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시클로덱스트린과 소염진통제간의 포접복합체에 관한 연구 (II): 2-히드록시프로필-β-시클로덱스트린이 이부프로펜 좌제의 방출에 미치는 영향

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Inclusion Complex of Analgesic and antiinflammatory Agents with Cyclodextrins (II) : Effect of 2-Hydroxypropyl-β-cyclodextrin on the Release of Ibuprofen Suppository

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Ibuprofen, a nonsteroidal antiinflammatory, analgesic and antipyretic drug, has several limitations in clinical application because of low solubility in water and gastrointestinal irritation. Effect of ibuprofen/2-hydroxypropyl-β-cyclodextrin (HPβCD) inclusion compound on release of suppository was investigated. Complex formation was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy. The release of ibuprofen from suppository base in vitro was significantly increased by the complexation with HPβCD. The release of ibuprofen from hydrophilic base was faster than that from hydrophobic base. In vivo studies, the release rate of ibuprofen from suppository was accelerated after rectal administration in complex form. This results suggested that ibuprofen/HPβCD complex can be practically used for suppository to have faster effect of ibuprofen with reduced side effect.

Keywords—Ibuprofen, Hydroxypropyl-β-cyclodextrin, Inclusion compound, Complexation, Suppository, Rectal absorption

Ibuprofen, (±)-2-(p-isobutylphenyl)propionic acid, is an efficient nonsteroidal antiinflammatory, analgesic and antipyretic agent. It is widely used for the treatment of rheumatoid arthritis, osteoarthritis and acute pain in musculoskeletal disorders. It is very important for antiinflammatory agents to elicit rapid therapeutic effect and to maintain effective serum concentration. Ibuprofen is very slightly soluble and poorly wettable in water. Generally, it also causes vomiting, nausea, gastric irritation, epigastric pain, heartburn, abdominal discomfort, rare dermathemia and hemophthalmia after oral administration.[1] For this reason, it has been studied the other formulations to enhance solubility, dissolution rate and bioavailability and to reduce the side effect due to overdose.

After it is revealed that cyclodextrin has an inclusion capability of hydrophobic molecules, there were a number of researches about the new formulation using cyclodextrin.[2-5] Cyclodextrin is a homologue of cyclic oligosaccharide derivatives, which are also known as Schardinger dextrin or as cycloamylose. The interior of the cavity is hydrophobic whereas the exterior is rather hydrophilic. By this particular conformation various kinds of guest molecules occupy the hydrophobic cavity in the

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cyclodextrin. These inclusion compounds have new pharmaceutical properties resulting in the improvement of solubility, stability and bioavailability.[2-5] Although cyclodextrin complex is water-soluble, β-cyclodextrin is sparingly soluble in water. The low solubility of β-cyclodextrin can be improved by chemical modification including alkylation and hydroxylation of β-cyclodextrin. These modification may disrupt the hydrogen bonding network and modify the crystalline β-cyclodextrin into amorphous mixture of isomers leading to increased solubility of such derivatives. In recent years hydroxypropyl-β- cyclodextrin (HPβCD) has been extensively applied in pharmaceutical formulation.[6-9] In a previous study[10], we also used HPβCD to improve dissolution rate and bioavailability of ibuprofen.

Although ibuprofen complex can reduce the side effect somewhat in oral administration, it cannot get rid of all adverse effect such as gastric irritation, nausea, and vomiting completely. Rectal administration is an example of other routes.[11-14] Suppository is a solid dosage form used for rectal or vaginal administration of therapeutic agents. The active ingredient is dispersed in an inert matrix generally composed of a rigid or semirigid base. As suppositories do offer a number of advantages such as to reduce hepatic first-pass elimination and to enhance drug bioavailability, they are recently becoming an important formulation for systemic delivery. A number of antiinflammatory agents as analgesics, antipyretics, sedatives and hypnotics are now being sold in suppository form.

In this study, we observed an effect of HPβCD on release of ibuprofen from suppository as a vehicle to overcome the poor aqueous solubility of drug. Complex formation was confirmed using nuclear magnetic resonance spectrometer. We examined the dependence of release on the base used and investigated the effect of complex on rectal absorption of ibuprofen in rat.

Experimental

Materials

Ibuprofen (Dong-A Pharm. Ind. Co., Korea), flurbiprofen (Whan-In Pharm. Ind. Co., Korea), heparin (Korea Green Cross Co., Korea) and witepsol H-15 (Han-Dok Pharm. Ind. Co., Korea) were used. HPβCD was purchased from Sigma Chemical Co. (U.S.A.). Polyethylene glycol (PEG) 1500 and 4000 were from Osaka Chemical Co. (Japan). All other chemicals were analytical reagent grade or HPLC grade.

Measurement of NMR Spectra

Ibuprofen was dissolved in pD 11 D_2O adjusted with NaOD and mixed with HPβCD solution. ^{1}H-NMR and ^{13}C-NMR spectra were determined at room temperature with a high-field NMR spectrometers (Varian unity 300). All chemical shifts were assigned relative to tetramethylsilane which was used as an external reference.

Preparation of Suppository

The inclusion complex was prepared at the 1 : 1 molar ratio of drug to HPβCD. Practically, 206.28 mg (1 mM) of ibuprofen and 1500 mg (1 mM) of HPβCD were dissolved in 5 m*l* of 5% ammonia water at room temperature. The solution was evaporated with rotary evaporator under reduced pressure at 40°C for 2 hr. The product was washed out with ethylether to remove the residual ibuprofen which did not participate in complexation. Physical mixture was prepared by simply blending ibuprofen and HPβCD in a mortar then transferring to a vacuum desiccator until ready for use. Suppositories were prepared by the fusion method. Witepsol H-15 and mixture of polyethylene glycol 1500 and 4000 were used as an example of hydrophobic and hydrophilic bases, respectively. Intact ibuprofen (30 mg/g base) or corresponding complex (248.1 mg/g base) was added to the oleaginous base melt at 50°C and water-soluble base melt at 60°C, respectively, and then thoroughly mixed. Each mixture was cooled to 30-35°C, dispersed and

promptly poured into aluminium suppository molds. After aging for at least 12 hours at 5°C, the moldings obtained were used in this study.

Measurement of the Apparent Partition Coefficient

The apparent partition coefficient of ibuprofen between aqueous phase and suppository base was determined. After shaking 5 m*l* of saline containing 5 mg ibuprofen or corresponding complex, the solution mixed with 5 m*l* of molten witepsol H-15 was stored in shaking incubator at 37°C for 20 minutes. The remaining amount of ibuprofen in the aqueous phase was analyzed by high performance liquid chromatography (HPLC). The partition coefficient was defined as the ratio of the equilibrium concentration of ibuprofen in the organic phase (C_o) to that in the aqueous phase (C_w).

$$\text{Partition Coefficient} = \frac{C_o}{C_w} = \frac{(C_t - C_w)}{C_w}$$

where C_t is the initial concentration of ibuprofen in aqueous phase.

***in vitro* Release of Ibuprofen from Suppository**

The release of ibuprofen from suppositories containing ibuprofen alone and its complex, respectively, were measured using a suppository release apparatus (Toyama Sangyo Co., Osaka, Japan) according to the method of Muranishi *et al.*[15] Each suppository was placed in the cylindrical chamber, which was lined on the inside with a Milipore membrane filter (3.0 μm) as a barrier for diffusion of the suppository base. The chamber was placed into a flask containing 300 m*l* of normal saline. The stirring rate of the release phase was set at 100 rpm and temperature was maintained at 37°C. The rotation rate of the steel rod in the suppository chamber was 25 rpm. At an appropriate time interval, 3 m*l* of sample were withdrawn from the receiver and assayed for ibuprofen by HPLC. The volume in the vessel was refilled with the equal volumes of saline solution after each sampling.

***in vivo* Absorption of Ibuprofen**

Male Sprague-Dawley rats weighing 130-150 g were obtained from Taehan Laboratory animal research center. Water and food (Jeil Co., Korea) were freely supplied for more than two weeks under a temperature-controlled environment (20-25°C). Rats weighing 230-280 g were fasted with free access to water for 48 hours prior to the experiment. Under light ether anesthesia, the femoral vein and artery were cannulated with polyethylene tubing (PEG 50, Clay Adams, N.J., U.S.A.) for drug administration and blood sampling, respectively. After animal awoke in about 20-30 minutes, suppositories containing ibuprofen or its complex were administrated at dose 4 mg/gk of rat body weight to each rat. After administration of suppository, the anus was closed with a glue to avoid the leak of melted suppository. During the experiment, the temperature of rats were maintained constantly. Blood samples were collected through the polyethylene tubing from the femoral artery at appropriate intervals and centrifuged immediately for 4 minutes at 12000 rpm. Plasma samples were frozen below -20°C until analyzed. The assay was performed within 3 days.

Analytical Procedure of Ibuprofen in Plasma

The extraction procedure of Albert *et al.*[16] for the determination of ibuprofen was used with minor modification. To 200 μ*l* of plasma, 0.1 m*l* of 2.5 μg/m*l* flurbiprofen (internal standard) and 0.1 m*l* of 1M HCl solution were added. The solution was vortex-mixed for 1 minute and extracted with 2 m*l* of a mixture of isooctane and isopropyl alcohol (85 : 15v/v%) by shaking vigorously for 10 minutes on a rotary shaker. After centrifugation at 2000 rpm for 10 min, organic layer was transferred into another tube, then evaporated to dryness under reduced pressure at 50°C. The samples were reconstituted using 500 μ*l* of mobile phase. Aliquots of 10 μ*l* were injected into the HPLC system. The linearity of concentration by HPLC was established from 0.5 to 20 μg/m*l* ($r>0.995$). We used mo-

ment analysis method for determination of pharmacokinetic parameters. For statistical evaluation, C_{max}, t_{max} and AUC were used. Statistical differences were assumed to be significant when $p<0.05$ (Student's t-test).

Results and Discussion

NMR Spectra of Inclusion Compound

In previous paper[10] we observed the characteristics of ibuprofen/HPβCD complex by thermal analysis, infrared spectroscopy and x-ray diffraction. In this study NMR spectroscopy was used to resolve the exact structure and geometry of inclusion compound. Figure 1 shows the ^1H NMR spectra of ibuprofen dissolved in D_2O in the absence and presence of HPβCD. In the presence of HPβCD, the signals of the aromatic protons (δ: 7.2-7.5) are shifted upfield, indicating greater shielding of the aromatic proton of ibuprofen when the aromatic ring is included inside the cyclodextrin ring.[17] The ^{13}C NMR spectra of ibuprofen and its complex were shown in Figure 2. In this figure aromatic carbon of ibuprofen (δ: 126-140) was shielded because of influence of cyclodextrin on the ring current of ibuprofen and aliphatic carbon (δ: 20, 43, 47) experienced the downfield shift due to diminished rotational freedom of movement.[18] From these results, we could suggest that the aromatic portion of ibuprofen occupied the cavity of HPβCD.[19]

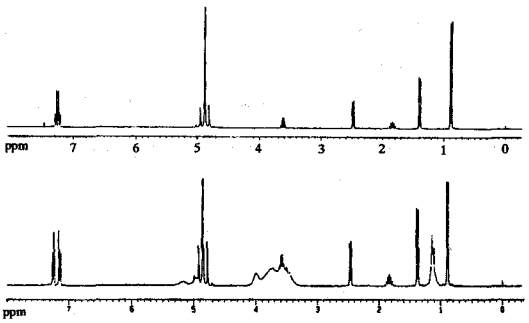

Figure 1—^1H-NMR spectra of ibuprofen in the absence of HPβCD (upper) and in the presence of HPβCD (down).

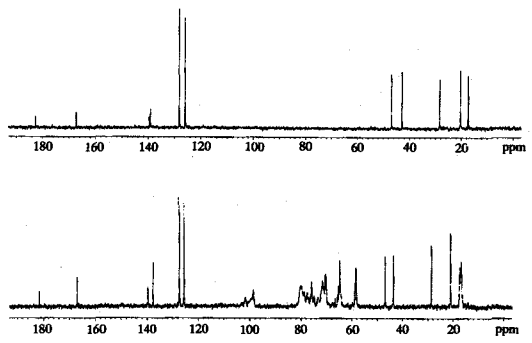

Figure 2—^{13}C-NMR spectra of ibuprofen in the absence of HPβCD (upper) and in the presence of HPβCD (down).

Measurements of the apparent partition coefficient

The apparent partition coefficient of ibuprofen between the witepsol H-15 and saline was 4.5 and the corresponding value of complex was 2.0. The decreased partition coefficient of complex indicates the hydrophilicity of HPβCD comparing to ibuprofen alone. Dollo *et al.*[20] observed the transfer rate of drug between aqueous and organic phases. They found that a decrease in transfer rates to organic phase in the case of complex was observed showing that cyclodextrins were able to retain drug in the aqueous phase. We can also infer that the release rate of complex from witepsol suppository is faster than that of ibuprofen alone and complex can be transferred easily into rectal fluid because of its hydrophilic property.

in vitro Release

The release profiles of ibuprofen alone, inclusion complexes and physical mixture from the witepsol H-15 base suppositories were shown in Figure 3. It is evident that the release of ibuprofen from suppository was remarkably enhanced by the complexation with HPβCD. The enhanced release rate is probably due to increased solubility, decreased crystallinity and improved wettability of inclusion compound in suppositories.[5,10] The release of drug from suppository is known to be influenced by various factors: drug-vehicle interaction, vehicle com-

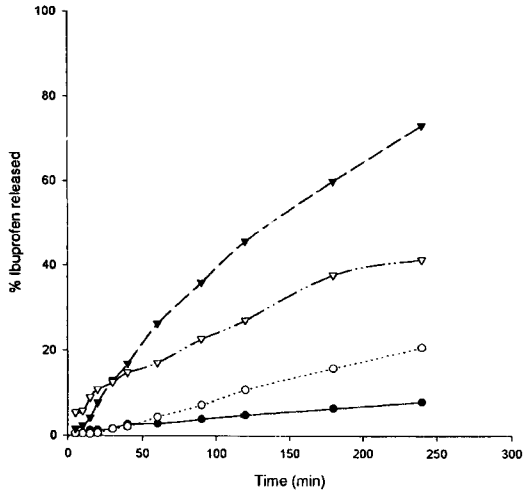

Figure 3—Release profiles of ibuprofen from witepsol H-15 base suppository. Key: ●; ibuprofen, ○; physical mixture, ▽; β-cyclodextrin complex, ▼; 2-hydroxypropyl-β-cyclodextrin complex

position, solubility, partition coefficient and particle size of drug in vehicle.[12-15] Among these factors, the formation of inclusion compound contributes to increase the solubility. Suppository prepared with physical mixture of ibuprofen and HPβCD was shown faster release than ibuprofen alone, and this increase was the cause of ease wettability of HPβCD. While β-cyclodextrin was slightly soluble, the inclusion compound of β-cyclodextrin showd enhanced release of ibuprofen from suppository. Figure 4 shows the release profile of ibuprofen from PEG base suppository. Because of high hydrophilicity of PEG the release of ibuprofen was fast in case of complex and physical mixture of ibuprofen and HPβCD. Ibuprofen suppository without cyclodextrin shows slow release profile. The release from PEG base was faster and larger than that from the witepsol H-15 base. The small release of ibuprofen from witepsol H-15 base suppository may be due to its highly hydrophobic property which limits the contact with water on the contrary to PEG.

***in vivo* Absorption**

Figure 5 shows the blood concentration after rectal administration of ibuprofen alone and its complex. According to the concentration-time curves, we obtained the pharmacokinetic parameters using moment program analysis method. Some pharmacokinetic parameters are shown in Table I. The areas under the plasma concentration-time curves and the peak plasma concentration following the administration of

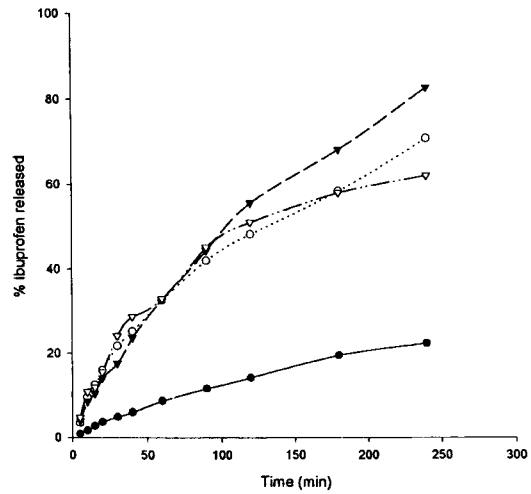

Figure 4—Release profiles of ibuprofen from polyethylene glycol base suppository. Key: ●; ibuprofen, ○; physical mixture, ▽; β-cyclodextrin complex, ▼; 2-hydroxypropyl-β-cyclodextrin complex

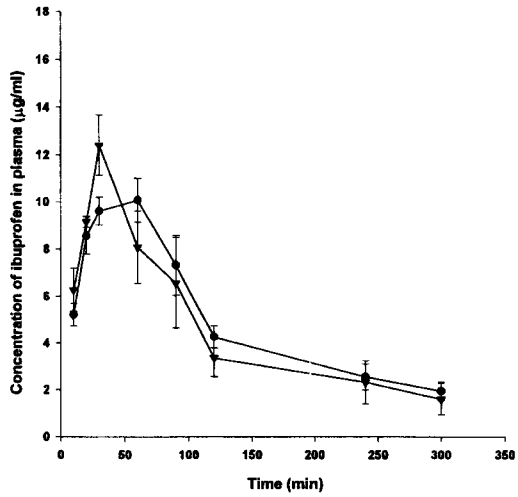

Figure 5—Mean plasma concentration of ibuprofen after rectal administration of ibuprofen or complex. Key: ●; ibuprofen, ▼; 2-hydroxypropyl-β-cyclodextrin complex

Table I—*Pharmacokinetic Parameters Obtained from Plasma Concentration Curves of Ibuprofen Suppositories*

	AUC[a]	C_{max}(μg/ml)[b]	t_{max}(min)
Ibuprofen	1595±140	11.2±0.5	60
Complex	1563±267	12.4±1.3	30

[a] AUC means the area under curve obtained by administration of suppository containing ibuprofen or complex.
Non-significantly different between two values ($p > 0.05$).
Data are expressed as mean±S.E. (n=3).

[b] C_{max} means the maximum plasma concentration obtained by administration of suppository containing ibuprofen or complex.
Non-significantly different between two values ($p > 0.05$).

[c] t_{max} means the time to reach Cmax obtained by administration of suppository containing ibuprofen or complex.

ibuprofen and the complex form was not significantly different. Comparing the ibuprofen alone and complex suppositories, time to reach maximum concentration t_{max} in complex suppository was shorter than that of ibuprofen alone. This means that ibuprofen suppository prepared with complex was faster absorbed than that of ibuprofen alone probably due to the fast dissolution rate of the complex. From these results, it can be stated that HPβCD can be used as a drug carrier because it provides a high release rate of suppository.

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References

1) P.A. Insel, *Analgesic-antipyretics and antiinflammatory agents*: In *Goodman & Gilman's the Pharmacological Basis of Therapeutics*, A.G. Gilman, T.W. Rall, A.S. Nies and P.Taylor (Eds.), McGraw Hill, Inc., New York, U.S.A., pp. 664-668 (1991).
2) Y. Hamada, N. Nambu and T. Nagai, Interactions of α- and β-cyclodextrin with several non-steroidal antiinflammatory drugs in aqueous solution, *Chem. Pharm. Bull.*, **23**, 1205-1211 (1975).
3) K. Ikeda, K. Vekama, and M. Otogiri, Inclusion complexes of β-cyclodextrin with antiinflammatory drugs fenamates in aqueous solutions, *Chem. Pharm. Bull.*, **23**, 201-208 (1975).
4) M.M. Sanghavi, K.B. Choudhari, R.S. Matharu and L.Viswanathan, Inclusion complexation of lorazepam with β-cyclodextrin, *Drug Dev. Ind. Pharm.*, **19**, 701-712 (1993).
5) D.D. Chow and A.H. Karara, Characterization, dissolution and bioavailability in rats of ibuprofen-β-cyclodextrin complex system, *Int. J. Pharm.*, **28**, 95-101 (1986).
6) T. Cserhati and J. Hollo, Interaction of taxol and other anticancer drugs with hydroxypropyl-β-cyclodextrin, *Int. J. Pharm.*, **108**, 69-75 (1994).
7) C. Chen, F. Chen, A. Wu, H. Hsu, J. Kang and H. Cheng, Effect of hydroxypropyl-β-cyclodextrin on the solubility, photostability and *in vitro* permeability of alkannin/shikonin enantiomers, *Int. J. Pharm.*, **141**, 171-178 (1996).
8) T. Cserhati, E. Forgacs and J. Szejtli, Inclusion complex formation of antisense nucleotides with hydroxypropyl-β-cyclodextrin, *Int. J. Pharm.*, **141**, 1-7 (1996).
9) M.A. Vandeli, G. Salvioli, A. Mucci, R. Panini, L. Malmusi and F. Forni, 2-Hydroxypropyl-β-cyclodextrin complexation with ursodeoxycholic acid, *Int. J. Pharm.*, **118**, 77-83 (1995).
10) I.J. Oh, J.G. Park, Y.B. Lee and S.C. Shin, Inclusion complex of analgesic and antiinflammatory agents with cyclodextrin (I): Enhancement of dissolution of ibuprofen by 2-hydroxypropyl-β-cyclodextrin, *J. Kor. pharm. Sci.*, **23**, 11-18 (1993).
11) T. Kondo, T. Irie and K. Uekama, Combination effects of α-cyclodextrin and xanthan gum on rectal absorption and metabolism of morphine from hollow-type suppositories in rabbit, *Biol. Pharm. Bull.*, **19**, 280-286 (1996).
12) A. Hidetoshi and K. Takashi, Use of water-soluble β-cyclodextrin derivatives as carriers of antiinflammatory drugs biphenylacetic acid in rectal delivery, *Yakugaky Zasshi*, **112**, 65-72 (1992).
13) H.R. Jeon, D.W. Park, S.M. Lee, H.W. Yi and Y.W. Choi, Effects of suppository bases and additives on rectal absorption of ibupro-

fen lysinate, *J. Kor. Pharm. Sci.,* **24**, 145-153 (1994).
14) S.J. Hwang, S.B. Park and G.J. Rhee, A comparative study on the pharmaceutical properties of rectal suppository containing omeprazole complexes, *J. Kor. Pharm. Sci.,* **25**, 227-237 (1995).
15) S. Muranishi, Y. Okubo and H. Sezaki, Manufacture and examination of apparatus for drug release from suppositories, *Yakuzaigaku,* **39**, 1-7 (1979).
16) K.S. Albert, W.R. Gillespie, A. Raabe and M. Garry, Determination of flurbiprofen in human serum by reverse phase high performance liquid chromatography with fluorescence detection, *J. Pharm. Sci.,* **73**, 1823-1825 (1984).
17) A.K. Roy and J.K. Guillory, The effect of cyclodextrins on the aqueous stability of cyclopentolate hydrochloride, *Int. J. Pharm.,* **138**, 37-43 (1996).
18) G. Bettinetti, F. Melani, P. Mura, R. Monnanni and F. Giordano, Carbon-13 nuclear magnetic resonance study of naproxen interaction with cyclodextrin in solution, *J. Pharm. Sci.,* **80**, 1162-1169 (1991).
19) I.J. Oh, M.Y. Lee, Y.B. Lee, S.C. Shin and I. Y. Park, Structure and geometry of ibuprofen/2-hydroxypropyl-β-cyclodextrin inclusion compound, *in preparation of manuscript.*
20) G. Dollo, P.L. Corre, F. Chevanne and R.L. Verge, Inclusion complexation of amide-typed local anaesthetics with β-cyclodextrin and its derivatives. II. Evaluation of affinity constants and in vitro transfer rate constants, *Int. J. Pharm.,* **136**, 165-174 (1996).