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In Vivo Pharmacokinetic Studies of Prodrugs of Ibuprofen

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In vivo pharmacokinetic studies of N-Mannich base derivatives of ibuprofenamide as prodrugs were performed on rabbits. Ibuprofen and both the prodrugs (IBMB-M and IBMB-P) were administered orally and at different time intervals blood samples were collected and assayed for ibuprofen and ibuprofenamide by HPLC method. From the plasma concentration-time profile; $(C_p)_{max}$, t_{max}, AUC and the time required to achieve minimum effective concentration were calculated. N-Mannich base prodrugs first get hydrolyzed to ibuprofenamide which in turn gets hydrolyzed to ibuprofen by the enzyme amidase. The $(C_p)_{max}$ and AUC values of IBMB-M were found to be more compared to IBMB-P. In both the cases ibuprofen started appearing after 2 h and it required minimum 4 h to get the ibuprofen in therapeutic range. Both the prodrugs released ibuprofen slowly which gave sustained effect. IBMB-M provided ibuprofen in therapeutic range for 48 h and IBMB-P for 24 h.

The non-steroidal antiinflammatory agents have major drawbacks of causing gastrointestinal ulcerogenicity. The prodrug approach was used to get a safer NSAID, where the drug containing –COOH or –OH group is converted to prodrug. The prodrugs of ibuprofen were prepared as N-Mannich base derivatives of ibuprofenamide using either morpholine or piperidine as amine component. Two prodrugs of ibuprofen were synthesized. These were, N-(morpholinomethyl) ibuprofenamide hydrochloride (IBMB-M) and N-(piperidinomethyl) ibuprofenamide hydrochloride (IBMB-P)[1]. The *in vitro* kinetic studies of the prodrugs were performed in aqueous buffers at different pH values in simulated gastric and intestinal fluids and in human plasma at 37°. The results showed that hydrolysis took place in two steps. First the N-Mannich base was hydrolyzed to ibuprofenamide which was pH-dependent and then ibuprofenamide was converted to ibuprofen which was enzymatically controlled[2].

The prodrug behaves differently under *in vitro* and *in vivo* conditions because many biological factors play an important role in bioavailability and release rate of drug from prodrug during *in vivo* studies. The ideal way to observe the appearance of drug from prodrug is by actual studies in humans. But as the prodrugs are new drugs, it is not feasible to perform *in vivo* studies directly on humans[3-7]. Rabbit was selected

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as an animal model to study the release pattern of prodrug as there are some physiological similarities of rabbits with humans. The purpose of this study is to determine the availability of drug ibuprofen and ibuprofenamide from prodrugs IBMB-M and IBMB-P; and the time required to achieve minimum effective concentration.

Four adult rabbits of either sex each weighing 3.0-3.5 kg were used in a cross over study. The protocol of the cross-over study was approved by the IAEC. Rabbits were fasted overnight but water was allowed *ad libitum*. Before administering the dosage form, control blood samples were obtained from marginal ear vein of all the rabbits. The ibuprofen and IBMB-P were given in suspension form (2% methylcellulose). IBMB-M was given in solution form. The drug ibuprofen (25 mg/kg) and molar equivalent of prodrug (equivalent to 25 mg/kg ibuprofen) were administered orally via Ryle's tube (intubation tube). After drug administration, 2 ml of blood samples were collected at time intervals in the test tube containing heparin. The plasma fractions were then assayed for ibuprofen and ibuprofenamide. Graphs of plasma concentration (µg/ml) of ibuprofenamide/ibuprofen vs. time(h) were plotted (figs. 1-3).

The concentration of ibuprofen and ibuprofenamide was determined by HPLC method[2]. Whole blood samples were centrifuged at 1000 rpm for 15 min, the plasma was separated out using Pasteur pipette. To 0.5 ml of plasma, 2 ml of acetonitrile was added for protein precipitation, it was vortex mixed for 60 s and then centrifuged for 15 min at 2000 rpm. The supernatant was passed through C_{18} elute filter. The concentration of ibuprofen and ibuprofenamide in the filterate was determined using HPLC. The standards were prepared daily from fresh plasma spiked with known quantities of ibuprofen and ibuprofenamide. A solvent system acetonitrile: water (containing 1% acetic acid) 55: 45 was used at the flow rate of 1.4 ml/min. The drug was analyzed at 230 nm. Under these conditions ibuprofenamide had an elution time 4.9 min while that of ibuprofen was 7.4 min.

The *in vivo* studies of the prodrugs were performed on rabbits. The appearance of ibuprofenamide (IBA) and ibuprofen (IBU) from the prodrugs was observed. The prodrug as Mannich base was first hydrolyzed to ibuprofenamide, which in turn was hydrolyzed to ibuprofen. The conversion of prodrug to ibuprofenamide was pH dependent. The analysis of the plasma samples was done by HPLC method.

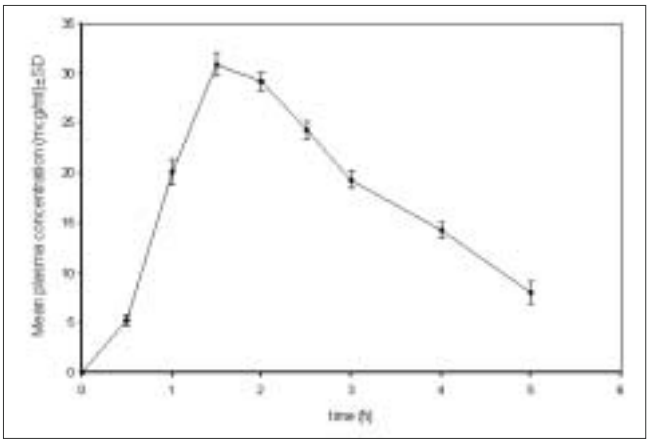

Fig. 1: Mean plasma concentration time profile of IBU.
The plot shows plasma concentrations of ibuprofen (-●-) at different time intervals. Each value represents the mean±SD of four subjects. Each subject was given 25mg/kg of ibuprofen.

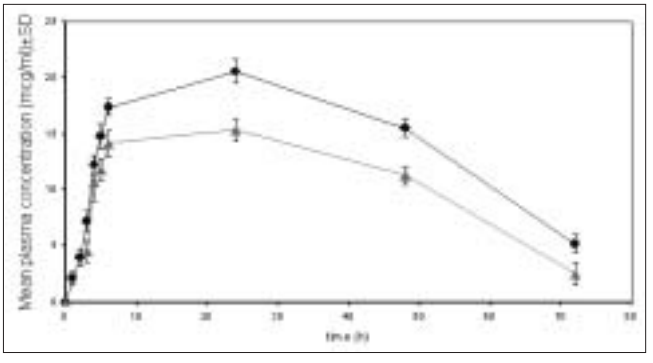

Fig 2: Mean plasma concentration-time profile of ibuprofen and ibuprofenamide showing release rate kinetics of IBMB-M
The graph shows the mean plasma concentrations ± SD of ibuprofen (—▲—) and ibuprofenamide (—●—) at different time intervals. Each value represents the mean±SD of four subjects. Each subject was given IBMB-M equivalent to 25 mg/kg ibuprofen.

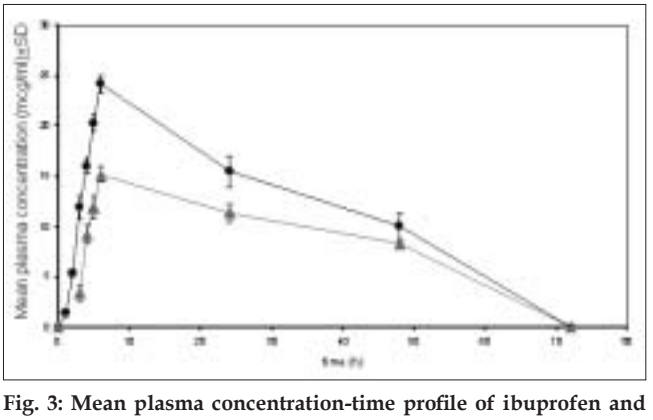

Fig. 3: Mean plasma concentration-time profile of ibuprofen and ibuprofenamide showing release rate kinetics of IBMB-P.
The graph shows the mean plasma concentrations±SD of ibuprofen (—▲—) and ibuprofenamide (—●—) at different time intervals. Each value represents the mean±SD of four subjects. Each subject was given IBMB-P equivalent to 25 mg/kg ibuprofen.

For the comparison purpose ibuprofen (25 mg/kg) was administered orally to the rabbits in the suspension form, and $(C_p)_{max}$ and t_{max} was determined. The $(C_p)_{max}$ was found to be 30.91 µg/ml and t_{max} was found to be 1.5 h. After 2 h, the plasma level of ibuprofen started declining. Molar equivalent of IBMB-M to 25 mg/kg of ibuprofen was administered orally to rabbits in solution form, as IBMB-M is a water-soluble prodrug.

The ibuprofenamide (IBA) started appearing after one hour and the plasma levels of IBA were found to be too low (2.16 µg/ml). After 6 h, the plasma level of IBA reached to 17.38 µg/ml and after 24 h, the plasma levels were at maximum of 20.57 µg/ml. But after that plasma level of IBA started declining; the plasma level was found to be 15.52 µg/ml after 48 h, and 5.2 µg/ml after 72 h. The $(C_p)_{max}$ was found to be 20.57 µg/ml and t_{max} was achieved within 24 h.

The ibuprofen started appearing after 2 h. After 4 h the plasma level was found to be 10.75 µg/ml. The therapeutic effective concentration of IBU is 10 µg/ml. So after 4 h, the minimum effective concentrations of ibuprofen were achieved. The plasma levels of ibuprofen were increased to 14.14 µg/ml after 6 h. After 24 h, the $(C_p)_{max}$ was achieved to 15.35 µg/ml. Even after 48 h, the ibuprofen was present in therapeutic range 11.27 µg/ml. But after 72 h, the concentration was found to be very low.

The results showed that as IBMB-M is water soluble, it did not require dissolution time. From previous kinetic studies we know that the hydrolysis rate of IBMB-M to IBA was high at acidic pH of stomach. That is why the ibuprofenamide started appearing after one hour, but the concentration was very low. There was a lag time for appearance of ibuprofen. The ibuprofen started appearing only after 2 h. Here comes the role of amidase enzyme, which is present in liver. The conversion of ibuprofenamide to ibuprofen requires amidase enzyme, so as and when ibuprofenamide was hydrolyzed from prodrug (Mannich base), it was converted to ibuprofen by amidase enzyme.

IBMB-P was given to rabbits in suspension form, so dissolution rate was also one of the factors. But from previous studies it is known that at acidic pH the dissolution rate of IBMB-P is high, so dissolution rate should not affect the absorption of prodrug. From release rate data, it was found that IBMB-P was hydrolyzed to IBA. After 1 h, the plasma concentration of IBA was found to be 1.42 µg/ml and after six hours 24.22 µg/ml.

From the previous kinetic studies it was found that the hydrolysis rate of IBMB-P was less in acidic pH but at pH 7.4 of plasma, the hydrolysis rate was high. So in the plasma, $(C_p)_{max}$ 24.22 µg/ml of IBA was achieved after 6 h. After 24 h, the concentration was little less, 19.5 µg/ml and after 48 h it was found to be 10 µg/ml. But after 72 h, no IBA could be detected.

Ibuprofen started appearing after 2 h, as seen with IBMB-M. The peak plasma level was achieved after 6 h. The drug concentration was reached to 9.29 µg/ml after 4 h, which was very close to therapeutic concentration. After 24 h, the ibuprofen plasma level was 11.39 µg/ml, which was in therapeutic range but after 48 h; the concentration was found to be less than therapeutic concentration. After 72 h, the ibuprofen could not be detected.

The graph of plasma concentration (µg/ml) vs. time (h) was plotted. The area under curve (AUC) was calculated for both the prodrugs IBMB-M and IBMB-P. The AUC of IBMB-M was more compared to IBMB-P. In case of IBMB-M; the AUC of IBA was 1072.34 µg h/ml and for ibuprofen, 784.91 µg h/ml. And for IBMB-P, the AUC of ibuprofenamide was 935.68 µg h/ml and for ibuprofen 608.82 µg h/ml.

From these results, we can conclude that both the prodrugs release ibuprofen slowly, which gave sustained effect. Ibuprofen started appearing after 2 h and it required at least 4 h to get ibuprofen in the therapeutic range.

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