



SYNTHESIS AND EVALUATION OF MUTUAL PRODRUG OF ASPIRIN AND CHLORZOXAZONE

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ABSTRACT

Aspirin chlorzoxazone ester linked mutual prodrug was synthesized with the aim of improving the therapeutic index through prevention of gastrointestinal irritation and bleeding. The structure of the synthesized ester prodrug was confirmed by IR and ¹H NMR spectroscopy and their purity was established by elemental analysis, HPLC and TLC. The release of ASP as well as CZX, from the ester prodrug was studied. A validated analytical HPLC method for the estimation of the ASP, and the prodrug was developed. The kinetics of ester hydrolysis was studied in four different non-enzymatic buffer solutions, at pH 3, 4, 5 and 7.4 as well as in experimental plasma. Study of skeletal muscle relaxant and anti-inflammatory properties in comparison with the reference compounds has shown that both skeletal muscle relaxant and anti-inflammatory activities were present at the same doses of the investigated compounds. The ester was found to be less irritating to gastric mucosal membrane than the parent drugs. These results suggest that the synthesized prodrug is characterized by better therapeutic index than the parent drugs.

Keywords: Aspirin, Chlorzoxazone, Prodrug, Ulcerogenicity, NSAIDs, Chemical and enzymatic stability.

INTRODUCTION¹⁻⁷

Non-steroidal anti-inflammatory drugs (NSAIDs) are associated with gastrointestinal side effects particularly stomach ulceration, bleeding and perforation. The side effects produced by NSAIDs are believed to be involved by two different mechanisms: a local action exerted by direct contact of drug with gastric mucosa and generalized systemic action following absorption, now believed to be by inhibition of the COX-1. Acetylsalicylic acid when applied directly to the gastric mucosa produced more lesions as compared to the IV route. The same type of action is found with ASP¹⁻⁷.

For many years several attempts have been made to develop bioreversible or prodrug of non-steroidal anti-inflammatory drugs (NSAIDs) containing carboxylic function in order to depress upper gastrointestinal (GI) irritation and bleeding. Esterification of carboxylic acid functional group of NSAIDs would suppress GI toxicity without adversely affecting their anti-inflammatory activity. In addition to biotransformation of the prodrug to the parent compound at its target sites of activity may be used to achieve rate and time controlled drug delivery of the active entities².

Prodrug has been the concept of retro metabolic drug design that incorporates targeting, metabolism and duration of action consideration into the design process¹⁻³. The carboxylic groups of NSAIDs can be temporarily masked and its direct effect on gastric mucosa can be minimized. Ester prodrugs of naproxen have been synthesized using N-hydroxy methyl succinamide and N-hydroxymethyl isatin as promoities to reduce their GI irritation and improve bioavailability. Prodrug of several NSAIDs such as diclofenac, ASP, ketoprofen etc have been synthesized using 1, 4-dihydro-1-methylpyridine-3-carboxylate as a carrier to treat Alzheimer's disease⁸.

Our aim is to reduce the gastric irritation due to direct contact of the drugs with the gastric mucosa and increased absorption. For this work a potent anti-inflammatory drug associated with GI irritation was selected as a model drug for carboxylic acid derivative. CZX is used as skeletal muscle

relaxant. The rational of this work is to couple ASP with CZX to achieve many advantages related to synergistic effect with reduced GI irritation. Generally these two drugs are prescribed by the physician in combination but that doesn't mean this combination reveals the side effects associated with each other. Coupling of both compounds as a mutual prodrug results in a potent anti-inflammatory compound with reduction of the main adverse local effects related to the activity of NSAID.

MATERIALS AND METHODS

IR spectra were recorded on a FT-IR and UV spectra on a ¹H and ¹³C NMR spectra were recorded on a , operating at 75.5 MHz for the ¹³C nucleus. Samples were measured in DMSO-d₆ solutions at 20 °C in 5-mm NMR tubes. Chemical shifts (ppm) are referred to TMS. Melting points were recorded on a melting point apparatus provided by Aditi associates, Mumbai-68. GC-MS of the compound was recorded from Shivaji University, Kolhapur, Maharashtra, India. Chemicals used for various procedures are obtained from Loba chemicals, Mumbai, India. Reagents used for various preparations of buffers were of analytical grade. Fresh double distilled water from glass apparatus was used in preparation of all the solutions, mobile phase was prepared from HPLC grade methanol obtained from Loba chemicals, Mumbai, India. Human plasma was procured from the local blood bank. ASP & CZX pure drug is obtained as a gift sample from Emcure pharmaceuticals Ltd, Bhosari, Pune.

Synthesis of ester prodrug⁹

The synthesis of the title compound is illustrated in the fig. 1. First ASP was reacted with thionyl chloride to give the acid halide of the ASP. In the second step place (0.77 mol) of dry CZX, (0.84 mol) of pure dimethyl aniline and of anhydrous ether in a flask, set the stirrer in motion and heat the mixture to gentle refluxing on a water bath. Add (0.79 mol) of recrystallized acid chloride of ASP. After vigorous refluxing cool the mixture immediately to room temperature, add about

10 ml of distilled water and continue the stirring until all precipitated solid has dissolved. Separate the ether layer and extract it with 10% sulphuric acid until the acid extract does not become cloudy when rendered alkaline with sodium hydroxide. Finally, wash with 15 ml of saturated solution of sodium hydrogen carbonate and dry the ethereal solution with 1 gm of anhydrous sodium sulphate overnight. Remove the ether by distillation through an efficient fractionating column and distil the residue through an efficient fractionating column and collect the final product. The yield is 62%.

Solubility study¹⁰

Solubility of compounds was found out in methanol, Ethanol, Chloroform, Acetone and Ether. Compound synthesized was added to solvent (5 ml) in a vial which was tightly closed and kept in rotating shaker over-night speed (50 rpm) at constant temp for overnight. It was ensured that equilibrium was established. The solvents were filtered and filtrate was taken in evaporating dish. The solvent was evaporated off and the weight of the residue was determined. The solubility of ASP-CZX in respective solvents was calculated as mg/ml.

Determination of partition co-efficient¹⁰:

Partition co-efficient of synthesized ASP-CZX was determined in three systems i.e. octanol: water, Octanol: Hydrochloric acid buffer (pH1.2) and Octanol: Phosphate buffer (pH 7.4) at 25°C temperature. Synthesized compound (10 mg) was added to 10 ml of aqueous phase and 10 ml of octanol. The mixture was shaken for 1 hr and left for 2 hrs at 25°C. The two layers were separated out using separating funnel. Prodrug concentration in aqueous phase, in hydrochloric acid buffer (pH1.2) and phosphate buffer (pH7.4) was determined from calibration curve plotted of the drug in the respective solvents. The partition co-efficient was calculated as

$$K = \text{Conc. (organic phase)} / \text{Conc. (aqueous phase)}$$

In vitro hydrolysis of ASP-CZX prodrug:

ASP-CZX prodrug was determined by isocratic reversed-phase HPLC procedures using Jasco 1080 equipment with UV detector and a 20 µl loop injection valve. For analysis, a reversed-phase C₁₈ column 250 mm x 4.6 mm with internal diameter 5µm was used as stationary phase. For aqueous kinetic study mobile phase containing water : methanol (20:80 v/v) was selected. The flow rate of 1 ml/min was maintained and column effluent was monitored at 264 nm. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standard chromatogram under the same conditions.

Hydrolysis study in aqueous buffer¹¹⁻¹⁵:

The hydrolysis kinetics of the prodrug was studied at pH 3, 4, 5, and 7.4 using acetate and phosphate buffers. The total buffer concentration was 0.02 M and constant ionic strength of 0.5 m for each sample was maintained by adding KCl. The total buffer volume was 90 ml. The mixture was equilibrated at 37°C for 1 hr. To this mixture 100 mg of drug was dissolved in 10 ml of methanol and the mixture agitated by an overhead stirrer. At selected time intervals of 15, 30, 60, 120 and 180 min, 1 ml of the solution was removed and diluted with mobile phase up to 10 ml and 20 µl of this solution was injected for direct analysis by HPLC. At pH 7.4

the sample were withdrawn at time intervals of 0.5, 1, 2, 3, 4, 5 and 6 hrs.

Enzymatic hydrolysis¹¹⁻¹²:

We procured the whole human blood from local blood bank. It is necessary to remove the anticoagulant like EDTA it is necessary to add acetic acid. So 10 ml of whole human blood is first treated with 1 ml of 0.1% acetic acid. Then it centrifuged for 30 minutes at 5000 rpm to separate RBCs from blood. Then the supernatant is collected and treated with 1 ml of HPLC grade methanol. This is again centrifuged for 15 minutes at 5000 rpm for separation of proteins. It is then passed through the 45µ syringe filter. The supernatant is then used for hydrolysis study.

The enzyme hydrolysis of prodrug was studied in human plasma diluted to 80% with 50mM phosphate buffer at pH 7.4 at 37°C. The reaction was initiated by adding 1ml of the stock solution (100 mg of prodrug in 10 ml of methanol) 9 ml of preheated plasma. The solution was kept in water bath at 37°C. At appropriate time intervals samples of 1ml was withdrawn and diluted to 10 ml with methanol in order to deproteinize the plasma. After immediate mixing and centrifugation for 5 min at 5000 rpm 20 µl of the clear supernatant was analyzed by HPLC for remaining ester prodrug and the values of rate constants and half lives were calculated by using standard formulae.

In vivo studies¹⁶⁻¹⁹:

Animal experiments were carried out at Department of pharmacology, Bharati Vidyapeeth's college of pharmacy Kolhapur, Maharashtra, India. Approval no. (BVCPK/CPCSEA/IAEC/04/2008).

Anti-inflammatory activity:

The anti-inflammatory activity was evaluated using carrageenan-induced paw edema method on rat. Rats weighing 150-200gm were divided in three groups of six animals in each. Group I serves control group without using drug, group II received ASP 20 mg/kg, group III receives prodrug, where the dose was molecularly equivalent to the ASP. The drug was given intra-peritoneal to the animal by 0.5% of sodium CMC and each animal received a dose of 1 ml. Thirty minutes of after administration of the drug, each animal receives an injection of 0.1 ml of carrageenan by sub-plantar route in its left hind paw. The measurement of the hind paw volume was carried out using Plethysmometer before any treatment (V₀) and in any interval (V_t) after the administration of the drugs. All the results are expressed as Mean ± S.E.M.

Ulcerogenic activity:

Albino rats weighing 150-250gm were fasted overnight with access to water. The test compound (100mg/kg) were suspended in 5% methyl cellulose and given orally. The dosing was continued for four days where the rats have free access for food and water. Rats were sacrificed 8 hrs post last dosing. Their stomachs were removed opened along the greater curvature and washed with saline. The presence or absence of gastric irritation is determined. The presence of a single or of multiple lesions (erosion, ulcer or perforation) is considered to be positive. The severity of gastric lesion was measured along its greatest length, the overall total length was designated as the 'ulcer index' (UI).

Skeletal muscle relaxant activity:

Rota-rod method:

Male or female mice with body weight between 25 and 30 gm were used. The animals were first tested for their ability to stay on the moving rod. Those which can stay for more than 1 minute were selected for the study. The synthesized drugs and the standard compounds were given intra-

peritoneal in form of suspension in 0.2% acacia. Thirty minutes later the mice were kept on the rotating rod for 1 minute. The rod was kept revolving with constant speed of 2 rotations per minutes. The number of animals falling from the roller during this time is counted. The test compounds were compared with the standard compound and the percent reduction time is calculated for comparison.

Table 1 Solubility of the synthesized mutual prodrug

Solvent Used	Solubility of Prodrug (in mg/ml)
Ethanol	18
Methanol	34
Chloroform	2.6
Acetone	16.6
Ether	8.6

Table 2 Partition coefficient of mutual prodrug

System Used	Partition coefficient
Octanol: Water	2.3492
Octanol: Hydrochloric acid buffer (pH 1.2)	3.2444
Octanol: Phosphate buffer (pH 7.4)	2.4315

Table 3 System suitability parameters

Parameters	ASP	CZX	ASP-CZX	FEN
Theoretical plates	1535.8	2816.2	3468.2	2919.4
Asymmetry	0.94	0.76	1.19	1.15
Retention	2.06	3.67	5.2	10.8
Resolution	7.1			
			5.86	
			9.64	
Linearity(µg/ml)	20-120	20-120		
LOD (µg/ml)	0.3026	0.3028		
LOQ (µg/ml)	1.009	1.008		

Table 4 Concentration of ASP-CZX prodrug at various time intervals in plasma

Time in minutes	% remaining conc. of ASP-CZX
1	95.81
5	72.45
10	65.31
15	48.11

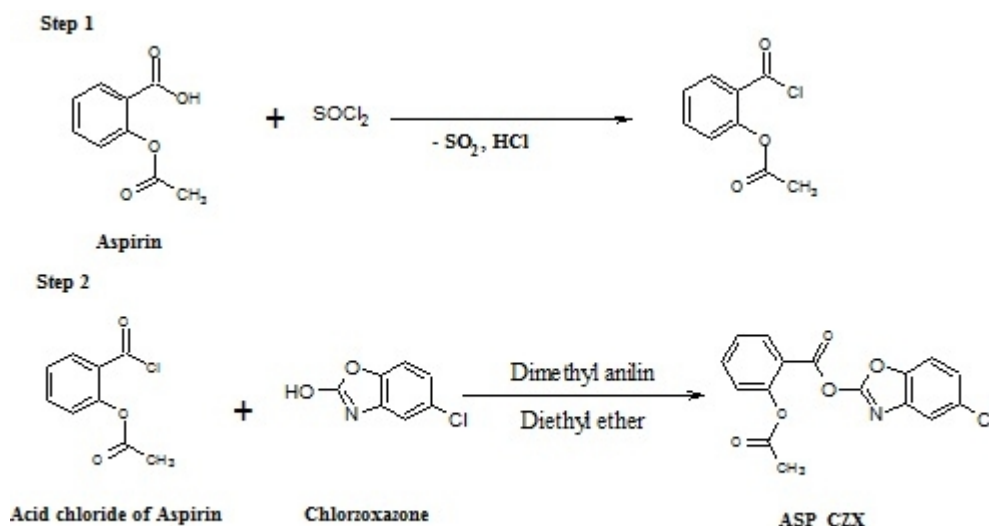


Fig.1. Scheme

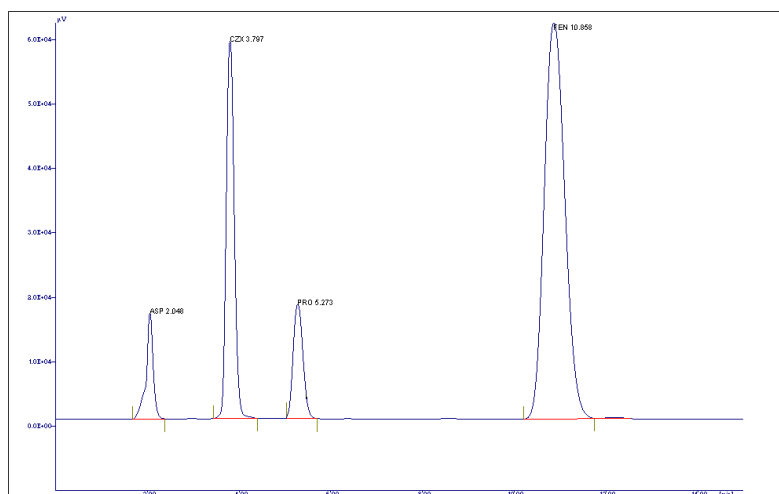


Fig. 2 Chromatogram of aspirin (ASP), chlorzoxazone (CZX), prodrug (ASP-CZX) and fenofibrate (FEN)

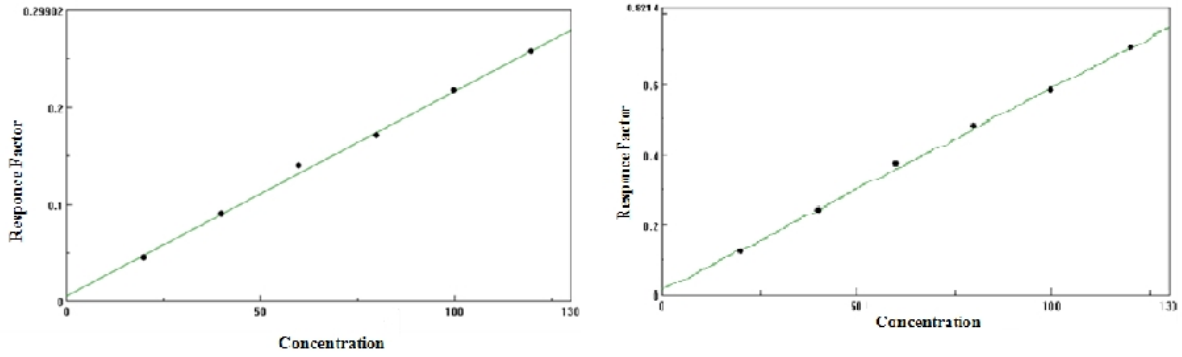


Fig. 3 Calibration curve for ASP and CZX

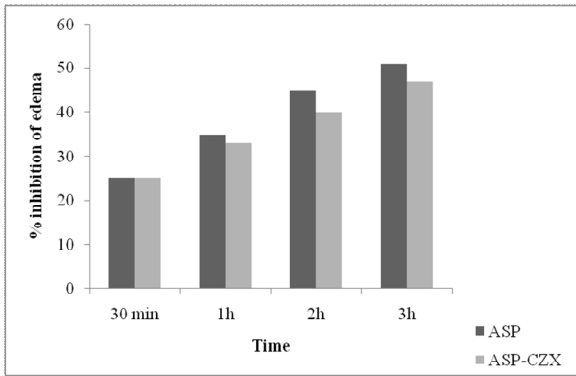


Fig. 4 Comparison of reduction of Paw volume of the Mutual Prodrug and ASP

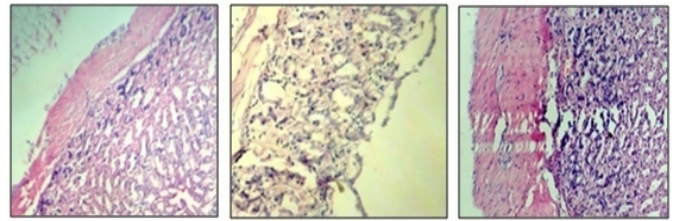


Figure 5 Histological photographs of rat stomach Control (A) aspirin (B) Prodrug(C)

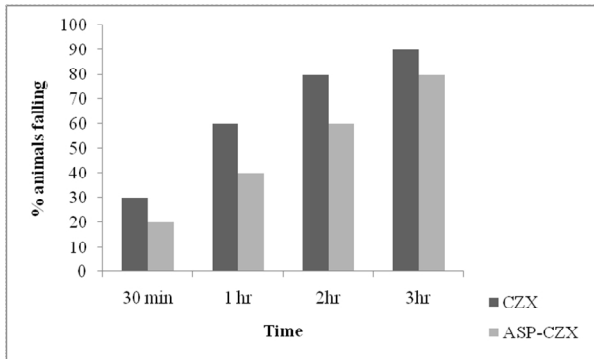


Fig 6 Skeletal muscle relaxant activity: Comparison of percent reduction in time of mutual prodrug and parent drug

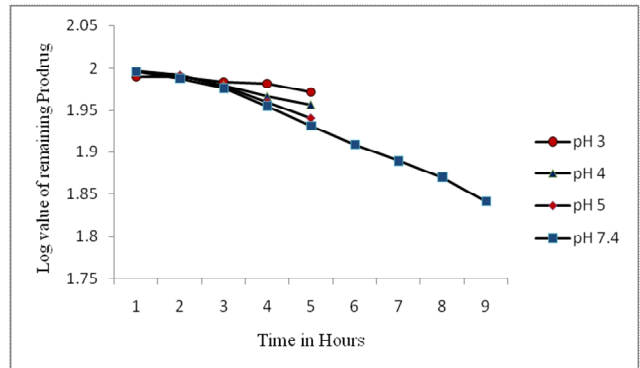


Figure 7 Hydrolysis of ASP-CZX mutual prodrug at different pH

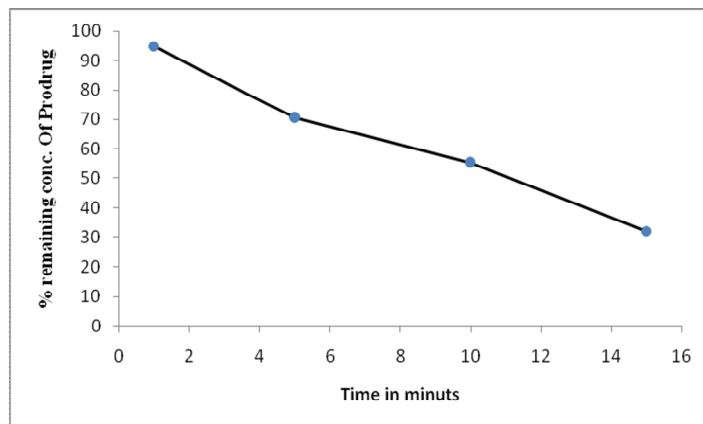


Figure 8 Enzyme hydrolysis study of ASP-CZX mutual prodrug

RESULTS AND DISCUSSION:**Results for the solubility study and Partition coefficient:**

Solubility studies are carried out in various solvents. All the results are given in table no. 1. The result shows that the mutual compound has the maximum solubility in methanol. In table no. 2 results for the partition coefficient study were given.

Spectroscopic interpretation:

The synthesized compound is characterized by IR, NMR. The ASP-CZX ester prodrug was purified by re-crystallization using chloroform and obtained in 71% yield.

¹H NMR (C²HCl₃, 400 MHz): δ 7.5-6 (m, CH aromatic); δ 6.9-6.8 (4H, m, COO-CH₃); δ 8.12 (s, NH).

IR (KBr): 1070 cm⁻¹ (CO-O-C str); 1200 cm⁻¹ (C-O str aliphatic); 1259 cm⁻¹ (C-O str aromatic); 1301 cm⁻¹ (C-N str); 1379 cm⁻¹ (CH bend); 1730 cm⁻¹ (C=O str); 2961 cm⁻¹ (CH str); 2870 cm⁻¹ (CH₃ str); 3335 cm⁻¹ (N-H str)

Hydrolysis study of prodrug:

Before proceeding towards the hydrolysis study of the synthesized drugs, HPLC method was developed for separation of ASP, CZX and ASP-CZX with fenofibrate (FEN) as an internal standard.

The essential pre-requisite for success in the use of prodrug of NSAIDs is that the masked compounds should be acid-stable to prevent the direct contact effect with the gastric mucosa as well as the local inhibition of the prostaglandins. Furthermore, the chemical stability studies were carried out

in aqueous buffer in order indicates whether the prodrug hydrolyze in aqueous medium and to what extent.

The chemical degradation of ester prodrug of ASP followed first order kinetics, and quantitatively converted to ASP as revealed by HPLC analysis of the reaction solution. To examine the degradation of ester prodrug in pH as of stomach, pH 3, 4, 5 and 7.4 were selected, because the mean fasting stomach pH of adult is approximately 2 and increases to 4-5 following ingestion of food. NSAIDs are not recommended to be taken in fasting state; consequently pH 3, 4 and 5 were selected to mimic the appropriate clinical range. An assay time of 3 h was selected, after which time stomach emptying would normally be effectively complete.

The mutual prodrug is stable at pH 3, that suggest that the prodrug is stable at acidic conditions of stomach. The first criterion of prodrug is fulfilled by the synthesized prodrug.

Similar way the enzymatic hydrolysis study of the synthesized prodrug was carried out. 1 ml of the sample was diluted with the mobile phase and the degradation of the prodrug was quantified with help of the pre-established HPLC method.

In-vivo study**Anti-inflammatory activity:**

Inhibition of swelling in carrageenan induced rat edema in paw brought about by oral administration of the drug. The percentage of swelling inhibition was calculated using eq. 2

$$\text{Percent reduction of paw volume} = \frac{[\text{Paw volume of control} - \text{Paw volume of test}]}{\text{Paw volume of control}} \times 100$$

.....Equation no. 1

The prodrug showed better activity compared to free ASP. The maximum anti-inflammatory activity was observed at 1hr and remained practically constant after 3 hrs. The anti-inflammatory activity of ASP decreases with time. Statistical variance showed that the anti-inflammatory activity of ASP-CZX was effective in comparison with the control group. However there is difference in anti-inflammatory activity of ASP and ASP-CZX was observed over long period. Thus mutual prodrug approach of was proved to be suitable for ASP.

Ulcerogenic activity:

The ulcerogenic effect of ASP and ASP-CZX were studied at three doses viz. 25, 50, and 100 mg/kg. The histological photographs shows that the animal treated with aspirin has damaged stomach pattern fig 5 (B) where as the animal treated with prodrug fig 5 (C) has almost same histology as that of the control Fig 5 (A). Less number of ulcers were seen at all doses in animals treated with ASP-CZX compared with the animals treated with ASP. These findings suggested successful masking of carboxylic function of the ASP.

In- vitro hydrolysis of prodrug

Since the carboxyl group of ASP is essential for the therapeutic action, the prodrug of a prolonged action was designed in a form where drug moiety can be released in its original state with time. Therefore, the release of ASP from ASP-CZX prodrug was studied in vitro in order to evaluate

the possible time span in which the drug could be available. The prodrug quantitatively dissociates and release ASP so selected for in vivo study. A validated analytical HPLC method for the estimation of the ASP, CZX and ASP-CZX was developed. The developed chromatographic method was validated for selectivity, precision, accuracy, suitability, linearity and range. Also, the applied chromatographic procedure was validated to separate selectively, the ester, CZX and ASP from the biogenic materials i.e. plasma and the hydrolyzed extract. The kinetics of ester hydrolysis was studied in different buffer solutions (pH 3, 4, 5 and 7.4), without enzymes. Also, the effect of activated esterase enzymes present in plasma was investigated. The data for chemical hydrolysis fits pseudo first order kinetics. As expected, the rate of prodrug hydrolysis was found to be highly pH-dependent while the prodrug was hydrolyzed at higher pH (7.4) with higher rate. The hydrolysis of the ester was tested in the experimental plasma, to investigate the effect of activated esterase enzyme on the drug.

In-vivo evaluation

Skeletal muscle relaxant, anti-inflammatory and ulcerogenic activities of the prodrug were studied in comparison to equivalent doses of ASP and CZX. The skeletal muscle relaxant activity was investigated in mice according to reported method. The capacity of retaining on the rotating rod and the differences between the capacities and those of controls are used as a measure of the degree of skeletal

muscle relaxant activity (Figure 6). As a general pattern, the skeletal muscle relaxant activities of the prodrug show improvement over time. The maximum skeletal muscle relaxant activity is reached after about 1 h for prodrug. The observed latent muscle relaxant activity may results from prodrug bio-availability and / or hydrolysis to parent drug. The effectiveness of NSAIDs in acute inflammation can be estimated by using intraplantar injection of carrageenan in the rat. Swelling of the injected paw was measured at 30, 60, 90, 120 and 180 minutes using a mercury plethysmometer. The pre-treatment with intraperitoneal resulted in a significant reduction in swelling that lasted about 3 hr post-carrageenan injection. The anti-inflammatory activity of prodrug significantly improves over time. This means that the prodrug is devoid of anti-inflammatory activity and that the observed latent activity results from hydrolysis to the parent drug. An equimolar dose of prodrug reduced inflammation in a pattern approximately similar to ASP.

The ulcerogenic activity of the prodrug, as a representative example for the synthesized oral delivery system, was tested in comparison with parent drugs (ASP and CZX) following oral administration for 4 days in rat. As could be seen from the gross observation, linear or oval-shaped lesions were found mainly in the corpus of the stomach. The group subjected to prodrug had significantly fewer lesions than the ASP group. The examination of the stomach specimens of the treated experimental animals by microscope affords a highly precise method for investigation of the ulcerogenic potential of NSAIDs. Fig. 5 shows stomach specimens of rat treated with a chronic dose of prodrug (Fig. 5C); the parent drugs ASP (Fig. 5B) and the control group (Fig. 5A) which receive only the vehicle. The ASP treated group (Fig. 5B) characterized by complete damage of the mucous layer besides ulceration of the sub-mucosal cells. These effects are not observed in the prodrug treated group (Fig. 5C) or control group (Fig. 5A). The observations afford good evidence for the safety of the suggested oral delivery system of NSAIDs compared with the traditional use of the parent drugs.


CONCLUSION

The in vitro and in vivo evaluation of the synthesized ASP-CZX ester prodrug revealed improvement in the therapeutic index of the parent drugs aspirin. The derivative was characterized by prodrug profile, adequate chemical stability, and reduced ulcerogenic liability. The prodrug retained the anti-inflammatory and skeletal muscle relaxant activities of the parent drugs.

REFERENCES:

1. D. V. Derle, K. N. Gujar and B. S. H. Sagar, Adverse effects associated with use of NSAIDs an Overview, Indian Journal of Pharmaceutical Sciences, 2006 July – August, 409-414. <http://dx.doi.org/10.4103/0250-474X.27809>
2. D. Bhosle, S. bharambe, Neha Gairola and Suneela S. Dhaneshwar, Mutual prodrugs concepts: fundamentals and applications, Indian Journal of Pharmaceutical Sciences, 2006 May – June, 286-294.
3. Deepika Nagpal, R. Singh, Neha Gairola, S. L. Bodhankar and Suneela S. Dhaneshwar, Mutual azo prodrug of 5-aminosalicylic acid for colon

- targeted drug delivery: synthesis, kinetic study and pharmacological evaluation, Indian Journal of Pharmaceutical Sciences, 2006 March - April 171-178.
4. Yadav M.R., Halen P.K., Chagti K.K., Hemalatha B. Y., Giridhar R., A novel approach towards therapeutic optimization of diclofenac Ars Pharm; 2005 46 (3): 263-277.
5. Alain Vigroux and Michel Bergon, Synthesis of Prodrugs and a Mutual Prodrug of CZX and Acetaminophen Based on a Masked Benzoxazolone; Bioorganic & Medicinal Chemistry Letters, 1995 Vol. 5, No. 5, 427-430. [http://dx.doi.org/10.1016/0960-894X\(95\)00049-Y](http://dx.doi.org/10.1016/0960-894X(95)00049-Y)
6. Farghaly A. Omar Eur, Cyclic amide derivatives as potential prodrugs. Synthesis and evaluation of N-hydroxymethylphthalimide esters of some non-steroidal anti-inflammatory carboxylic acid drugs. J. Med. Chem. 1998 33 123-131. [http://dx.doi.org/10.1016/S0223-5234\(98\)80037-8](http://dx.doi.org/10.1016/S0223-5234(98)80037-8)
7. M. Zovkoa, B. Zorca, M. Lovreka, B. Boneschans Macromolecular prodrugs. IX. Synthesis of polymer-fenoprofen conjugates; International Journal of Pharmaceutics, 2001 228, 129–138. [http://dx.doi.org/10.1016/S0378-5173\(01\)00822-5](http://dx.doi.org/10.1016/S0378-5173(01)00822-5)
8. S.S. Dhaneshwar et al., Dextran: A promising macromolecular drug carrier, Indian Journal of Pharmaceutical Sciences, 2006 November-December, 705-714. <http://dx.doi.org/10.4103/0250-474X.31000>
9. H. G. Vogel's Drug discovery and evaluation, pharmacological assay, 2002 2nd edition.
10. V. Bhosale, G. P. Agrawal and P. Mishra, Preparation and evaluation of directly compressible forms of mutual prodrugs of ASP, Indian Journal of Pharmaceutical Sciences, 2006 July – August, 425-431.
11. Lalit Kumar Wadhwa, Pritam Dev Sharma, Glycolamide esters of 6-methoxy-2-naphthylacetic acid as potential prodrugs - physicochemical properties, chemical stability and enzymatic hydrolysis, International Journal of Pharmaceutics, 1995, 118, 31-39. [http://dx.doi.org/10.1016/0378-5173\(94\)00315-V](http://dx.doi.org/10.1016/0378-5173(94)00315-V)
12. M.S.Y. Khan, Mymoona Akhter, Synthesis, pharmacological activity and hydrolytic behaviour of glyceride prodrugs of ASP, European Journal of Medicinal Chemistry, 2005, 40, 371–376. <http://dx.doi.org/10.1016/j.ejmech.2004.11.009> PMID:15804536
13. Nithar R. Chatterjee, Amol A. Kulkarni, Santosh P. Ghulekar, Synthesis, pharmacological activity and hydrolytic behavior of ethylenediamine and benzathine conjugates of ASP European Journal of Medicinal Chemistry, 2007, 1-5.
14. Peng Wang a,c, Meiling Qi b, Lihe Liu c, Lin Fang Determination of ASP in dog plasma by liquid chromatography and application in pharmacokinetic studies of an ASP prodrug in dogs Journal of Pharmaceutical and Biomedical Analysis, 2005, 38, 714–719. <http://dx.doi.org/10.1016/j.jpba.2005.02.013> PMID:15967300
15. Xiangguo Zhao, Xinyi Tao, Dongzhi Wei, Qingxun Song, Pharmacological activity and hydrolysis behaviour of novel ASP glucopyranoside conjugates European Journal of Medicinal Chemistry, 2006,41,1352–1358.<http://dx.doi.org/10.1016/j.ejmech.2006.05.014> PMID:16806590
16. Didier M. Lambert, Rationale and applications of lipids as prodrug carriers, European Journal of Pharmaceutical Sciences, 2000, 11 Suppl. 2, S15–S27. [http://dx.doi.org/10.1016/S0928-0987\(00\)00161-5](http://dx.doi.org/10.1016/S0928-0987(00)00161-5)
17. P. Jakgid, K. Mlinarid Majerski, B. Zorc, M. DumiU, Macromolecular prodrugs. VI. Kinetic study of poly [cql-(N-2-hydroxyethyl-DL-aspartamide)]-ketoprofen hydrolysis International Journal of Pharmaceutics, 1996,135,177-182.[http://dx.doi.org/10.1016/0378-5173\(96\)04469-9](http://dx.doi.org/10.1016/0378-5173(96)04469-9)
18. B.M. El Haj, A.M. Al Ainri, M.H. Hassan, R.K. Bin Khadem, M.S. Marzouq The GC/MS analysis of some commonly used non-steroidal anti-inflammatory drugs (NSAIDs) in pharmaceutical dosage forms and in urine; Forensic Science International, 1999,105,141–153. [http://dx.doi.org/10.1016/S0379-0738\(99\)00106-1](http://dx.doi.org/10.1016/S0379-0738(99)00106-1)
19. Francesco Paolo Boninaa, Carmelo Pugliaa, Tony Barbuzzia, Paolo de Caprariib, c c d Francesco Palagiano, Maria Grazia Rimoli, Antonella Saija In vitro and in vivo evaluation of polyoxyethylene esters as dermal prodrugs of ketoprofen, naproxen and diclofenac European Journal of Pharmaceutical Sciences, 2001, 14, 123–134. [http://dx.doi.org/10.1016/S0928-0987\(01\)00163-4](http://dx.doi.org/10.1016/S0928-0987(01)00163-4)

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