IJPSR (2010), Vol. 1, Issue 9 (Suppl.) **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 May, 2010; received in revised form 17 July, 2010; accepted 24 August, 2010

SYNTHESIS AND CHARACTERISATION OF IBUPROFEN, KETOPROFEN PRODRUG FOR TASTE MASKING USING β- CYCLODEXTRIN

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Keywords:

Ibuprofen,
Prodrug,
Taste masking,
Cyclodextrin

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ABSTRACT

The Profens have indicated the gastrointestinal side effects due to the acidic moiety of the profen. The Profens are bitter in taste and it is omitted by mostly children. Therefore an attempt has been made to prepare the ester Prodrug using ethyl alcohol and butyl alcohol and its bitter taste is masked by using β-Cyclodextrin. The prepared prodrugs are characterised by melting point, UV and IR spectroscopy. The bitter taste intensity was evaluated using volunteers by comparison of test samples with standard solutions containing quinine at various concentrations.

INTRODUCTION: Gastrointestinal (GI) side effects constitute the most frequent off all the adverse reactions of nonsteroidal anti inflammatory drugs (NSAID) [1]. Even though Ibuprofen is very potent and widely used among other clinically used NSAIDs, Literature is abundant with its gastric and other side effects because of free carboxylic group. These reactions range, in both severity and frequency leading to GI bleeding, ulceration and haemorrhage [2, 3]. The major factor in the development of GI ulceration and haemorrhage induced by NSAIDs is the inhibition of prostaglandin synthesis, as the endogenous prostaglandins are known to have cytoprotective action on the gastric mucosa[4].

It has also been accepted that GI lesions produced by NSAIDs are the result of two different mechanisms; a direct contact effect and a generalised systemic effect, which may be manifested after absorption following intravenous dosing. This type of damage could be prevented if the carboxylic acid group functionally be masked and therefore the use of Prodrug has been postulated as an approach to decrease the GI toxicity due to the direct contact effect. Some amide conjugates and a few ester derivatives of ibuprofen have been reported with reduced ulcerogenic tendency [5, 6]. But the search for a better Prodrug with reduced side effects still continues.

The purpose of this investigation was to synthesize various ester Prodrug using ethyl alcohol and butyl alcohol and characterize by physicochemical, spectral (UV.IR) and elemental analysis in order to establish their assigned structures. Furthermore, The Profens are bitter in taste and is omitted by mostly children so an attempt has been made that the prepared prodrugs are masked by using β- Cyclodextrin. Reduction of bad taste by beta-Cyclodextrin (CD) is a long known method [7, 8]. There are two theoretical possibilities (a) the CD enwraps the bad tasting molecule (= inclusion Complexation), impeding its interaction with the taste buds or (b) the CD interacts with the gate keeper proteins of the taste buds, paralysing them. All taste sensation (sweet, salt, sour, bitter,) would be extinguished, as long as the adhered CDs are not removed from the taste buds. The bitter taste of a substance disappears in the presence of CD, only when the drug molecule which causes the bitter taste is complexed by an appropriate CD molecule. These complex molecules are strongly hydrated on their outer surface; therefore, they do not get attached to the taste-bud receptors on the tongue in oral cavity [9]. The bitter taste intensity was evaluated using volunteers by comparison of test samples with standard solution containing quinine sulphate.

MATERIALS & METHODS: Ibuprofen, Ketoprofen was gifts from Cipla Bangalore, India, NIPL Mumbai, India. β- Cyclodextrin purchased from Sigma Aldrich, Bangalore, India, Quinine sulphate purchased from S.D. Fine Chemicals, Mumbai, India. All Other chemicals used were of analytical grade. All melting points were determined in open capillary tubes and are uncorrected.

Synthesis of Ibuprofen, Ketoprofen alkyl esters: Ibuprofen alkyl ester was synthesised by a general method for esterification. Ibuprofen, Ketoprofen (1.5g) was solubilized respectively in 25ml of alcohol in a round-bottom flask. Sulphuric acid was added as a catalyst for esterification. The mixture was refluxed for 4h at about 70° C for Ibuprofen, Ketoprofen ethyl ester, and at 80° C for Ibuprofen, Ketoprofen butyl ester with stirring. Sodium acetate (0.5g) was added to quench the catalyst and the residual alcohol was removed by vacuum evaporation. The crude products were purified by silica gel column chromatography by eluting with petroleum ether/ethyl acetate (9:1 v/v). The identities of the

products were determined UV and IR spectra. Ibuprofen, Ketoprofen ethyl ester and Ibuprofen, Ketoprofen butyl esters were obtained in the yield of 90, 91; and 92, 91% respectively. The nature, melting Point and percent yield , The UV - λ_{max}, UV molar absorptivity and IR characteristics (C=O stretching vibration) are reported in **Table 1**.

Characterisation of Prodrug: Melting Point (mp): Veego VMP-PM digital melting point apparatus, uncorrected.

For UV spectrophotometric characterisation, solution of ester prodrugs of Ibuprofen (250 µg/ml) and Ketoprofen (10µg/ml) were prepared in methanol and water (3:1) and scanned in the range of 200 – 400nm. FT-IR spectra of Ibuprofen ester Prodrug and ketoprofen ester Prodrug were obtained using the KBr disc technique.

Hydrolysis Studies: Hydrolysis studies were carried Out in aqueous buffer so as to study whether the Prodrug hydrolyse in aqueous medium and what extent, or not, suggesting the fate of the Prodrug in the system. Hydrolysis kinetics of the synthesized Ibuprofen, Ketoprofen ester prodrugs were studied in aqueous buffer solution at pH 7.4. Under experimental conditions the target compounds hydrolysed to release the parent drug as evident by UV analysis. At constant pH and temperature the reaction displayed strict first order kinetics as the K_{obs} was found to be fairly constant. The data are given in **Table 2**.

To examine the degradation ester prodrugs in pH as that in stomach, pH 1.2 was selected. An assay time of 2h was selected, after which time stomach emptying would normally be effectively complete. The Ibuprofen, Ketoprofen ester prodrugs did not hydrolyse to release the parent compound suggesting that they are stable at the gastric pH. At pH 7.4 the ibuprofen, ketoprofen ester prodrugs hydrolysed to parent compound indicating that the Prodrug will undergo hydrolysis in the system easily.

TABLE 1: NATURE, MELTING POINT AND % YIELD, UV AND IR CHARACTERISATION OF ESTER PRODRUGS OF IBUPROFEN AND KETOPROFEN

Prodrugs	Nature	Melting Point	% Yield	λmax (nm)	E (L/cm. mole)	C = O Str. vibration (Cm^{-1})
Et Es IBU	Solid	46 -48	90.45	263.7	254	1736.09
Bt Es IBU	Solid	47-48	91.34	263.5	302	1736.09
Et Es KBU	Solid	48-50	92.43	253.2	29013	1732.23
Bt Es KBU	Solid	48-49	91.32	253.6	28707	1734.16

TABLE 2 HYDROLYSIS OF IBUPROFEN, KETOPROFEN ESTER PRODRUG A MEAN OF THREE SETS OF EXPERIMENTS

Compound	K_{obs} [a] ± SD Phosphate buffer (pH 7.4)	Hydrochloric acid buffer (pH 1.2)	$t_{1/2}$(min) phosphate buffer (pH 7.4)
Et Es IBU	0.005 ± 0.006	----	84.5
Bt Es IBU	0.007 ± 0.004	----	82.5
Et Es KBU	0.005 ± 0.007	----	84.4
Bt Es KBU	0.006 ± 0.005	----	83.6

Anti- inflammatory activity: The inhibition of swelling in carrageenan – induced edema[10] in rat paw about by oral administration of the drugs is shown in table-3. The percentage of swelling inhibition was calculated using the equation;

Inhibition % = $\{[(V_t - V_0)_{control} - (V_t - V0)_{treated}] / (V_t - V_o)_{control}\} \times 100$

Wherein, V_o and V_t release to the average volume in the hind paw of the rats (n=6) before any treatment and after anti-inflammatory agent treatment, respectively.

All the Four ester prodrugs showed better activity compared to the free parent drug. The maximum anti-inflammatory activity was observed at 3h and remained practically constant up to 6 - 8 h. The anti-inflammatory activity of free ibuprofen, ketoprofen decreased with time. Statistical significance testing using one way analysis of variance showed that the anti-inflammatory activities of the parent drug and its ester prodrugs were effective in comparison with the control group.

Analgesic Activity: The percent protection in mice [11] brought about by administration of the drug is shown in **Table 3**. All the Ethyl, Butyl ester prodrugs showed analgesic activity compared to ibuprofen. The percent protection was calculated using equation;

Protection % = 100 – [number of writhing in test/ number of writhing in control x 100].

Ulcerogenic study: The ulcerogenic [12] effect of ibuprofen and Ester prodrugs was studied at a dose of 100mg kg^{-1}. It was observed that the ulcerogenic dose for the Ester Prodrug was approximately four times the dose of ibuprofen. All the animals treated with Ethyl, Butyl Ester Ibuprofen and Ketoprofen compared with animals treated with ibuprofen. All the animals treated with Ethyl, Butyl Ester Ibuprofen and Ketoprofen did not develop ulcers as they did not hydrolyse in gastric pH. These findings suggest successful masking of the carboxylic function of ibuprofen.

TABLE 3: PHARMACOLOGICAL PROFILE OF IBUPROFEN, KETOPROFEN ESTER PRODRUGS

Compound	Oral dose mg/kg^{-1}	Anti inflammatory activity (%inhibition of oedema)[a]		Analgesic activity (% analgesia)[a]	Ulcer index[b]
		3h	24h		
Ibuprofen	20	51.5	34.56	23.42	13.54 ± 0.45
Et Es IBU	20	48.05	37.04	28.54	Nil
Bt Es IBU	20	53.43	32.36	35.75	Nil
Ketoprofen	10	57.05	70.00	25.56	16.45±0.23
Et Es KBU	10	35.04	65.34	34.53	Nil
Bt Es KBU	10	41.12	63.45	36.45	Nil

[a] Statistical analysis was performed with ANOVA Followed by t-test, $P < 0.001$.
[b] Dose: 100 mg kg^{-1} for ulcerogenic activity

Preparation of physical mixture: The following system of Et, Bt Es IBU, Et, Bt Es KBU and CD were prepared in 1:25 molar ratio.

Physical mixture (PM): The physical mixture of Et, Bt Es IBU, Et, Bt Es KBU and CD was obtained by mixing individual components geometrically,

that had previously been sieved through sieve no 44, together with a spatula.

Fourier Transform Infra-red Spectroscopy (FTIR)
FTIR transmission spectra were obtained by using KBr discs by means of hydrostatic press. The scanning range was 400 to 4000cm^{-1} .The characteristics peaks were recorded.

Differential Scanning calorimeter (DSC) was performed using Differential Scanning Calorimeter (Mettler Toledo, DSC 822). Samples were heated in an open aluminium pans at a rate of 5 ° C per min^{-1} under a nitrogen flow of 40 ml/min.

Preparation and Evaluation of the Dry Suspension: Dry suspension powder containing equivalent of 100 mg of Et, Bt Es IBU and 50 mg of Et, Bt Es KBU were prepared from Et, Bt Es IBU, Et, Bt Es KBU and Physical mixture. Sodium carboxy methyl cellulose (HVP) was used as suspending agents. Citric acid monohydrate was used as Ph modifier. The following procedure was applied to prepare a suspension powder. The smallest amount of physical mixture was mixed with the same amount of another excepient, following the principle of geometric dilution. To prepare the reconstituted suspension, an appropriate 10 ml of water was added to the suspension powder and stirred with glass rod until a homogenous product was obtained (**table 4 & 5**).

Angle of Repose: For measurement of angle of repose of suspension powder, they were passed through a funnel on the horizontal surface. The height (h) of the heap formed was measured with cathetometer and the radius (r) of the cone base was also determined. The angle of repose (Φ) was calculated from following equation:

$$\Phi = \tan^{-1}(h/r)$$

Sedimentation Characters: To study the sedimentation in suspension, the sedimentation volume was determined as function of time. The sedimentation Volume, F is defined as the ratio of the final, equilibrium volume of the sediment, Vu to the total volume Vo before settling, as expressed in the following equation:

$$F= (Vu/Vo)$$

In this study, the sedimentation volume was determined as function of time. 10 ml suspension (height = 12cm) was decanted in a cylinder of 10 ml with diameter of 1.5 cm. After 1h, the sedimentation volume F was determined.

Gustatory Sensation Test: Gustatory sensation test was carried out according to the method described by Mou-young et al., [13]. Twenty healthy male human volunteers in the age group of 23-27 years were selected based on quinine sensitivity test. The non-taster and super taster were rejected. 1 g of Et, Bt Es IBU and Et, Bt Es KBU of each respectively dispersed in 100 ml water for 15 sec. For comparison of pure Et, Bt Es IBU and Et, Bt Es KBU was subjected to taste evaluation by the panel.

Immediately after the preparation, each volunteer held about 1 ml of the dispersion in the mouth for 30s. After expectoration, bitterness level was recorded. A numerical scale was used with following values: 0 = tasteless, 0.5 = very slightly bitter, 1 = slightly bitter, 1.5 = slight to moderate bitter, 2 = moderately bitter, 2.5 = moderate to strong bitter, 3 = strongly bitter, 3+ = very strong. This numerical scale was validated by testing samples randomly. The oral cavity was rinsed with distilled water three times to avoid bias. Wash out period between testing different samples was 15 min (**table 6**).

TABLE 4: FORMULATION OF SUSPENSION POWDER

Drug/excepients	Per Cachet					
	Et Es IBU	Bt Es IBU	Et Es KBU	Et Es KBU	IBU	KBU
IBU (g)	---	---	---	---		0.050
KBU (g)	---	---	---	---		0.025
Physical mixture (g)	0.175	0.175	0.0875	0.0875	---	---
Xanthan gum (g)	0.002	0.002	0.002	0.002	0.002	0.002
Microcrystalline cellulose (g)	0.064	0.064	0.064	0.064	0.064	0.064
Citric acid(g)	0.006	0.006	0.006	0.006	0.006	0.006
Methyl Paraben (g)	0.002	0.002	0.002	0.002	0.002	0.002
Sunset Yellow FCF (g)	0.001	0.001	0.001	0.001	0.001	0.001
Total Filled weight per cachet(g)	0.250	0.250	0.250	0.250	0.250	0.250

TABLE 5: PHYSICAL PROPERTIES OF SUSPENSION POWDER

Parameters	Et Es IBU	Bt Es IBU	Et Es KBU	Et Es KBU	IBU	KBU
Angle of repose (Θ) ± SD[a]						
F Value after reconstitution ± SD[a]	36.32±0.53	37.78±0.46	38.14±0.4	37.56±0.43	37.68±0.52	37.45±0.41
pH after (reconstitution)	4.6 - 4.7	4.6- 4.7	4.6 – 4.7	4.6 -4.8	4.6 – 4.7	4.6 – 4.8

[a] Values represent the mean ±SD of three experiments

TABLE 6: BITTERNESS SCORE EVALUATION BY A PANEL OF TWENTY HUMAN VOLUNTEERS

Formulation	Number of Volunteers rating the preparation as							
	0	0.5	1	1.5	2.0	2.5	3.0	3+
IBU						1	17	2
KBU						1	17	2
Et Es IBU	20							
Bt Es IBU	20							
Et Es KBU	20							
Et Es KBU	20							

Gustatory Sensation test for Suspension Powder: The prepared suspension powders were subjected to taste evaluation by the same panel of twenty selected volunteers. for formula 1,2 10% of panel rated it as very strongly bitter, 85% strongly bitter and 5% moderate to strong bitter while all the other formula 3,4,5,6 was rated as tasteless by 100% of volunteers of panel (**Table 6**).

CONCLUSION: The Study conclusively demonstrated the complete masking of bitter taste of prepared Et, Bt Es IBU, Et, Bt Es KBU with Cyclodextrin in suspension. The FTIR and DSC studies indicated inclusion complexation in Physical mixture. The taste masking is due to CD enwraps bitter tasting drugs, impeding its interaction with the taste buds. Further the sweet taste of CD imparted additive effect.

ACKNOWLEDGEMENT: The authors express their gratitude to Shri Mahant Devendra Dass Ji Maharaj, Chairman, Shri Guru Ram Rai Institute of Technology and Sciences, Dehradun, Uttarakhand, India, for providing the facilities necessary to carry out the research work.

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