and half-life time ($t_{1/2}$), and statistically significant on the extent of absorption (peak excretion rate), as well as the biological availability of the drug (AUC_{0-24h} and total amount excreted). On the other hand, for β-CyD, the effect on duration of action of the drug ($t_{1/2p}$) is highly significant and the effect on the overall biological availability of the drug (AUC_{0-24h} and total amount excreted) is significant.

3. Experimental

3.1. Materials

Furosemide (Hoechst' Germany) α-CyD, β-CyD, γ-CyD, DM-β-CyD and TM-β-CyD (Sigma Chemical Co., USA). Acetonitrile and ethyl acetate (HPLC grade) were purchased from Merck, Darmstadt, Germany. All other chemicals were of analytical grade.

3.2. Methods

3.2.1. Preparation of complexes

Furosemide-CyD complexes were prepared by the kneading method [21]. Furosemide was kneaded like a paste with small amount of water to which CyD in equimolar concentration was then added without a solvent. The paste was ground in a mortar, then dried under vacuum in the presence of phosphorus pentoxide as a drying agent at room temperature.

3.2.2. Assessment of bioavailability

The bioavailability of furosemide and its CyD complexes was assessed in six healthy human volunteers. They had an average age of 28 years (range 25–35 years) and an average weight of 65 kg (range 55–100 kg). All subjects were instructed not to take any drugs for one week prior to and during the trial. After an overnight fast, each subject received a single dose (20 mg/70 kg b.w) of furosemide or the equivalent amount of its inclusion complex in CyD, packed in a gelatin capsule in a cross-over design system with 200 ml of water. Standard breakfast was offered 4 h after taking the drug. A washout period of at least 7 days was allowed between the trials.

3.2.2.1. Urine sample

Complete emptying of the bladder was ensured just before taking the drug and also each urine sample collection. Urine samples were collected at 1, 2, 3, 4, 6, 9, 12, 15, 18 and 24 h post dosing. The samples were analyzed for the drug by HPLC.

3.2.2.2. HPLC assay

Standard concentrations: A stock solution was prepared by dissolving 10 mg of furosemide in 100 ml acetonitrile. A series of standard concentrations were made by adding 5, 10, 25, 50, 100, 150, and 250 µl of the stock solution to 1 ml of a fasting human urine, and mixed thoroughly, to give the following standard solutions: 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, and 25.0 µg/ml.

Sample treatment: Each of standard and test urine samples were extracted with 3 ml ethyl acetate in a vortex mixer for 1 min, and phase separation was achieved by centrifugation. From each sample, 2.0 ml of organic layer was transferred to a test tube for removal of solvent by evaporation using a temperature-regulated sand bath. Each sample residue was reconstituted in 0.3 ml mobile phase (water/acetonitrile/acetic acid 58 : 42 : 1), then vortexed for 1 min and then injected into the HPLC-equipment (Waters 600E Multisolvent Delivery System, equipped with a Model U6K injector and 484 Waters tunable absorbance detector, Waters Assoc., Milford, MA, USA). The column used was Lichrosorb 5 RP-18 250 × 4.6 mm and 5 µm particle size (Phenomenex, USA) and a guard pak precolumn module with Bondapak C18 inserts (Waters Assoc., Milford, MA, USA). The flow rate was 1.5 ml/minute and the detector wavelength was at 272 nm. The column temperature was controlled at 40 °C by a temperature control module (TCM Waters Assoc. Milford, MA, USA) and the injection volume was 50 µl.

The calibration was carried out with prepared urine samples (range from 0.5–25 µg/ml). The percent recoveries were found to be 114,98 and 105%, for 1, 5 and 25 µg/ml, respectively. Linear least square regression line of the constructed standard curve was computed, using the Baseline 810 computer program (Waters Assoc. Milford, MA, USA), and the correlation coefficient was found to be 0.9965.

3.3.3. Pharmacokinetic analysis

Pharmacokinetic parameters of furosemide and its CyD complexes were determined from excretion rate-time data. The peak excretion rate, peak excretion time and the half peak time ($t_{1/2p}$) which is the time span during which the excretion rate of the drug is equal to or higher than half the peak excretion rate, were obtained directly from the excretion rate-time data. The area under the the excretion rate-time curve up to 24 h (AUC_{0-24h}) was determined using the linear trapezoidal rule [22]. The apparent elimination rate constant (k_{el}) was calculated by the technique of least squares regression from the data for the last three points of excretion rate-time curve or the cumulative amount of unchanged drug excreted in urine up to time infinity [23].

The elimination half-life time ($t_{1/2}$) was calculated according to the equation:

$$t_{1/2} = \ln 2 / k_{el}$$

All the pharmacokinetic parameters were analyzed statistically by the ANOVA "F" test [20].

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Pfefferminzöl/Kümmelöl-Fixkombination bei nicht-säurebedingter Dyspepsie-Vergleich der Wirksamkeit und Verträglichkeit zweier galenischer Zubereitungen

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In einer prospektiven, randomisierten, multizentrischen, referenzkontrollierten Doppelblindstudie wurden die Äquivalenz der Wirksamkeit und die Verträglichkeit zweier galenisch unterschiedlicher Fixkombinationen aus Pfefferminzöl/Kümmelöl bei 223 Patienten mit nicht-säurebedingter Dyspepsie (Dyspepsie vom Dysmotilitätstyp oder essentielle/idiopathische Dyspepsie, auch in Kombination mit Colon irritabile) untersucht. Prüfpräparat war eine Wirkstoffkombination in magensaftresistenter Kapsel mit 90 mg Pfefferminzöl und 50 mg Kümmelöl[3], Referenzpräparat eine Kombination in schnellfreisetzender Darreichungsform mit 36 mg Pfefferminzöl und 20 mg Kümmelöl[4]. Als Zielgröße wurde die „Differenz der Schmerzintensität zwischen Therapiebeginn und -ende (Tag 29)“ anhand einer visuellen Analogskala (0 = nicht vorhanden, 10 = extrem stark) ermittelt. Die Auswertung von 213 Patienten (n = 108 für das Prüfpräparat, n = 105 für das Referenzpräparat) ergab, ausgehend von einer mittleren Schmerzintensität bei Therapiebeginn von 6,1 Punkten in der Gruppe, die die magensaftresistente Zubereitung erhielt, und 5,9 Punkten in der Referenzgruppe, eine statistisch signifikante Abnahme in beiden Therapiegruppen (−3,6 bzw. −3,3 Punkte; $p < 0{,}001$; t-Test für verbundene Stichproben, zweiseitig). Entsprechend der Arbeitshypothese konnte eine äquivalente Wirksamkeit beider Zubereitungen dokumentiert werden ($p < 0{,}001$; t-Test auf Äquivalenz, einseitig). Auch bezüglich der Verbesserung der untersuchten Begleitvariablen waren beide Gruppen vergleichbar, bei der „Schmerzhäufigkeit“ erwies sich die magensaftresistente Zubereitung als signifikant besser wirksam ($p = 0{,}04$; t-Test auf Unterschied, zweiseitig). Die Verträglichkeit beider Präparate war gut. Bedingt durch die magensaftresistente Galenik trat die Nebenwirkung „Aufstoßen mit Pfefferminzgeschmack“ unter dem Prüfpräparat trotz der höheren Dosierung seltener auf.

Peppermint oil/caraway oil-fixed combination in non-ulcer dyspepsia
Equivalent efficacy of the drug combination in an enteric coated or enteric soluble formulation

223 patients with non-ulcer dyspepsia (dysmotility type dyspepsia or essential/idiopathic dyspepsia, also in combination with irritable bowel syndrome) were included in a prospective, randomised, reference- and double-blind controlled multicentre trial to compare two different preparations of a fixed combination of peppermint oil and caraway oil. The aim of the trial was to evaluate the equivalence of the efficacy and tolerability of these two preparations. The test formulation consisted of the drug combination in an enteric coated capsule containing 90 mg peppermint oil and 50 mg caraway oil[3], while an enteric soluble formulation containing 36 mg peppermint oil and 20 mg caraway oil[4] was used as the reference. The main target item defined was the “difference in pain intensity between the beginning and the end of therapy”, measured by the patient on a visual analogue scale (0 = no pain, 10 = extremely strong pain). In 213 patients (n = 108 on the test preparation, n = 105 on the reference preparation) with mean pain intensity baseline measurements of 6.1 points in the test preparation group and 5.9 points in the reference group a statistically significant decline in pain intensity was observed in the two groups (−3.6 resp. −3.3 points; $p < 0.001$; two-sided one-sample t-test). Equivalent efficacy of