약제학회지(1997), 제27권 제 1 호
J. Kor. Pharm. Sci., Vol. 27, No. 1, 11-14(1997)

Comparison of Diclofenac Sodium and Diclofenac Sodium-β-cyclodextrin Complexation on Gastric Mucosal Injury in Rats

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(Received December 12, 1996)

디클로페낙나트륨 및 디클로페낙나트륨과 β-시클로덱스트린 포접물의 흰쥐 위 점막 손상 비교

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(1996년 12월 13일 접수)

This laboratory has recently reported the solubility and in vivo absorption enhancement of diclofenac sodium by β-cyclodextrin complexation. The acute gastroduodenal mucosa injury provoked by administration of 34 mg/kg and 68 mg/kg of a diclofenac sodium (DS) and equivalent dose of new formulation [diclofenac sodium-beta-cyclodextrin complexation(DS-β-CD)] was evaluated and compared. Microscopic examinations, performed after 18-hrs treatment, demonstrated that DS-β-CD was less gastrolesive than DS. The drop in gastrophy after a single dose of the assigned drug was considerably greater for DS than for DS-β-CD, which registered similar values to control. Since gastrophy is an expression of the anatomy-functional integrity of the gastric barrier, the results indicate that DS-β-CD exerts less direct acute damage on the gastric mucosa. Therefore, when administered short-term, DS-β-CD appears to be less gastrolesive than the standard DS formulation.

Keywords—Diclofenac sodium, β-cyclodextrin, Absorption, Complexation, Mucosal injury

2-[(2,6-Dichlorophenyl)amino]phenylacetic acid mono sodium salt(diclofenac sodium) is a non-steroidal antiinflammatory drug(NSAID) used in the treatment of rheumatic diseases. In clinical studies involving patients with various rheumatic disorders, diclofenac sodium has been found to exert a therapeutic effect similar to or better than that of indomethacin, acetylsalicylic acid, phenylbutazone and ibuprofen. However, the major limitation to the use of the NSAIDs is their determinant effects on the mucosa of the gastrointestinal tract, particularly the stomach. This injury commonly referred to as 'NSAID gastrophy', represents the single most frequent adverse reaction to medication.[1] In another recent study, Graham et al[2] reported that hemorrhagic lesions of the gastric mucosa occurred in >20% of osteoarthritis patients taking NSAID. Despite extensive research, the pathogenesis of NSAID-induced damage to the gastric mucosa is still not fully understood. It is generally accepted that inhibition of prostaglandin synthesis is an important component of the ulcerogenic mechanism.[3] However, Ligumsky et al.[4] demonstrated that gastric prostaglandin synthesis in rats could be inhibited by up to 95% without the development of hemorrhagic erosions, suggesting that inhibition of prostaglandin synthesis was unlikely to be the sole mechanism for the ulceration. Furthermore,

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since the precise physiological role of prostaglandin in gastric mucosal defense is not completely understood.

The cyclodextrin molecule can be considered a capsule of molecular size that is able to form an inclusion complex with the molecule of another substance. Cyclodextrin complexation held together by physical forces instead of covalent bonds[5] allows the modification of the physical and chemical properties of a drug, and hence its pharmacokinetic and possibly pharmacodynamic properties.[6] Complexing a very poorly water soluble drug such as diclofenac sodium with β-cyclodextrin increases its rate of dissolution and solubility.[7]

In the present study we employed microscopic evaluation to compare acute gastric damage provoked by DS and equivalent dose of DS-β-CD complexation.

Materials and Methods

DS and DS-β-CD were kindly provided from Asia Pharmaceutical Ind. Co. (Korea) and formalin was obtained from Yakuri Pure Chemical (Japan).

Male Sprague-Dawely rats (180 to 220 gm) were fasted 48 hours before induction of the gastric lesions, but they were allowed free access to water. The test animals(10 in each group) were dosed by oral administration with 34 or 68 mg/kg DS and equivalent dose of DS-β-CD (1:3 molecular ratio) in two equal doses with 12 hours interval between the doses.8) A control group of 10 rats were given 1 ml of aqueous solution alone. After 18 hours the rats were killed by decapitation and their stomachs were removed and opened along the greater curvature and washed with a direct stream.

The specimens were fixed with 10% neutral formalin and processed through usual tissue preparation methods for microscopic examination. At least, 5 tissue blocks were obtained from each specimen. Severity of inflammatory reactions were graded by numbers of inflammatory cells to normal, mild (inflammatory cells less than 50/10 HPF; high power field), moderate (inflammatory cells between 50 and 100/10 HPF)and severe grades (inflammatory cells more than 100/10 HPF).

Results and Discussion

At dose of 68 or 34 mg/kg of DS or equivalent dose of DS-β-CD, the ulceration was compared and presented (Table I). DS at dose of 68 mg/kg produced grossly mucosal lesions which were found in 5 out of 10 specimens ranging from 1 to 4 after administration of DS. Microscopically the lesions showed hemorrhagic necrosis, mucosal erosion and inflammatory reaction (Figure 1a). Adjacent mucosa and submucosa were infiltrated mainly by acute inflammatory cells composed of neutrophils and eosinophils (Figure 1b). However, the treatment of equivalent amount of DS complexed with β-cyclodextrin showed that mucosal lesions were not detected grossly and microscopic findings were mild infiltration of inflammatory cells including lymphocytes and some eosinophils (Figure 1c).

Table I—*Mucosal Injury Expression After Administration of Normal, Diclofenc Sodium and Diclofenac Sodium-β-cyclodextrin Complexes*

Group	Mucosal erosion	Inflammation	Hemorrhage	Congestion
Group 1 (DS, : 68 mg/kg)	5/10	+++	++	++
Group 2 (DS-β-CD: 68 mg/kg)	0/10	+	+	+
Group 3 (DS, : 34 mg/kg)	2/10	++	++	++
Group 4 (DS-β-CD: 34 mg/kg)	0/10	-	-	-
Control	0/10	-	-	-

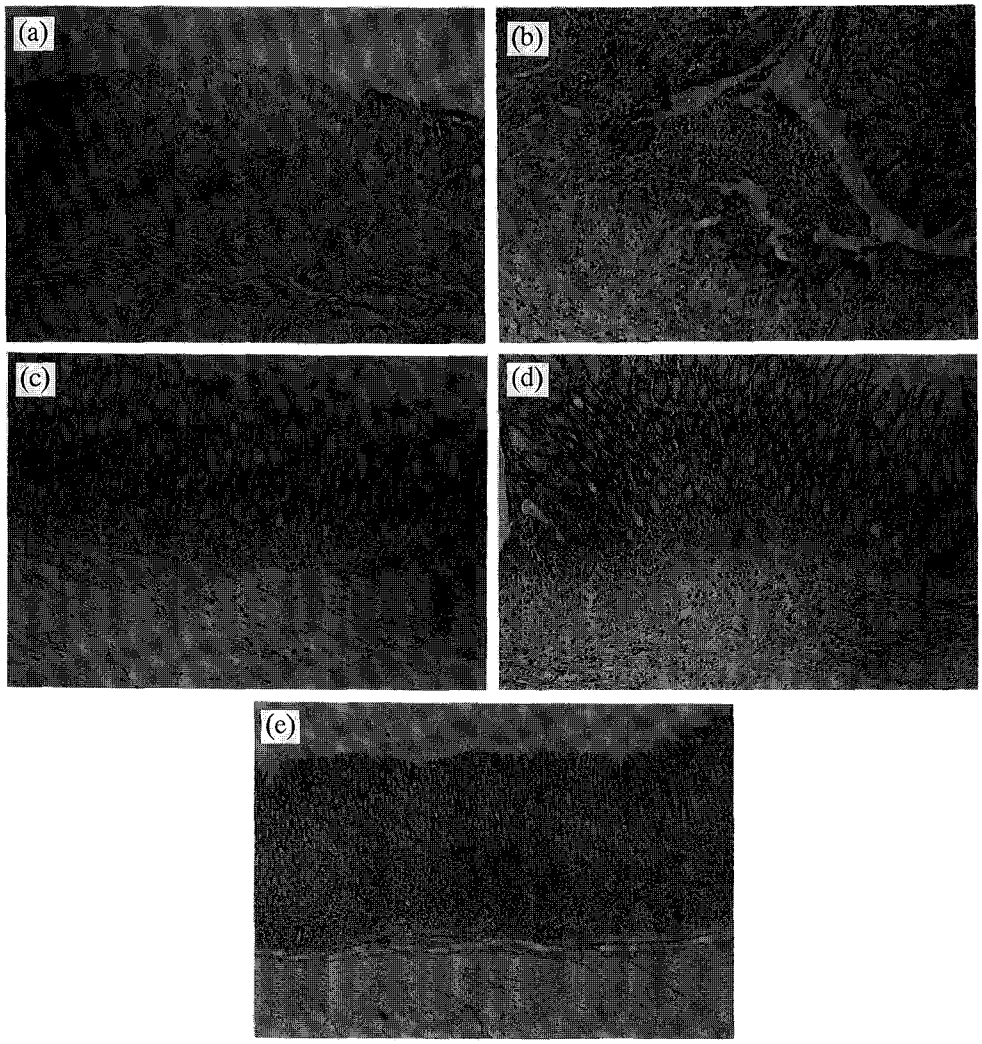

Figure 1—(a) Hemorrhagic necrosis of mucosa. (b) Heavy infiltration of acute inflammatory cells. (c) Mild infiltration of inflammatory cells in mucosa and submucosa. (d) Moderate infiltration of inflammatory cells. (e) Normal mucosa.

Compared with rats given 68 mg/kg DS or DS-β-CD, animals at dose of 34 mg/kg DS showed less mucosal injury than those of 68 mg/kg DS. Macroscopic mucosal lesions were found in 2 out of 10 specimens ranging from 1 to 2 (Table I). Microscopic findings were similar to group 1 except for less severity of inflammatory reaction (Figure 1d), whereas group 4 and control group did not show significant pathologic changes of mucosa (Figure 1e).

The present data show that DS-β-CD complex provokes fewer acute ulceration lesions than standard formulation in rats. The percentage of rats with ulcers was increased as the dose of the drug was increased. β-cyclodextrin is an oligosaccharide with a molecular weight of 1155 derived from the digestion of starch by *Bacillus Macerans* amylase.[9] It appears to have no pharmacological effect and little, if any, intrinsic toxicity.[4] Because the internal milieu of cyclodextrin is hydrophobic and has the appropriate dimensions to accommodate the NSAID molecule, it easily forms inclusion complexes with these lipophilic drugs.[10]

Formulations of diclofenac sodium that limit the contact of the drug with the gastric mucosa may theoretically reduce the incidence of mucosal injury. The main effect of complexing diclofenac sodium molecule in the β-cyclodextrin is that, as it accelerate absorption of the drug, plasma levels of DS reach the peak earlier than the standard formulation.6) This is likely reduce the contact time of diclofenac sodium with the gastric mucosa.

In conclusion, our data indicate that DS-β-CD induces less acute gastric damage than DS sodium when given short-term, even although it provides no information on chronic gastromucosal lesion of the drug.

References

1) J. L. Wallace and D. N. Granger, Pathogenesis of NSAID gastropathy : are neutrophils the culprit? *TiPS*, **13**, 129-131 (1992).
2) D. Y. Graham, N. M. Agarawal and S. H. Roth, Preventation of NSAID-induced gastric ulcer with misoprostil : multicenter, double-blind, placebo-controlled trial. *Lancet*, **2**, 1277-1280 (1988).
3) J. L. Wallace, C. M. Keenan and D. N. Granger, Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *J. physiol.*, **259**, G 462-467 (1990).
4) M. Ligumsky, E. M.Golanska, D. G. Hansen and G. L. Kauffmann, Aspirin can inhibit gastric mucosal cyclo-oxigenase without causing lesions in the rat. *Gastroenterology*, **84**, 756-761 (1983).
5) J. Szejtli, Cyclodextrins : properties and applications. *Drugs Invest.*, 2 ; Suppl. 4, 11-21 (1990).
6) K. D. Rainford. NSAID Gastropathy : novel physicochemical approach for reducing gastric mucosal injury by drug complexation with cyclodextrins. *Drug Invest* 2 ; Suppl. 4, 3-10 (1990).
7) K. T. Lee, J. H. Kim, J. I. Kim, S. J. Kim, H. K. S. H. Seo, Solubility and in vivo absorption enhancement of diclofenac sodium by β-cyclodextrin complexation. *J. Kor. Pharm. Sci.*, **26**(3), 167-174 (1996).
8) M. S. Al-Ghamdi, A. S. Dissanayake, Z. A. Cader and S. Jain, Tenoxicam-induced gastropathy in the rat : a comparison with piroxicam and diclofenac sodium, and the inhibitory effects of ranitidine and sucralfate. *J. Int. Med. Res.*, **19**, 242-248, (1991).
9) S. Stadler. *A foercast for application of cyclodextrins in the pharmaindustry*. In Proc 1st Int Symp on Cyclodextrins. J. Szejtly(ed). Budapest/Dordrech, Holland, Reidel, 377-387 (1981).
10) K. Ukemia, T. Imai, T. Irie, T. Hiayama, Improvement of dissolution and suppository release characteristics of flubiprofen by inclusion complexation with heptakis(2,6-diO-methyl)-beta-cyclodextrin. *J. Pharm. Sci.*, **74**, 841-845 (1985).