Acta Pharmaceutica Turcica
XLIII (3,4) (169-172) 2001

MODULATION OF ANTI-INFLAMMATORY DRUGS' ULCEROGENICITY *VIA* SOLID DISPERSION WITH SKIMMED MILK ON THE EXAMPLE KETOPROFEN

ANTİENFLAMATUAR İLAÇLARIN ÜLSEROJENİTESİNİN AZ YAĞLI SÜT İLE HAZIRLANAN KATI DİSPERSİYON YOLUYLA MODÜLASYONUNA ÖRNEK OLARAK KETOPROFEN

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Ketoprofen (KTP) is an analgesic, anti-inflammatory and antipyretic agent which is not soluble in water and causes serious gastrointestinal (GI) disorders. It is used 100-200 mg in 2 or 4 divided doses perorally after meals. Its maximum dose is 200 mg daily. In the present study, solid dispersion (SD) of KTP with skimmed milk (SM) was prepared in order to improve the solubility and modulate GI side effects such as ulcerogenicity. Investigations have shown that by using SD of KTP, ulcerogenic activity on rat stomach was modulated significantly.

Key words: *Anti-inflammatory drugs; Solid dispersion; Skimmed milk; Ulcerogenicity; Ketoprofen*

Ketoprofen (KTP) suda çözünmeyen ve ciddi gastrointestinal (GI) yan etkilere yol açabilen analjezik, antienflamatuar ve antipiretik bir ilaçtır. Peroral yoldan öğünlerden sonra 2 veya 4 kısma bölünerek 100-200 mg kullanılır. Günlük maksimum dozu 200 mg dır. Bu çalışmada ketoprofenin az yağlı süt ile katı dispersiyonu hazırlanarak çözünürlüğünün artırılması ve ülserojenite gibi GI yan etkilerinin azaltılması amaçlandı. Az yağlı süt kullanılarak hazırlanan ketoprofen katı dispersiyonu ile sıçan mideleri üzerindeki ülser yapıcı etkinin azaldığı tespit edildi.

Anahtar kelimeler: *Antienflamatuar ilaçlar; Katı dispersiyon; Az yağlı süt; Ülserojenite; Ketoprofen*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin synthesis by blocking cyclo-oxygenase enzymes. Therefore these drugs cause gastric irritation and ulceration (1). It has been reported that gastric ulceration was decreased by using amino acid salts of analgesic and anti-inflammatory drugs as aspirin and indomethacine (IND). The pH of amino acid solutions are mildly basic. This basic characteristic prevents the formation of ulcer by neutralizing the gastric acid caused by these drugs (2-4). SD of NSAIDs as KTP and IND were prepared both to improve their solubility and to modulate the GI irritation by virtue of their effect on GI mucosa (5). SDs have been used to improve the solubility and dissolution rates of poorly soluble drugs. Generally water soluble polymers as PVP, PEG and phosphatidyl choline(PC) are used as the carriers of SDs (5,6). In addition, in order to improve the solubility of NSAIDs, addition of surface active agents, formation of water soluble salts (3) and to enhance dissolution and absorption rate, reduction of particle size have been attempted (7). For reducing the particle size, SDs (8,9) have been used.

KTP is a water-insoluble, analgesic and anti-inflammatory drug which causes local or systemic ulcers formation in the GI tract (1). The main objective of this study was to modulate the ulcerogenicity of KTP. For this purpose, SM was used as the carrier in SD and investigated for its modulation on ulcerogenicity of KTP by using rat sto-

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machs and inhibition % of ulcer was calculated in terms of index (10).

Materials

Ketoprofen (Sigma) and skimmed milk (Pınar) (Ingredients of 100 ml SM: protein:3.3g, fat:0.1g, carbohydrate:4.7g, minerals:0.7g) were used as purchased. Tripan blue (E. Merck) and other reagents and chemicals were of analytical grade.

Apparatus: Microscope with a stereoscopic loop (triocular-side lighting) Olympus, SZH 10-11 Japan; Pentax Macro camera 100 with F/2-8 object lense; Lyovac GT 2 (Leybold Heraeus) was used for lyophilization; Ultrasonic disintegrator was of Measuring And Scientific Equipment LTD, England.

Methods

KTP and SD of KTP with SM were investigated for their ulcerogenic effects on stomachs of rats.

1. Preparation of SM powder: Frozen skimmed milk (25 ml) was lyophilized at an input temperature of -16^{0}C under vacuum conditions (0.03 mbar). Based on preliminary studies, the lyophilization time was fixed at 72 h to reduce the water content to 3%. 25 ml SM yielded 2.615 g (n=5) powder which was later sieved through 250 µm mesh.

2. Preparation of the SDs: 500 mg KTP was suspended in 25 ml SM and mixed in a water bath (50^{0}C) for 30 minutes by using a magnetic stirrer. It was then frozen by keeping in a nitrogen bath and lyophilized and the product (SD) was sieved through 250 µm mesh.

3. Studies on ulcerogenic activity of KTP and its various formulations: Rats (Wistar albino) of 200 g were deprived of food with free access to water for 24 h prior to experiments. Rats were divided into six groups with three male and three female in each group, for intraperitoneal (ip) and peroral administration (per.adm.) separately.

Blank: 0.5 ml of 1 % carboxymethylcellulose (CMC) solution containing 0.02% Tween 80 was used for each ip and per.adm.

Control: 0.5 ml of KTP (50 mg.kg^{-1}) in blank solution was used for each ip and per.adm.

Test: SD of KTP with SM was suspended in blank solution and administered for each ip and peroral routes at 50 mg.kg^{-1} KTP in a volume of 0.5 ml. 24 hours after drug administration and 10 minutes prior to sacrifice, 1 ml 1.5% Tripan blue in saline was injected into the tail vein of rats. The stomach of each rat was removed and immersed into 1 % formalin solution. The stomach was then incised along the greater curvature and the length (mm) of each mucosal ulcer developed as dark spots upon blue base in the glandular portion was measured under a dissecting microscope (5x). The sum of the length of each mucosal ulcer per rat was used as the ulcer index. The Student-Newman-Keuls test was used to compare each group and to determine the statistical significance of the data.

As ulcer indexes, the ratio of total ulceration areas in rat stomachs to rat numbers were used. % inhibition in ulcerogenicity was calculated according to the formula:

$$\%\,\text{inhibition} = \frac{(\text{Control group index} - \text{Test group index}) \times 100}{\text{Control group index}}$$

Control group index= Amount of mucosal lesions in rat stomachs injected KTP over rat number.

Test group index= Amount of mucosal lesions in rat stomachs injected SD of KTP with SM over rat number.

The pictures of rat stomachs were taken by Pentax Macro Camera 100 with F/2-8 object lense.

Results and discussion

Table 1 shows the ulcer indexes obtained after the administration of SD of KTP prepared with SM (containing 50 mg.kg^{-1} KTP) given 0.5 ml ip and found as 16.848 whereas the ulcer index of plain drug KTP was found as 22.696. Therefore inhibition was calculated to be 31.17 %. On the other hand, in case of per.adm. ulcer indexes were calculated as 11.268 and 17.461 for SD of KTP and KTP administered 0.5 ml respectively. Inhibition was found as 41.55 % (Table 2).Statistical evaluations of the experiments are shown in table 3.

Table 1.Effect of SD of KTP with SM given ip on KTP-induced gastric ulcers in rats.

Treatment	Number of rats	Ulcer index (mm)	Inbibition %
Blank (1% CMC)	6	3.934 ± 1.192	-
Control (KTP)	6	22.696 ± 9.709	-
Test (SD)	6	16.848 ± 6.759	31.17

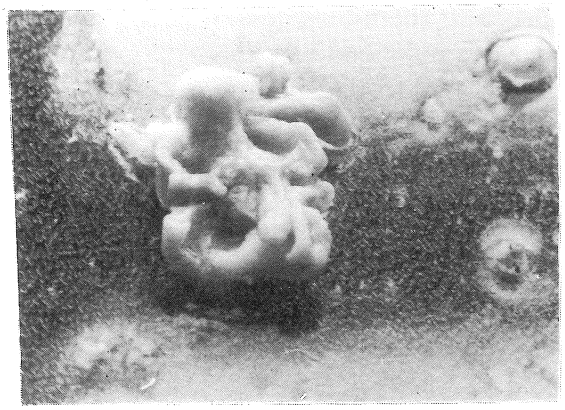
Table 2.Effect of SD of KTP with SM given by per.adm. on KTP-induced gastric ulcers in rats.

Treatment	Number of rats	Ulcer index (mm)	Inbibition %
Blank (1% CMC)	6	2.558 ± 1.581	-
Control (KTP)	6	17.461 ± 7.420	-
Test (SD)	6	11.268 ± 5.228	41.55

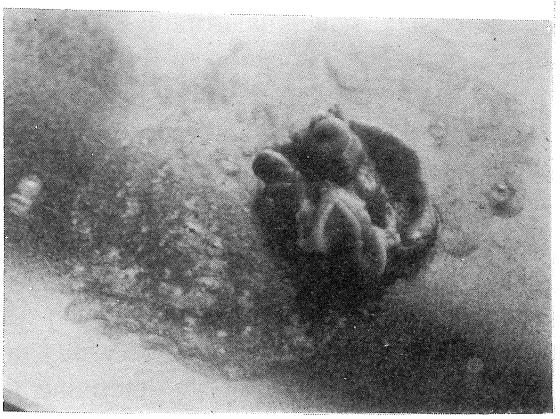
Table 3. Evaluation of the results statistically (One way Anova).

Route	Control	KTP	SD
I.P.	3.9 ± 1.9A	22.7 ± 6.1B	16.8 ± 5.6A
Per. adm.	2.6 ± 1.2A	17.5 ± 4.9B	11.3 ± 2.7A

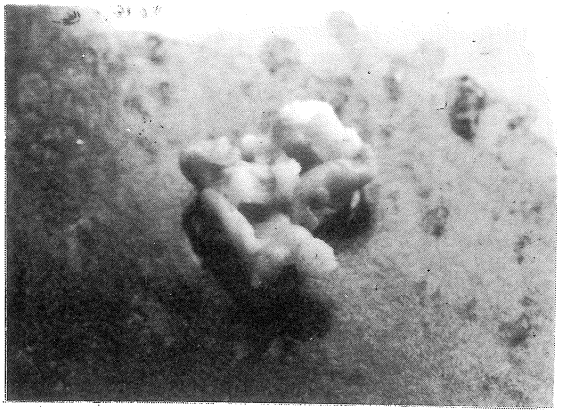
Data are as mean ±SEM. Analysis of variance test was used. For comporison Student-Newman-Keuls test was used.



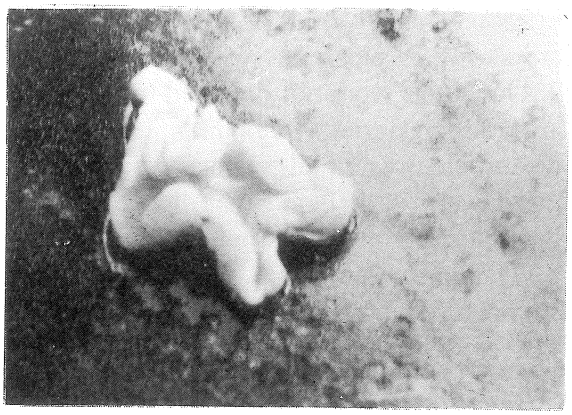
Picture 1. Stomach of a rat used as blank (i.p).



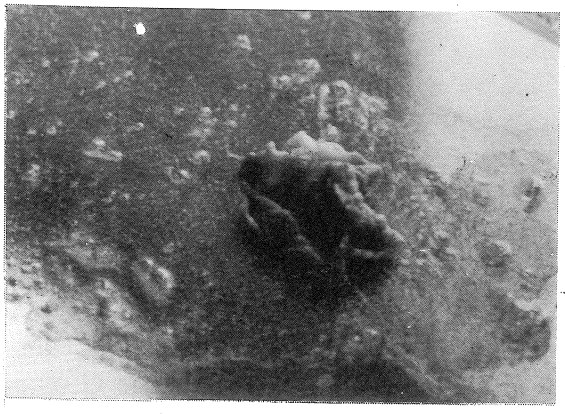
Picture 2. Stomach of a rat administered KTP alone (i.p).



Picture 3. Stomach of a rat administered SD of KTP with SM (i.p).



Picture 4. Stomach of a rat used as blank (per. adm.).



Picture 5. Stomach of a rat administered KTP alone (per.adm.).

Picture 6. Stomach of a rat administered SD of KTP with SM (per. adm.).

In a similar study a widely used analgesic and anti-inflammatory agent indomethacin (IND), which is irritant to the stomach, and solid dispersion of IND prepared with SM (containing 40mg.kg^{-1} IND) were administered sc and ulcerogenic effects were investigated and ulcer indexes were found as 8.47 and 2.48 for IND and SD of IND respectively. Inhibition % was also found as 70.72 in case of SD of IND(10). These results seem to be in accordance with the ones obtained from this recent study.

In studies performed to determine the ulcerogenicity of drugs, inhibition degree was found to be affected by the administration route, structure of the drugs and stress ulcer formation in rats during the experiment.

Stomachs of rats after ip and per.adm. are shown in pictures 1-6. In pictures 1 and 4, blue areas were not detected on stomachs after administering the blank solution, whereas in pictures 2 and 5 it was observed that there were dark blue areas due to ulcerogenic lesions in the stomachs of rats given KTP alone. Pictures 3 and 6 show the stomachs of rats which received KTP with SM as SD and the least ulcer areas and pale blue areas of the stomachs. According to these results, it could be concluded that in case of peroral administration, lesser ulcerogenic activity with SD of drug-SM might be expected. A water soluble complex of drug-carrier was assumed to have formed. This formation could be more absorbable, there-fore less resident in the stomach and con-sequently less local ulcerogenicity than plain drug occurs.

Acknowledgement

The authors thank to the Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Istanbul, for their kindness in providing the microscopic measurements and depicting pictures.

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Accepted: 15.01.2001