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Hydrolysis Kinetics Studies of Mutual Prodrugs of Ibuprofen

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The hydrolysis kinetics of mutual prodrugs of ibuprofen was investigated using high performance liquid chromatographic method. The hydrolysis kinetics of prodrugs was studied to access their utility as a prodrug. These kinetic studies were performed in 0.1N hydrochloric acid (pH 1.2), 10% rat gastric mucosal homogenate, phosphate buffer (pH 7.4), 10% rat intestinal homogenate, 80% human plasma (pH 7.4) and 10% rat liver homogenate at 37°. The influence of pH on the chemical stability of prodrugs was also studied in phosphate buffer (pH 3.6 to7.2). The results indicate that these derivatives have undergone pH-dependent hydrolysis and rate of hydrolysis of the prodrugs has been increased in phosphate buffer (pH 7.4) as against 0.1N hydrochloric acid (pH 1.2). The rate of hydrolysis of prodrugs showed an increase in 10% rat gastric mucosal and 10% rat intestinal homogenate. But the kinetic studies performed in 80% human plasma and 10% rat liver homogenate showed about five to six fold increase in hydrolysis as compared to other fluid models.

A basal requisite for the usefulness of the prodrug approach is ready availability of chemical derivative types which satisfy the prodrug requirements, the most of these being reconversion of the prodrug to the parent drug *in vivo*. Prodrug reconversion in the intestinal lumen can be utilized as an oral drug delivery strategy when drugs in question have poor stability at acidic pH or cause gastric irritation. In this regard, a prodrug can be made that is stable or undergoes very slow hydrolysis to the parent drug in the pH range of stomach and undergoes hydrolysis to the parent drug at intestinal pH. The hydrolysis of prodrug can also take place enzymatically[1].

A number of pharmaceutical substances have ester or amide as functional groups which may undergo hydrolysis in solutions or in aqueous suspensions. Hydrolytic reactions involve nucleophilic attack on labile bonds such as lactam, ester, amide, imine and so on, by water on the drug in the solution and it follows first order kinetics[2,3]. Earlier reports revealed that, in order to access the suitability of a prodrug, it is necessary to study its kinetics and mechanism of hydrolysis in aqueous media, human plasma, rat gastric mucosal homogenate, rat intestinal homogenate and rat liver homogenate[4-13].

Therefore the present work was aimed at studying the hydrolysis kinetic to ascertain whether enzymes of GIT, liver and plasma would be able to catalyse the conversion of the prodrugs to the parent drugs or not. The hydrolysis of prodrugs was studied in 80% human plasma (pH 7.4), 10% rat gastric mucosal homogenate (0.1N HCl), 10% rat intestinal homogenate (pH 7.4), 10% rat liver homogenate (pH 7.4) and pure aqueous buffer solution. All the experiments were carried out at 37°. The kinetic studies were also performed at different pH values which represent the wide range of pH of gastrointestinal tract. The two mutual prodrugs of ibuprofen synthesized were IBU-PA (conjugate of ibuprofen with paracetamol) and IBU-SAL (conjugate of ibuprofen with salicylamide). The synthesis and characterization of the two prodrugs has been published in our earlier communication[14].

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MATERIALS AND METHODS

Kinetics studies of prodrugs in 0.1 N hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.4):

Accurately weighed prodrugs (10 mg) were dissolved initially in 5 ml of methanol, in a 10 ml volumetric flask and the volumetric flask was kept in a constant temperature bath at 37° for 10 min. The contents were then transferred to a vessel of dissolution apparatus (USP dissolution apparatus II i.e. paddle method)[15] containing 995 ml of 0.1N hydrochloric acid or phosphate buffer (pH 7.4). The 0.1N hydrochloric acid and phosphate buffer (pH 7.4) were prepared as per IP[16]. The contents of the vessel were stirred continuously at 100 rpm for 2 h and aliquots of 10 ml were withdrawn at various time intervals. An equal aliquot of fresh 0.1N hydrochloric acid or phosphate buffer (pH 7.4) was added to the vessel immediately.

The aliquots withdrawn were extracted three times with 5 ml of organic solvents (chloroform for IBU-PA and ether for IBU-SAL). The organic phases were mixed and washed three times with distilled water (3 ml). The water extracts were discarded. Organic phase was evaporated to dryness. The residue was dissolved in acetonitrile and diluted suitably to estimate the prodrugs by HPLC method. Rate of hydrolysis was calculated and corresponding half lives obtained from the identity: $t_{½}=0.693/k$[17,18].

Method of analysis:

HPLC was performed on an instrument of M/s Shimadzu, Japan, equipped with dual piston reciprocating pump (model LC–10 AT vp), rheodyne injection system (model 7125 with loop capacity of 20 μl), UV/Vis photodiode array detector (model SDP–MIOA vp) and stainless steel column (Luna 5 micron, 250 x 6.4 mm, C_{18}, Phenomenex, Inc. USA). HPLC grade acetonitrile (M/s Ranbaxy) was used as solvent. The flow rate was maintained at 1 ml/ min. The detection was performed at 240 nm. The retention time of ibuprofen, IBU–PA and IBU–SAL was 3.19 min, 3.85 min and 3.84 min, respectively. The amount of prodrug in the sample was calculated and % of prodrug was determined.

Kinetics studies of prodrugs in 80% v/v human plasma:

Hydrolysis kinetics of prodrugs was studied at 37° in phosphate buffer (pH 7.4) containing 80% v/v human plasma. In a 10 ml volumetric flask, 10 mg of prodrug was dissolved in 5 ml methanol and the volumetric flask was kept in a constant temperature bath at 37° for 10 min. The contents were transferred to a 250 ml beaker containing 95 ml of 80% v/v human plasma (pH 7.4). The contents of the beaker were stirred continuously and aliquots of 2 ml were withdrawn at various time intervals and equal aliquots of fresh 80% v/v human plasma (pH 7.4) were replaced in the beaker immediately. The samples so withdrawn were shaken and centrifuged for 10 min at 2500 rpm. The amount of prodrug in supernatant liquid was determined by HPLC method as above. The rate of hydrolysis and half lives of prodrugs were calculated.

Kinetics studies of prodrugs in 10% w/v rat gastric mucosal homogenate:

Hydrolysis kinetics of prodrugs was studied in 0.1N hydrochloric acid containing 10% w/v rat gastric mucosal homogenate. Wistar rats (150–175 g) were sacrificed and stomach was removed. The stomach was opened and gastric mucosa was removed with surgical blade and transferred to a tared beaker. The gastric mucosa was homogenized with the help of tissue homogenizer. A 10% w/v of gastric mucosal homogenate was then prepared with 0.1N hydrochloric acid and this fluid model was used for hydrolysis studies[19].

In 10 ml volumetric flask, 10 mg of prodrug was dissolved in 2 ml methanol and the volumetric flask was kept in a constant temperature bath at 37° for 10 min. The contents were then transferred to 250 ml beaker containing 48 ml 10% w/v rat gastric mucosal homogenate. It was kept on a rotating shaker (60 rpm) at 37° and aliquots of 2 ml were withdrawn at specific time intervals. Equal volume of fresh 0.1N hydrochloric acid containing 10 % w/v gastric mucosal homogenate was added to the beaker immediately. The samples were shaken and centrifuged for 10 min at 2500 rpm. The supernatant was decanted out and amount of prodrug in the sample was determined by HPLC method as above. The rate of hydrolysis and half lives of prodrugs were calculated. Animals were housed under standard laboratory conditions. All experimental protocols were approved by the Institutional Animal Ethics Committee.

Kinetics studies of prodrugs in 10% w/v rat intestinal homogenate:

Hydrolysis kinetics of prodrugs was studied in phosphate buffer (pH 7.4) containing 10% w/v rat (150–175 g) intestinal homogenate[19]. Wistar rats were sacrificed by cervical dislocation[20]. The intestine was removed and washed free of food matter and chopped. The pieces of intestine were taken in a tared beaker. A 10% w/v suspension of the intestine was prepared in phosphate buffer (pH 7.4). The intes-

tine was homogenized using a tissue homogenizer and the homogenate was used for hydrolysis studies. In 10 ml volumetric flask, 10 mg of prodrug was dissolved in 5 ml methanol and the volumetric flask was kept in a constant temperature bath at 37° for 10 min. The contents were then transferred to 250 ml beaker containing 95 ml of the intestinal homogenate. The same procedure as described with 10% w/v rat gastric mucosal homogenate was then followed. The rate of hydrolysis and half lives of prodrugs were calculated.

Kinetics studies of prodrugs in 10% w/v rat liver homogenate:

Hydrolysis kinetics of prodrugs was studied in phosphate buffer (pH 7.4) containing 10% w/v rat liver homogenate. Wistar rats (150–175 g) were sacrificed and liver was removed and chopped. A 10% w/v suspension was prepared as described in the previous section using phosphate buffer (pH 7.4)[19]. In a 10 ml volumetric flask, 10 mg of prodrug was dissolved in 5 ml methanol and the volumetric flask was kept in a constant temperature bath at 37° for 10 min. The contents were then transferred to a 250 ml beaker containing 95 ml of 10% w/v rat liver homogenate (pH 7.4) at 37°. The same procedure as described under 10% w/v rat gastric mucosal homogenate was then followed. The rate of hydrolysis and half lives of prodrugs were calculated.

Kinetics studies of prodrugs in aqueous buffers:

The kinetics of prodrugs was studied in phosphate buffer of pH range from 3.6 to 7.2. The phosphate buffers of pH 3.6, 4.0, 5.0, 5.5, 6.0, 6.5, 6.8, 7.0 and 7.2 were prepared according to IP[16]. The prodrug (10 mg) was triturated with the respective buffer solution and the final volume was made up to 10 ml with same buffer solution. These suspensions of prodrugs were filled in 10 ml capacity vials. The vials were sealed and kept in a thermostatic oven at 47°. After every two days up to 8 days, 1 ml of sample was withdrawn from each vial. The amount of prodrug in sample was determined by HPLC method as above. The degradation rate constant (k) was obtained from the plot of % residual prodrug versus time (min). The stability of prodrug at optimum pH values was obtained from the plot of logarithms of the rate constant (log k) versus the pH of suspension.

RESULTS AND DISCUSSION

Kinetic studies were performed at constant temperature in different fluid models. The perusal of the Table 1 and fig 1 and 2 showed that there has been an increase in the rate of hydrolysis of the prodrugs in phosphate buffer (pH 7.4) as against 0.1N hydrochloric acid (pH 1.2). This can be well justified on the basis of the nature of the functional groups i.e. ester group present in the prodrugs. And it is well known fact that the hydrolysis pattern of the ester in acidic medium is reversible and in alkaline medium it is non-reversible. The half life of IBU-PA in 0.1N hydrochloric acid (pH 1.2) was found to be 6079 min and in phosphate buffer (pH 7.4) was found to be 187 min while the half life of IBU-SAL was found to be 4682 min and 257 min in 0.1N hydrochloric acid (pH1.2) and phosphate buffer (pH 7.4), respectively. The plot of logarithms of amount of prodrug remaining [log (a-x)] against time gave linear expression indicating that the hydrolysis of prodrug in all fluid models followed first order kinetics.

The perusal of the Table 1 and fig 1 and 2 showed that there has been an increase in rate of hydrolysis in the gastric mucosal and intestinal homogenate fluid models. This observation can once again be well justified on the basis of presence of enzymes in these homogenates. The half lives of IBU-PA in 10% w/v rat gastric mucosal homogenate and in 10% w/v rat intestinal homogenate were found to be 4559 min and 163 min respectively, whereas the half lives of IBU-SAL in 10% w/v rat gastric mucosal homogenate and in 10% w/v rat intestinal homogenate were found to be 4077 min and 233 min, respectively.

Coming to the hydrolysis kinetics in 80% v/v human plasma (pH 7.4) and 10% w/v rat liver homogenate (pH 7.4) (fig. 1 and 2), it was observed that there was about five to

TABLE 1: HALF LIVES ($T_{½}$) OF PRODRUGS IN VARIOUS FLUID MODELS AT 37°

Fluid Model	$t_{½}$ (min)	
	IBU-PA	IBU-SAL
0.1 N hydrochloric acid (pH 1.2)	6079	4682
10% w/v rat gastric mucosal homogenate (pH 1.2)	4559	4076
Phosphate buffer (pH 7.4)	187	257
10% w/v rat intestinal homogenate (pH 7.4)	163	233
80% v/v human plasma (pH 7.4)	32	38
10% w/v rat liver homogenate (pH 7.4)	25	28

IBU-PA (conjugate of ibuprofen with paracetamol), IBU-SAL (conjugate of ibuprofen with salicylamide).

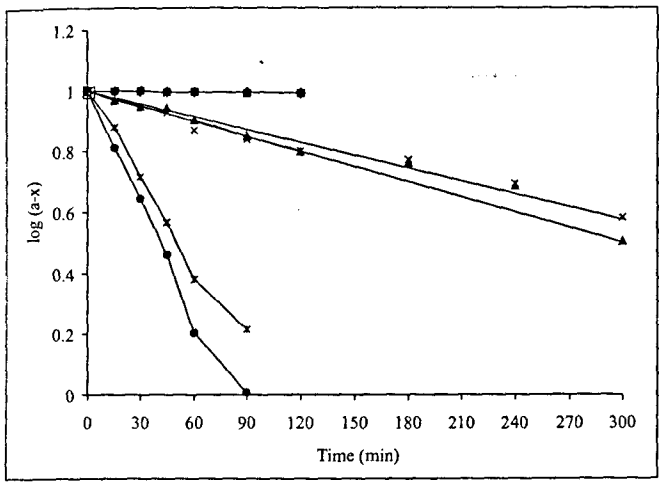

Fig. 1: Hydrolysis kinetics of IBU-PA.

Hydrolysis kinetics studies of IBU-PA were performed at 37° in 0.1N hydrochloric acid (pH 1.2) [-♦-], phosphate buffer (pH 7.4) [-▲-], 80% v/v human plasma (pH 7.4) [-∗-], 10% w/v rat gastric mucosal homogenate (pH 1.2) [-◘-], 10% w/v rat intestinal homogenate (pH 7.4) [-X-] and 10% w/v rat liver homogenate (pH 7.4) [-●-].

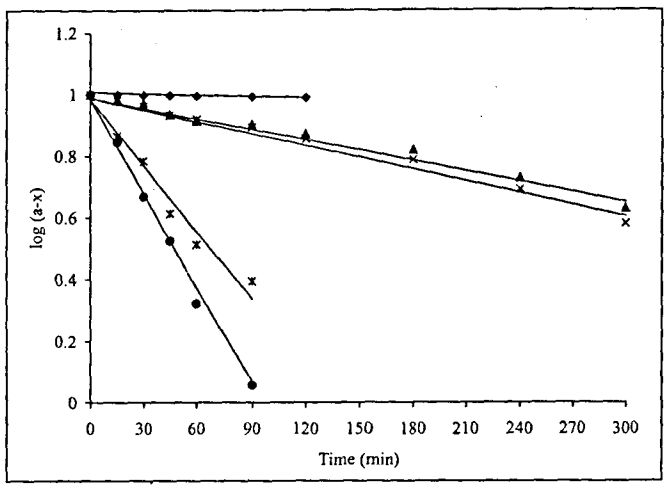

Fig. 2: Hydrolysis kinetics of IBU-SAL.

Hydrolysis kinetics studies of IBU-SAL were performed at 37° in 0.1N hydrochloric acid (pH 1.2) [-♦-], phosphate buffer (pH 7.4) [-▲-], 80% v/v human plasma (pH 7.4) [-∗-], 10% w/v rat gastric mucosal homogenate (pH 1.2) [-◘-], 10% w/v rat intestinal homogenate (pH 7.4) [-X-] and 10% w/v rat liver homogenate (pH 7.4) [-●-].

six fold increase in the rate of hydrolysis as compared to other fluid models. Increase in the hydrolysis rate can well be attributed to the presence of detoxifying enzymes present in liver. The half lives of IBU-PA in 80% v/v human plasma (pH 7.4) and 10% w/v rat liver homogenate (pH 7.4) were found to be 32 min and 25 min respectively, whereas the half lives of IBU-SAL in 80% v/v human plasma (pH 7.4) and 10% w/v rat liver homogenate (pH 7.4) were found to be 38 min and 28 min, respectively. Thus it can be concluded that the hydrolysis of prodrugs is pH as well as enzyme-dependent.

Kinetic studies of prodrugs were studied in phosphate buffers (pH 3.6 to 7.2). A linear relationship was observed,

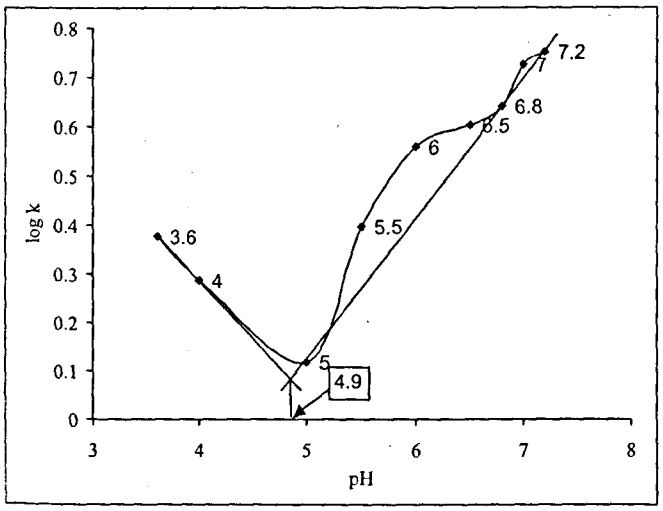

Fig. 3: pH-dependent degradation of IBU-PA in phosphate buffer.

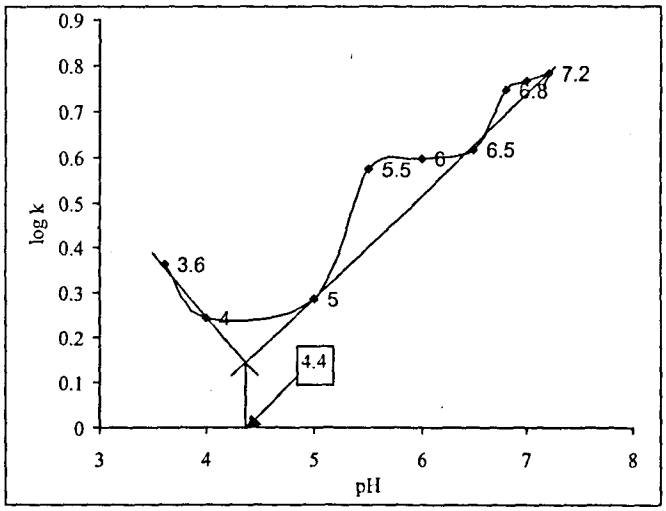

Fig. 4: pH-dependent degradation of IBU-SAL in phosphate buffer.

when the residual prodrug was plotted against time. This study indicated that the prodrug was degraded by zero order kinetics. The rate of degradation of prodrugs was found to increase with increase in pH. This may be attributed to the enhanced solubility of prodrugs, making more of the drug available for degradation. This indicated that the hydroxyl ion catalyzed hydrolysis of prodrugs (esters). Thus, the degradation rate was not dependent on the total concentration of prodrug, but only on the amount that was present in solution. The perusal of fig. 3 and 4 showed that the pH for optimum stability of prodrugs was found to be 4.9 for IBU–PA and 4.4 for IBU–SAL. These pH values are also within the safe physiologically tolerable limits.

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