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Formulation and Evaluation of Compressed Suppositories of Indomethacin

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Compressed suppositories in indomethacin were formulated employing PEG 4000 as base with a view to minimize the gastric irritation of the drug upon oral administration. Preliminary pharmacodynamic experiments displayed comparable antiinflammatory activity and reduced ulcerogenic potential of the drug upon rectal administration.The influence of disintegrants (primogel and potato starch) on the *in vitro* release of drug was studied employing USPXX basket and dialysis methods. Based on the *in vitro* drug release studies from dialysis method, fast release suppositories containing 5% primogel and 10% potato starch were further evaluated for pharmacokinetic performance in healthy human volunteers. The study indicated that disintegrants did not influence either the rate or the extent of absorption of indomethacin compared to plain suppositories. It was concluded that *in vitro* drug release by dialysis method could not simulate the *in vivo* conditions.

Indomethacin (CAS 53-86-1), a potent acidic nonsteroidal antiinflammatory agent (NSAIA), has been used effectively in the management of moderate to severe rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and acute gouty arthritis[1-3]. Like other acidic MSAIAs, indomethacin causes G.I. irritation, peptic ulceration, anorexia, dyspepsia and diarrhoea upon oral administration[4-6]. An alternate route of administration for patients with history of peptic ulceration and/or for those who are on chronic NSAIA therapy would be rectal route.

Conventional methods of formulating suppositories for rectal administration involve fusion of suppository base and use of moulds. Commonly cocoa butter is used as the base which undergoes polymorphism during the process of fusion. We have earlier reported the pharmacodynamic and pharmacokinetic evaluation of compressed suppositories of propranolol, naproxen and diclofenac sodium employing polyethylene glycol (PEG) 4000 as the base, which is stable in tropical climates[7-9]. The compression process offers additional advantage of enhancing the production rate. Present study was undertaken to develop compressed indomethacin suppositories using PEG 4000 as the base. The influence of disintegrants on the *in vitro* drug release and *in vivo* absorption of indomethacin from these suppositories was also evaluated.

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EXPERIMENTAL

Indomethacin was a kind gift from Tablets India Ltd., Madras, India. Polyethylene glycol (PEG) 4000, magnesium stearate, tale, potato starch, primogel (sodium starch glycollate) were of pharmaceutical grade. Methanol and acetonitrile were of HPLC grade and all other chemicals and reagents used were of analytical grade.

Animals:

Wistar rats of either sex weighing 120-140 g were procured from National Institute of Nutrition, Hyderabad, India. Animals were maintained in an air-conditioned animal house with 12 h light and dark cycle. Standard pelleted diet (Hindustan Lever Ltd., Bombay, India) was fed to the animals and water was provided ad libitum.

Formulation of indomethacin suppositories:

Suppositories of indomethacin were prepared by dispersing the drug in molten PEG 4000. The dispersion was stirred until the mass congealed and solidified. The

solidified mass was then pulverized and passed through 20 mesh screen. Disintegrants were added at this stage to the granules. The granules were lubricated with talc and magnesium stearate (1% each) and compressed into 0.8 g suppositories with one end convexed using a fly press. The hardness of all suppositories was in the range of 2.5-3.5 kg/cm^2. In total, there were seven formulations, 3 with primogel at 5, 10 and 15% 3 with potato starch at 5,10 and 15% and one with out any disintegrant. All seven formulations contained 25 mg indomethacin.

Pharmacodynamic studies:

The antiinflammatory activity of indomethacin from rectal suppositories was evaluated by employing the carrageenan-induced paw oedema model[10]. Rats were fasted overnight and were divided into 3 groups of six rats in each. Control group received vehicle solution orally. One test group served for oral administration of indomethacin (5 mg) as 1% gum acacia suspension. Second test group received indomethacin rectally as plain suppository. Carrageenan (0.1 ml, 1%) was injected in the subplantar region of left hind paw for all rats, 1 h after test drugs administration.

Paw volumes were measured at zero hour and at 3 h after the challenge of oedemogen using a digital plethysmometer (Ugo Basile, Italy) for all groups of rats. The per cent inhibition of paw volume in treated groups was calculated with respect to control group.

Rats were sacrificed by cervical dislocation in all groups 7 h after the administration of tests drugs. The stomach of each rat was removed, incised along greater curvature, washed with normal saline and observed under light stereo microscope for ulceration. The ulcer indices, in each group, were calculated according to the reported method[11], by calculating the individual scores in each rat and expressing the ulcer indices as mean±SD of six rats for each group. Control group rats did not show any ulceration. The percent inhibition of ulcerogenecity in rectal suppositories group was calculated by comparing with those of oral indomethacin group.

***In vitro* drug release studies:**

The *in vitro* release of indomethacin from different suppositories was studied employing USP XX basket method. A volume of 900 ml of 0.1 M phosphate buffer of pH 7.2 was used as dissolution medium. The temperature was maintained at 37±0.5°. A test suppository of indomethacin was placed in the basket which was immersed in dissolution medium and rotated at 100 rpm. Aliquots of samples (2 ml) were drawn at predetermined time intervals, each time replacing with the same volume of fresh dissolution medium. The samples were suitably diluted with dissolution fluid and analysed for indomethacin at 320 nm using double beam spectrophotometer (Shimadzu, Japan). The per cent drug released at different time intervals from each suppository formulation was calculated. The experiment was performed in quadruplicate and the mean percent drug released was plotted against time. From these graphs, $T_{50\%}$ (time for 50% drug release) were calculated.

The release of indomethacin from compressed suppositories was also investigated using dialysis membrane. A piece of pretreated dialysis tubing was tied at one end and 15 ml of phosphate buffer (pH 7.2) and a test suppository were placed in the bag. The other end was tied and the bag was suspended in 400 ml of phosphate buffer solution (pH 7.2). The dialysis medium was kept under stirring at 100 rpm and was maintained at a constant temperature of 37±0.5°. At regular time intervals, samples (2 ml) were withdrawn from dialysis medium and the volume of the medium was maintained constant through out the experiment. The samples were analyzed for drug content at 320 nm, after appropriate dilution with drug free dialysis medium, using double beam UV spectrophotometer (Shimadzu, Japan). The experiment was performed in quadruplicate and the percent of drug release at each time interval was plotted against time. From these plots $T_{50\%}$ (time for 50% drug release) values were calculated.

Pharmacokinetics human of indomethacin suppositories (50 mg):

Indomethacin suppositories containing 50 mg drug with (a) 5% primogel, (b) 10% starch and (c) no disintegrant were used for rectal administration. For oral administration, commercially available indomethacin capsules (IDICIN®, IDPL, Hyderabad) purchased from local pharmacy store were used.

The study was conducted as 4 way randomized crossover manner in 8 healthy male human volunteers. Complete physical and clinical examinations were done for the volunteers to ascertain their health. Volunteers were informed about the purpose of the study and a written consent was obtained from them that their participation in this study was voluntary. The study was approved by competent local ethical committee.

Volunteers participated in the study after an overnight fast. For oral administration, volunteers were instructed in take two capsules (2x25 mg) of indomethacin with 150 ml of water. Suppositories were used for rectal administration after complete evacuation of the bowel. All drug products were taken around 0800 h in a day. Blood samples (2 ml) were drawn from forearm vein at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 h after administration of formulations. Serum was separated immediately by centrifuging the blood samples and stored at -20° until analysed. The washout period for each treatment was one week. Indomethacin was estimated in the serum samples by employing a sensitive and extractionless HPLC technique[12] with minor modifications.

Estimation of indomethacin from serum:

To 0.5 ml of serum, 100 µl (10 µg) of naproxen (100 µg/ml in methanol) was added as internal standard. The samples were vortexed for 5 sec then 1 ml of methanol was added to precipitate serum proteins. The samples were vortexed for exactly 1 min. and centrifuged at 3000 rpm for 15 min. A volume of 25µl of supernatant was injected into HPLC system (Shimadzu LC-6A, Shimadzu, Japan) fixed with reverse phase C_{18} column (Machery-Nagel, Duren, Germany, 250 mm x 5 mm ID). The wavelength was set at 258 nm and analysis was run at 0.4 aufs. Mobile phase consisted of acetonitrile and water (50:50) the pH was adjusted to 3.2 with 85% phosphoric acid. The flow rate was 1 ml/min and chart speed was 5 mm/min. Calibration graph of indomethacin to naproxen (internal standard) peak area ratios, was found to be linear over the concentration range of 0.1 to 10 µg/ml of indomethacin in serum with correlation coefficients (r) more than 0.997 . The relative standard deviation was 3.85% indicating that the method is sufficiently precise and reproducible. The lower limit of detection of indomethacin with this method is 50 ng/ml of serum.

Pharmacokinetic and Statistical analyses:

Peak serum concentrations (C_{max}) and time of their occurrences (t_{max}) were read directly from the individual serum concentration time profiles. Other parameters such as AUC area under the curve ($AUC_{0-\infty}$) half life during the terminal phase ($t_{1/2}$) and mean residence time (MRT) were calculated using standard equations of noncompartmental analysis based on statistical moment theory[13].

Students 't' was used to evaluate the significance of the differences observed in antiinflammatory activity and ulcer indices after oral and rectal administration of indomethacin. A value of P less than 0.05 was considered significant. For evaluating the significance of the differences in pharmacokinetic parameters obtained after oral administration of indomethacin capsules and rectal administration of the three types of indomethacin suppositories, ANOVA with Dunnet's multiple comparison procedure was used. Significance was evaluated at 50% confidence level. For the estimation of bioequivalence between rectal suppositories and standard marketed oral capsule, the results from the ANOVA for logarithmic $AUC_{0-\infty}$ and C_{max} were used to calculate the (1-2 α) confidence interval for the mean parameter difference between each rectal suppository (test formulation) and oral capsules (reference product). A test preparation is considered bioequivalent if the 90% confidence interval for the difference was entirely within the 80% to 120% of the mean parameter value of the reference product[14]. The differences in the t^{max} values obtained after oral and rectal administration were evaluated using three one-sided Wilcoxan Rank Sum Tests[15].

RESULTS

All suppositories of indomethacin were found to have fine surface texture. No difficulty was experienced while preparing the suppositories with the proposed compression process. The length and diameter of the suppositories were 26 mm and 9 mm respectively. The weight range was 785 to 810 mg.

Pharmacodynamic studies:

The antiinflammatory activity expressed as percent inhibition of paw oedema volume and ulcer indices obtained after rectal and oral administration of indomethacin are represented in Figure 1. The antiinflammatory activity of indomethacin from rectal suppositories was comparable with that of oral indomethacin as evidenced by insignificant ($p>0.05$) differences in the mean inhibition of paw oedema volumes between rectal and oral indomethacin groups. Significant ($P<0.05$) reduction in ulcer index (82.11%) was observed upon rectal administration of indomethacin compared to oral indomethacin group.

***In vitro* drug release studies:**

The *in vitro* release profiles of compressed indomethacin suppositories obtained with USP XX basket and dialysis methods, respectively, are represented in Figures

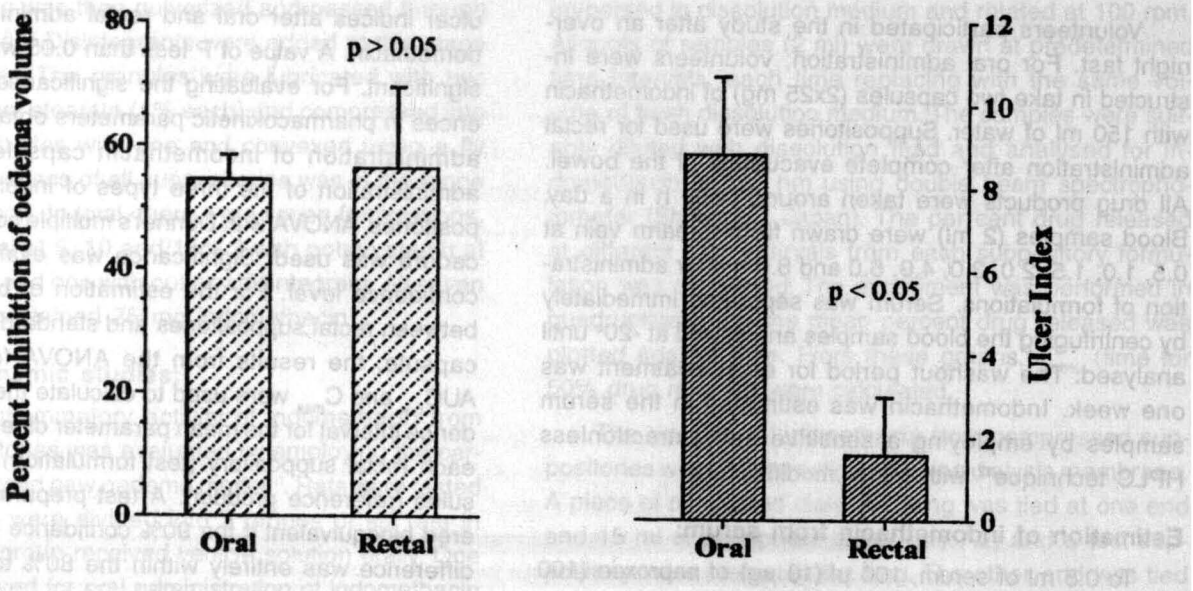

Fig. 1 : Antiinflammatory activity (% inhibition of rat paw oedema volume) and ulcer indices observed after oral and rectal administration of indomethacin (Mean±SD, n=6)

2 and 3. $T_{50\%}$ values obtained by both method are given in Table 1. The drug release was very fast from plain as well as suppositories by USP XX basket method. The release profiles from suppositories with disintegrants were not markedly different from those of plain suppositories as evident from insignificant differences in $T_{50\%}$ values.

The release of indomethacin from plain suppositories was slow by dialysis method compared to USP XX basket method. Addition of disintegrants significantly improved the release rate of the drug in contrast to USP XX basket method. Among the suppository formulations containing disintegrants, suppositories with 5% primogel and with 10% potato starch displayed fastest release of indomethacin as evident from their shorter $T_{50\%}$ values in dialysis method (Table 1). Based on these observations the above formulations were further evaluated for pharmacokinetic performance in healthy human volunteers.

Pharmacokinetic evaluation of Indomethacin (50mg) suppositories in healthy human volunteers:

The mean serum concentration-time profiles of indomethacin obtained after oral administration and after rectal administration are represented in Figure 4. The mean (±SD) values of all other parameters such as $t_{1/2}$, t_{max} and mean residence time (MRT) obtained after oral and rectal administration are given in Table 2. The t_{max}

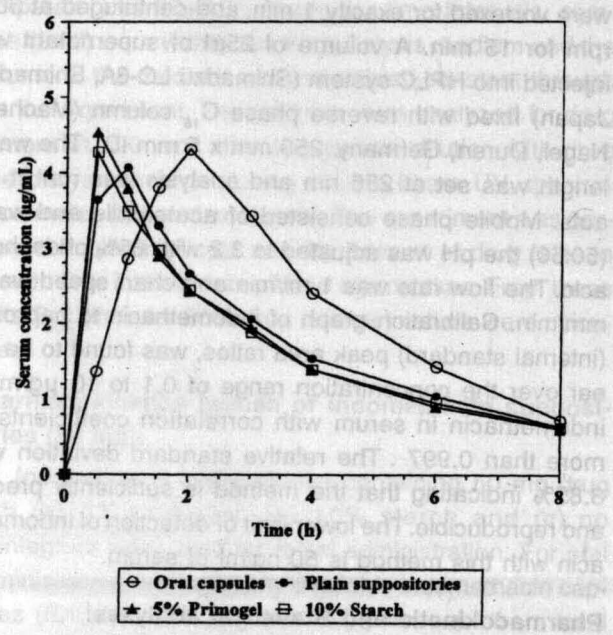

Fig. 2 : Mean serum concentration -time profiles of indomethacin (50 mg) administered orally (2 x 25 mg capsules) and rectally (as different suppositories) in healthy human volunteers (n=8) (SD bars are not represented for the sake of clarity)

values differed significantly ($P<0.05$) and peak concentrations were obtained, relatively faster (30 to 50 min) in case of rectal administration compared to marketed oral

indomethacin capsules (t_{max} oral = 2 h). The differences in t_{max} values among the three suppository formulations were insignificant (P>0.05). The differences in other pharmacokinetic parameters viz., C_{max}, $AUC_{0-\infty}$ $t_{1/2}$ and MRT were insignificant (p>0.05) among suppository formulations and also between rectal and oral administration of indomethacin. The percent bioavailabilities of indomethacin after rectal administration were 83.70, 80.38 and 78.87 for plain PEG 4000 suppositories (without disintegrants), suppositories with 5% primogel and suppositories with 10% starch respectively, calculated with reference to marketed oral capsules of indomethacin.

DISCUSSION

It has been well documented that Indomethacin was more toxic on the stomach by the oral route than by intravenous route, suggesting that direct tissue contact mechanism is more important factor in producing lesions of gastric mucosa[16]. Hence, rectal administration of Indomethacin is proposed in the present investigation to avoid the direct local contact of the drug with gastric mucosa. Compressed PEG 4000 suppositories containing 5 mg indomethacin were evaluated for preliminary pharmacodynamic (antiinflammatory) activity in an acute carrageenan-induced rat paw oedema model and ulcerogenic potential, simultaneously, in a single experiment. The results were compared with those of oral indomethacin given to rats as suspension. The per cent inhibition of rat paw oedema volume observed after rectal administration (56.26±12.86) did not differ significantly (p>0.05) from that observed after oral administration (54.78±3.75) indicating comparable antiinflammatory activity. There was significant (P<0.05) reduction in ulcer index upon rectal administration (1.58±1.40) compared to oral indomethacin (8.836±1.86) suggesting that avoiding tissue contact of indomethacin with gastric mucosa minimizes gastric ulceration. There was about 82.1% ulcer prevention activity of indomethacin upon rectal route which is therapeutically advantageous.

The release of indomethacin was very fast from all compressed suppositories by USP XX basket method ($T_{50\%}$<13 min). No significant differences in $T_{50\%}$ values were obtained between plain suppositories and suppositories containing disintegrants. The high solubility of PEG base and large volume of dissolution fluid might have resulted in very fast release and hence the effect of disintegrants could not be observed. Hence, USP XX basket method can not be used to evaluate the *in vitro* drug release from PEG based suppositories. This is in accordance with earlier report by Vidras et al. (1982)[17]. The same authors opined that dialysis in suitable method for evaluating the drug release from PEG based suppositories. Accordingly dialysis method was used to assess the *in vitro* release of indomethacin from fast release suppositories.

Table 1 : $T_{50\%}$ values for different fast release suppositories of Indomethacin obtained by using USP XX basket and dialysis methods

Suppository containing	USP XX basket method $T_{50\%}$ (min)	Dialysis method $T_{50\%}$ (min)
No disintegrant	12	120
5% Primogel	11	54
10% Primogel	10.5	81
15% Primogel	10	135
5% Potato starch	10.5	105
10% Potato starch	9.5	69
15% Potato starch	9.5	84

The release of indomethacin from plain suppositories was slow by dialysis method ($T_{50\%}$ = 2 h) compared to USP XX basket method (Table 1). Addition of disintegrants significantly improved the drug release rate as evident from lower $T_{50\%}$ values for fast release suppositories. Among the various concentrations of disintegrants use, suppositories containing 5% primogel and 10% potato starch displayed the fastest drug release as evident from $T_{50\%}$ values. Based on the in vitro release studies by dialysis method, suppositories containing 5% primogel and 10% starch were further evaluated for pharmacokinetic performance in healthy human volunteers.

The study was conducted as single dose randomised cross over desing in 8 healthy male human volunteers. The drug was rapidly absorbed after rectal administration and peak serum concentrations of the drug were observed around 30 min. The peak serum concentrations appear to be more reproducible between subjects with respect of peak heights and peak times following rectal administration as evidenced by the t_{max} values (Table 2). Low peak concentrations (C_{max}) values in blood are

Table 2 : Pharmacokinetic parameters (mean±Sd, n=8) obtained after administration of indomethacin orally as (2 x 25 mg) capsules and rectally as different suppositories in healthy human volunteers

Parameter	Oral capsules (2x25 mg)	Indomethacin (50 mg) suppositories containing No disintegrant	5% primogel	10% potato starch
C_{max} (µg/ml)	4.79±0.68	4.53±0.79	4.81±0.96	4.36±0.74
t_{max} (hr)	1.94±0.51	0.81±0.37	0.56±0.17	0.56±0.17
$t_{1/2}$ (hr)	2.34±0.42	2.53±0.31	2.60±0.31	2.65±0.11
$AUC_{0-\infty}$ (µg.hr/ml)	19.88±5.03	16.64±4.44	15.98±1.67	15.68±2.09
Percent Availability	100	83.70	80.38	78.87

usually seen when the suppository is expelled unintentionally during the study. However in the present study, no such situation was observed. Volunteers did not report any side effects in the study period during rectal administration.

The differences in C_{max} values were insignificant ($P>0.05$) among the three suppository formulations and the C_{max} value of each suppository formulation was comparable with that of oral indomethacin capsules. The t_{max} values did not differ significantly ($p>0.05$) among the suppository formulations. The differences in other pharmacokinetic parameters namely $AUC_{0-\infty}$, $t_{1/2}$ and MRT among suppository formulations were insignificant ($p>0.05$). It is known that C_{max} and t_{max} values are indicative of the rate of drug absorption. Since, insignificant differences were obtained for C_{max}, t_{max} and $AUC_{0-\infty}$ values among suppository formulations, it is clear from the present study that disintegrants did not enhance either the rate or the extent of absorption of indomethacin from fast release suppositories. Thus, though fast release characteristics were displayed by the suppositories containing disintegrants in *in vitro* dialysis experiments, they could not improve or enhance the absorption of the drug in *in vivo* dialysis experiments, they could not improve or enhance the absorption of the drug in *in vivo* experiments conducted in human volunteers. This may be due to small volume of rectal fluid which restricted the dissolution of the drug from suppository formulations inspite of the high invasion rate of the rectal mucosa. Accordingly the anticipated t_{max} value were not achieved even after addition of disintegrants. Hence, it is evident from our present study, that even dialysis method can not simulate the *in vivo* condition.

When pharmacokinetic parameters obtained after rectal administration were compared with those of oral indomethacin capsules, only t_{max} differed significantly ($P<0.05$) which is around 2 h for oral capsules compared to that of rectal suppositories (30-50 min). The prolonged time taken by the drug upon oral administration to give peak concentrations can be explained by the inter-individual variability in gastric emptying time, gastric pH and motility of GI tract and also due to the poor solubility of drug is aqueous gastric fluids.

Bioequivalence of all three rectal suppositories with oral indomethacin capsules was judged by using confidence interval approach[14]. The tested parameters $AUC_{0-\infty}$ and C_{max} of each rectal suppository formulation had limits of their 90% confidence interval for the mean clearly within±20% of the mean of reference parameters of standard oral marketed capsule. Thus, all the three suppositories fulfilled the criteria for bioequivalence with the oral marketed indomethacin capsule. The relative rectal bioavailabilities of indomethacin from plain PEG suppositories, suppositories with 5% primogel and suppositories with 10% starch were 83.7%, 80.38% and 78.87% respectively indicating good pharmacokinetic performance of indomethacin upon rectal administration.

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