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Prodrugs of Ibuprofen II – Kinetics of Decomposition of N-Mannich Base Prodrugs

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The kinetics of decomposition of N-Mannich base derivatives of ibuprofenamide was studied to assess their utility as a prodrug for ibuprofen. These kinetic studies were performed in aqueous buffers at different pH values, in simulated gastric and intestinal fluids and in human plasma at 37°. Kinetic studies in aqueous buffers at different pH showed that these derivatives undergone pH dependent hydrolysis. The pH-rate profile for these compounds had a sigmoidal shape. These N-Mannich bases were hydrolyzed to ibuprofenamide but in no case ibuprofen was released. Kinetic studies performed in simulated gastric and intestinal fluid showed that there was not much effect of gastric and intestinal constituents on the release rate. But the kinetic studies performed in human plasma showed that some amount of ibuprofenamide was converted to ibuprofen which might be due to the presence of proteolytic enzymes in human plasma.

The most important and basic requisite of prodrug approach to be useful in solving drug delivery problem is the reconversion of the prodrug to the parent drug *in vivo*. Ideally a prodrug should be converted to the active drug as soon as it reaches its target biological compartment. The rate of conversion of prodrug is the regulating step for exhibiting pharmacological action of the drug.

The kinetics of decomposition of a great number of N-Mannich bases in aqueous solution has been the subject of several studies[1-8]. At constant pH and temperature, the decomposition rates of N-Mannich bases followed strict first order kinetics and all reaction went to completion. The pH-rate profiles for most compounds have a sigmoid shape. The reaction mechanism proposed for the decomposition involves as determining step, a unimolecular N-C bond cleavage with formation of an amide (or imide) anion and immonium cation. The immonium cation then rapidly dissociates to formaldehyde and amine. In case of prodrug of ibuprofenamide as an N-Mannich base, the rate of hydrolysis is a very important parameter. The hydrolysis pattern of the prodrugs of N-Mannich bases is such that the N-Mannich bases should be hydrolyzed first to ibuprofenamide (intermediate compound) which subsequently is hydrolyzed to ibuprofen (final compound) indicating the involvement of a two step hydrolysis.

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As the decomposition rates of N-mannich bases is pH-dependent; the chemical kinetic studies were performed at constant temperature but differing pH to see the effect of pH on hydrolysis rate. At constant temperature, 37°, the kinetic studies were done at eight different pH values, which represent the wide range of pH of gastrointestinal tract. The studies were also performed in simulated gastric fluid and simulated intestinal fluid to observe the effect of fluid constituents on the hydrolysis rate of drug from prodrug. The two N-Mannich base prodrugs of ibuprofenamide synthesized were N-(morpholinomethyl) ibuprofenamide hydrochloride (IBMB-M) and N-(piperidinomethyl) ibuprofenamide hydrochloride (IBMB-P).

MATERIALS AND METHODS

Kinetic studies of prodrugs in aqueous buffers:

The kinetic studies were done at eight different pH levels such as 1.2, 3.5, 4.7, 5.3, 6.0, 6.8, 7.4 and 8.0. The synthesized prodrugs IBMB-M and IBMB-P in the concentra-

tion range of $3x10^{-3}$ M were incubated in aqueous buffer at 37°. The samples were withdrawn at 0, 24, 48, 72, 96, 120 and 168 h. The samples were analyzed by high performance thin layer chromatography (HPTLC). One millilitre of the sample was suitably diluted with methanol and applied on the plates for HPTLC analysis. The standards were applied on the same plate. The plates were developed and scanned. The total percentage of each ingredient was quantified.

Kinetic studies of prodrugs in simulated gastric and intestinal fluid:

Prodrugs IBMB-M and IBMB-P in the concentration range of $3x10^{-3}$ M were incubated in the simulated gastric/ intestinal fluid at 37°. Samples were withdrawn at appropriate intervals for 24 h and then analyzed by HPTLC method. Pseudo-first order rate constant (k) for the hydrolysis of N-Mannich bases were calculated from the slopes of the linear plots of the logarithm of the remaining N-Mannich base against time in h and the corresponding half-lives obtained from the identity: $t_{½} = 0.693/k$

Method of analysis:

The samples were applied using automatic sampler Camag Linomat IV on the silica gel 60 F_{254} precoated Aluminium plates (E. Merck) 20x10 cm, 0.2 mm thick. The samples were applied as 6 mm band and the distance of 6 mm maintained between the bands. They were developed in the Camag Twin Trough Chamber. For the mixture of ibuprofen+ibuprofenamide+IBMB-M, the mobile phase was methylene chloride:methanol:liquid ammonia::92:8:0.5 and for the mixture of ibuprofen+ibuprofenamide+IBMB-P, the mobile phase was methylene chloride:methanol:liquid ammonia::95:5:0.5. After development, the plates were removed from the chamber and dried. Plates were then scanned on TLC scanner II at 221 nm. Integration of peaks was done using Cats3 software programme.

Kinetics of decomposition in human plasma:

Ten milligrams of IBMB-M/IBMB-P was dissolved in 10 ml methanol. Five hundred micrograms of prodrug solution was added to human plasma and mixed well and total volume was made upto 10 ml with human plasma. It was kept in the incubator at 37°. Samples were withdrawn at 0, 4, 24, 48, 72 and 96 h. To 0.5 ml of sample, 2 ml of acetonitrile was added and it was vortex mixed for 60 s and then centrifuged for 15 min at 2000 rpm. Supernatant was decanted out and suitably diluted and passed through C_{18} ELUT cartridge filter. The filtrate was then injected to HPLC system.

The High Performance Liquid Chromatograph (TOSCH, Scientific Instrument Division, Japan) consisted of dual piston reciprocating pump (model CCPM), a rheodyne Injection system (model 7125) with loop capacity of 10 ml and variable wavelength detector (model UV 8010) and integrator SIC-12 was used. Stainless steel column (250x4.6 mm, 10 microns, Phenomenax Inc. U.S.A.) packed with C_{18} Hypersil was used.

For HPLC, a solvent system acetonitrile:water (containing 1% acetic acid) 55:45 was used. The flow rate was monitored at 230 nm. Under these conditions ibuprofenamide had an elution time 4.9 min while that of ibuprofen was 7.4 min. The amount of drug (ibuprofen+ibuprofenamide) in the sample was calculated and the % of ibuprofenamide and % of ibuprofen was determined.

RESULTS AND DISCUSSION

Kinetic studies were performed at constant temperature but differing pH to see the effect of pH on hydrolysis rate. For N-Mannich bases as prodrugs of amide of ibuprofen, two- step hydrolysis is required. N-Mannich bases should be hydrolyzed to ibuprofenamide (intermediate compound) which in turn hydrolyzed to ibuprofen (parent compound). But from the kinetic studies performed at different pH, it was found that in every case N-Mannich base got hydrolyzed to ibuprofenamide and in no case ibuprofen was released. There was only one step hydrolysis taken place.

The pseudo-first order rate constants were calculated from the slopes of linear plots of log % N-Mannich base remaining to be hydrolyzed against time. The log values of observed apparent first order constants were plotted against pH to see the influence of pH on the degradation rate of N-mannich base. The effect of pH on the rate of hydrolysis and half-lives of the prodrugs are shown in figs.1 and 2.

From pH-rate profile it was found that IBMB-M was hydrolyzed at lower pH values (pH 1.2-4.7) and as the pH increased the hydrolysis rate started decreasing. At pH 1.2 hydrolysis rate was $1.7x10^{-2}$ h^{-1} and $t_{1/2}$ was 40.76 h. As the pH increased to 6, the rate of hydrolysis was decreased to $2.76x10^{-4}$ h^{-1} and $t_{1/2}$ was increased to 251 h. But there was totally opposite pH rate profile was observed with IBMB-P (as compared to IBMB-M). At lower pH, very slow hydrolysis of prodrug was observed; as the pH increased the hydrolysis rate started increasing. The hydrolysis rate was maximum at pH 7.4. The k value was $8.4x10^{-2}$ h^{-1} and half-life was 20.38 h., whereas at pH 1.2, the k value was $5.98x10^{-4}$

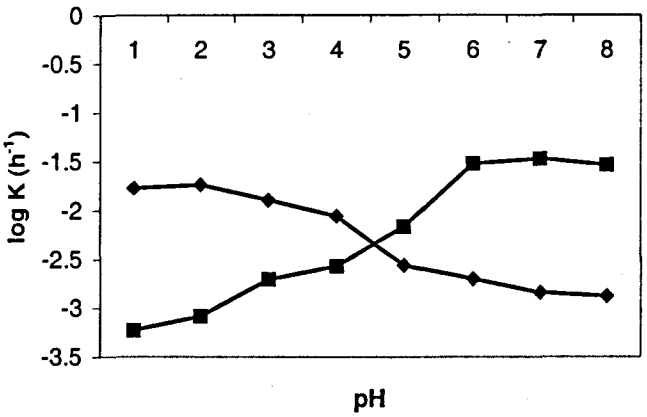

Fig. 1: pH- rate profile of IBMB-M and IBMB-P.

The influence of pH on the rate of hydrolysis of IBMB-M (-◆-) and IBMB-P (-■-).

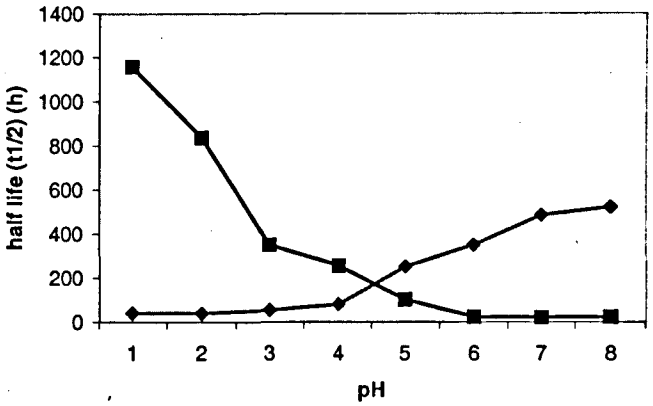

Fig. 2: Relationship between pH and half-life of IBMB-M (-◆-) and IBMB-P (-■-).

and half-life was 1158.8 h.

Kinetic studies of IBMB-M and IBMB-P were performed in simulated gastric fluid and simulated intestinal fluid to see the effect of constituents of gastric and intestinal fluids on hydrolysis rate. It was found that prodrug was hydrolyzed to ibuprofenamide but not to ibuprofen.

In simulated gastric fluid, the hydrolysis rate of IBMB-M was 4.37×10^{-2} h^{-1} and $t_{1/2}$ was 15.85 h. If we compare the hydrolysis rate in pH 1.2 buffer there was 2.5 times increase in the rate of hydrolysis in gastric fluid. But there was very little difference in the hydrolysis rate of IBMB-M in simulated intestinal fluid as compared to hydrolysis in pH 7.2 buffer. There was also the increase in rate of hydrolysis of IBMB-P in gastric fluid compared to the hydrolysis in pH 1.2 buffer. In gastric fluid the half-life was found to be 396 h. and in pH 1.2 buffer, the half-life was 1158.8 h. But there was not much difference observed in k value of simulated intestinal fluid and pH 7.4 buffer.

The hydrolysis of N-Mannich bases (IBMB-M, IBMB-P) is pH-dependent; it is not dependent on the enzymes. But the hydrolysis of ibuprofenamide to ibuprofen is enzymatically controlled; the amidase enzyme is responsible for conversion of amide to acid (ibuprofen). The amidase enzyme is only present in liver, some gastro-intestinal microorganisms and neoplastic tissues but not in plasma. This is the reason for slow hydrolysis of ibuprofenamide to ibuprofen in human plasma.

Kinetic studies of IBMB-M showed that after 96 h only 50% of IBMB-M got hydrolyzed to ibuprofenamide but very little quantity, only 9% of ibuprofen was hydrolyzed after 96 h. In case of IBMB-P, 100% of N-Mannich base got converted to ibuprofenamide and only 10% of ibuprofen hydrolyzed after 96 h.

The k value and $t_{1/2}$ values of IBMB-P in human plasma are comparable with the k and $t_{1/2}$ values in aqueous buffer pH 7.4. It showed a little effect of plasma constituents on hydrolysis of IBMB-M/IBMB-P to the ibuprofenamide but there was certainly some effect of plasma constituents on hydrolysis of ibuprofenamide to ibuprofen. The hydrolysis of ibuprofenamide to ibuprofen could be attributed to the presence of some proteolytic enzymes in plasma, which also act at amide linkage.

From these kinetic studies we can conclude that the N-Mannich bases of amide of ibuprofen can be successfully used as prodrugs. The hydrolysis of N-Mannich base to the ibuprofenamide is dependent on pH but the conversion of ibuprofenamide to ibuprofen is enzymatically controlled. The prodrugs behave in different manner under in vitro and *in vivo* conditions because many biological factors play an important role on the release rate of drug from prodrug during *in vivo* studies. Hence it is important that the release rate studies should be performed under *in vivo* conditions.

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