# Comparative Bioavailability Studies of Indomethacin from Two-Controlled Release Formulations in Healthy Albino Sheep

D. V. GOWDA*, H. G. SHIVAKUMAR
Department of Pharmaceutics, J. S. S. College of Pharmacy, S. S. Nagara, Mysore-570 015, India.

The objective of the study was to obtain the pharmacokinetic data of two controlled release formulations of (75 mg) indomethacin and to compare the relative bioavailability of the test formulation (product B cetostearyl alcohol microspheres) with standard formulation (product A–Microcid®SR 75 mg capsule). A single dose randomized (4×2) complete cross over study of the indomethacin (75 mg) was carried out on 8 healthy albino sheep. The study was carried out on two separate occasions with a washout period of 2 weeks. Blood samples were collected at predetermined time intervals. Plasma indomethacin concentrations and other pharmacokinetic parameters obtained were statistically analyzed. The results of the paired t-test for the comparison of pharmacokinetic data showed that there was no significant variation between the products A and B. Products did not show any significant difference between them with regard to the $T_{max}$, $C_{max}$, $AUC_{O-24}$, $AUC_{O-\infty}$, MRT, $K_a$, $K_{el}$, $t_{1/2}$, 3.0 h, 2054 ± 55.78 ng/ml, 9637 ± 132.87 ng h/ml, 9870 ± 129.22 ng h/ml, 4.76 ± 0.10 h, 0.3812 ± 0.002 $h^{-1}$, 0.2713 ± 0.004 $h^{-1}$, 2.55 ± 0.03 $h^{-1}$, respectively for product A and 3.5 h, 1929 ± 20.32 ng/ml, 8343 ± 40.04 ng h/ml, 8617 ± 46.88 ng h/ml, 4.98 ± 0.02 h, 0.3648 ± 0.002 $h^{-1}$, 0.2427 ± 0.010 $h^{-1}$, 2.86 ± 0.20 $h^{-1}$ for product B. From the dissolution studies and *in vivo* bioavailability studies, it was concluded that the products A and B are bioequivalent.



Indomethacin (IM) is an important indole acetic acid non-steroidal antiinflammatory drug commonly used in the treatment of rheumatoid arthritis and other severe inflammatory diseases[1,2]. IM is an inhibitor of PG synthesis and used for several inflammatory orthopathies[3], but in recent years IM has also been recommended as the treatment of choice for low birth weight infants with ductus arteriosus[4]. IM associated adverse effects are due to initial high plasma concentrations[5]. The occurrence of these adverse effects can be reduced by the use of controlled release formulations or by the concurrent administration of IM with probenacid[6,7]. Oral conventional dosage forms are administered three to four times to maintain adequate and effective therapeutic concentration in blood, which is responsible for occurrence of high initial peak plasma concentrations. However it fails to protect the patients against morning stiffness[8]. Thus the development of controlled release formulations of IM have several advantages over the other conventional dosage forms, *viz.*, reduction in occurrence of high initial peak plasma concentrations, protection against morning stiffness, prolonged duration of action, improved bioavailability, patient compliance and reduction in adverse effects[9,10]. Controlled release formulations can provide convenient treatment regimens as compared to the conventional formulations[11,12]. In the present investigation indomethacin microspheres and capsule were tested for *in vitro* dissolution. Further, comparative bioavailability of indomethacin from microspheres and capsule in healthy sheep was carried out. Plasma concentrations of IM were quantified by a modification of the HPLC method described for IM by Johnson *et al.*[13]

***For correspondence**
E-mail: dvg5@lycos.com

## MATERIALS AND METHODS

IM and mefanamic acid (MA), the internal standards were generously donated by Micro labs (Bangalore, India). All other chemicals and solvents were of analytical grade and were supplied by Ranbaxy, fine chemicals (New Delhi, India). Formulations (Microcid®SR 75 mg) and indomethacin loaded in cetostearyl alcohol microspheres are coded as product A and product B, respectively.

### *In vitro* release and ageing studies:

In the present study, *in vitro* drug release profiles for

products A and B were carried out using an USP XXI dissolution apparatus type II. Encapsulation of the microspheres was avoided, as dissolution of shell will add an additional parameter to the result. Dissolution studies were carried out for the formulation containing microspheres and compared with that of commercial formulation (Microcid SR® - 75 capsule) in 900 ml dissolution medium (2 h in pH 1.2 hydrochloric acid buffer and 6 h in pH 7.2 phosphate buffer). A small amount of Tween 80 (0.1%) was added to increase the wettability of microspheres. The dissolution media was maintained at 37° ± 0.5° and stirred at 100 rpm. Drug release from the formulations were determined by withdrawing 10 ml of sample using guarded pipette at 30 min time intervals for first 4 h and at 1 h interval for the remaining 4 h. Samples withdrawn were estimated after appropriate dilution. Release studies were carried out in triplicate. The drug release from the products A and B was calculated using standard drug release equations and compared with that of commercially available oral formulation, product A.

Effect of ageing on drug release studies were carried out taking products A and B which were stored in a dessicator at 25º and 11% relative humidity for a period of 8 w. Each product (100 mg) was taken on the 1, 2, 4, and 8 w and was subjected to *in vitro* drug release studies. Release studies were carried out in triplicate.

***In vivo* studies:**

A written approval was obtained from the Institutional Animal Ethical Committee of JSS Medical College Hospital and JSS College of Pharmacy, Mysore, India. Detailed verbal and written information on the study was provided to the Veterinary surgeon, in charge for Central Animal Facility, JSS Medical College Hospital and written consent was obtained. Four male and four female healthy adult albino sheep were included in this study. The age of the sheep were in the range of 6-8 (7.13 ± 0.64 mean ± SD) y and their body weight ranged between 30-35 (31.87 ± 1.8 mean ± SD) kg. Based on medical history, examination, and laboratory investigation, none of the subjects had any medical abnormality. Provisions were made for all observed signs and symptoms occurring during the study period to be recorded.

**Study design:**

The study was conducted as an open, randomized complete cross over design in which a single 75 mg dose of each products (A and B) was administered to fasted, healthy adult male and female sheep on two different occasions, separated by a wash out period of 2 w between dosing interval.

**Blood sampling:**

All the animals were reported to the pre-clinical trial laboratory from animal house at 7.00 a.m., after over night fasting of 10 h. After shaving near the neck region of sheeps, a 18 gauge (1.3×45 mm, 96 ml/min) cannula was inserted in to jugular vein and kept with heparinised saline lock for ensuing 24 h blood sampling. Test medication products A and B were administered to the subjects with banana and 200 ml of water. A light food was provided at 3 h followed by two standard meals at 7 and 11 h following the administration of drug. Blood sample of 5 ml each was collected at 0 h (pre-dose) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 16, 20, and 24 h post dose intervals. The samples were centrifuged at 1500 'g' for 10 min, the plasma was separated and stored at -20° prior to analysis. Any other type of food was not permitted after 12 h after the administration of test medication. All subjects remained ambulatory and strenuous physical activity was disallowed during the first 12 h of blood sampling. Plasma concentrations of drug from all the formulations and its corresponding commercial formulation were quantified by a modified HPLC method as described by Johnson *et al.*[13]

**Statistical data analysis:**

The pharmacokinetic parameters were calculated using the Quick calk, computer PK calculation programme[14]. The drug plasma concentration and pharmacokinetic parameters were subjected to paired t-test and analysis of variance at 95% confidence limit.

## RESULTS AND DISCUSSION

From the release studies, it was observed that, there was no significant release of drug at gastric pH. At the end of 8 h, drug release from products A and B at intestinal pH was found to be 94.80% and 99.41%, respectively. The cumulative percent drug release after ageing from products A and B was within the range (94%) and there was no significant change in the *in vitro* drug release was noticed after to 8 w of ageing.

The mean plasma concentration time profiles and comparative mean pharmacokinetic parameters of indomethacin following the administration of the two products (A and B) are shown in fig. 1 and Table 1. After oral administration, the highest mean $C_{max}$ values was

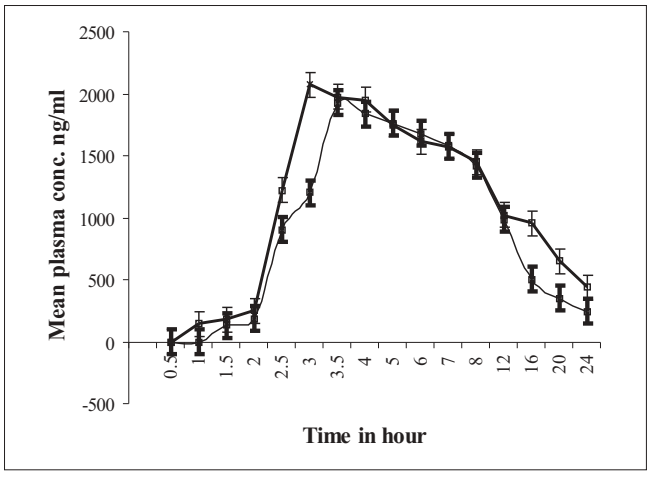


**Fig. 1: Mean plasma concentrations–time profiles of IM Plasma concentration curves for 8 subjects following oral administration of product A (▬) in the form of a capsule containing granules against product B (---) in the form of microspheres**

**TABLE 1: A STATISTICAL COMPARISON OF THE MEAN VALUES OF PHARMACOKINETIC PARAMETERS**

| Parameters | Product A | Product B | P value |
|---|---|---|---|
| $C_{max}$ | 2054 ± 55.78 ng/ml | 1929 ± 20.32 ng/ml | >0.05 |
| $T_{max}$ | 3.0 h | 3.5 h | >0.05 |
| $K_a$ | 0.3812 ± 0.002 $h^{-1}$ | 0.3648 ± 0.002 $h^{-1}$ | >0.05 |
| $K_{el}$ | 0.2713 ± 0.004 $h^{-1}$ | 0.2427 ± 0.010 $h^{-1}$ | >0.05 |
| $T_{1/2}$ | 2.55 ± 0.03 $h^{-1}$ | 2.86 ± 0.20 $h^{-1}$ | >0.05 |
| MRT | 4.76 ± 0.10 h | 4.98 ± 0.02 h | >0.05 |
| $AUC_{0-24}$ | 9637 ± 132.87 ng h/ml | 8343 ± 40.04 ng h/ml | >0.05 |
| $AUC_{0-\infty}$ | 9870 ± 129.22 ng h/ml | 8617 ± 46.88 ng h/ml | >0.05 |

A statistical comparison of the mean values of pharmacokinetic parameters derived from the concentration- time curves of product a and b following oral administration. Product A= Microcid®SR , Product B = Cetostearyl alcohol microspheres

observed for product A (2054 ± 55.78 ng/ml) compared to product B (1929 ± 20.32 ng/ml. However, the difference in the $C_{max}$ values recorded for both products was statistically insignificant. The peak plasma levels were observed to be within the range from 1978 ng/ml to 2086 ng/ml for product A, from 1912 ng/ml to 1955 ng/m for product B. Comparison of the mean concentration time curve of product A with that of product B, indicates that the product B associated with a significant lower peak plasma concentration than that of product A. In addition, the mean plasma concentrations of IM for both the products in all subjects were within the therapeutic window (300-3000 ng/ml)[15]. The time taken to reach peak plasma concentration $T_{max}$ was 3.5 and 3 h for products B and A, respectively. $T_{max}$ of IM was little higher in case of product B, but no statistical significant difference was found between two products. Mean $K_a$ for product A product B were found to be 0.3812 ± 0.002 $h^{-1}$ and 0.3648 ± 0.029 $h^{-1}$, respectively and the difference between the two products was insignificant.

Mean $K_{el}$ for product A and B were found to be 0.2713 ± 0.004 $h^{-1}$ and 0.2422 ± 0.010 $h^{-1}$, respectively. The difference between the values $K_{el}$ obtained from the two formulations was not statistically significant. Mean elimination half life $T_{1/2}$ for product A and product B were found to be 2.55 ± 0.03 $h^{-1}$ and 2.86 ± 0.20 $h^{-1}$, respectively, no statistical significant difference between them.

However, a small difference between both products related to $C_{max,}$ $T_{max,}$ $T_{1/2}$ and reduced fluctuations (peak to trough ratios) of the plasma concentrations. All these effects probably may be due to the dissolution rate limited drug release and hence absorption[16]. From the study it can be observed that reduced fluctuations combined with the elevated mean plasma concentration from both the products, offers advantage in protecting patients against morning stiffness.

Mean residence time (MRT) of products A and B was found to be 4.76 ± 0.10 h and 4.98 ± 0.02 h, respectively. The difference in mean values of MRT from the two formulations was statistically insignificant.

The mean $AUC_{0-24}$ values for products A and B was 9637 ± 132.87 ng h/ml and 8343 ± 40.04 ng h/ml. From the result, statistical analysis indicated that the product B exhibited a smaller and non-significant reduction in the AUC values. It was observed that the slow release of IM from the products A and B may be responsible for the decreased AUC values when compared to the reported conventional dosage forms[17]. The observed mean $AUC_{0-\infty}$ values for products A and B was 9870 ± 129.22 ng h/ml and 8617 ± 46.88 ng h/ml does not show any significant statistical difference between the products.

The individual and mean $AUC_{0-24}$ ratios (B/A), reflects the relative extent of absorption of product B with that of product A is presented in Table 2. The average values of the individual and mean $AUC_{0-24}$ ratio at 95% confidence limit, was within acceptable limits for bioequivalent products[18].

To obtain *in vitro-in vivo* correlation, absorption profiles were constructed for product A and B using the fraction absorbed *in vivo* was plotted against fraction dissolved *in vitro* is shown in fig. 2 (A and B). It was observed that the both products showed an adequate correlation[19] between cumulative fraction dissolved (CFD) *in vitro*,

**TABLE 2: RELATIVE BIOAVAILABILITY (AUC RATIO) OF PRODUCT B TO PRODUCT A**

| Subject codes | $AUC_{0-24}$ ratio (B/A) |
|---|---|
| $A_1$ | 1.16 |
| $A_2$ | 1.16 |
| $A_3$ | 1.14 |
| $A_4$ | 1.14 |
| $B_1$ | 0.85 |
| $B_2$ | 0.86 |
| $B_3$ | 0.88 |
| $B_4$ | 0.87 |

Relative bioavailability (AUC ratio) of product B to product A administered orally (75 mg) as controlled release products. Mean ± SD

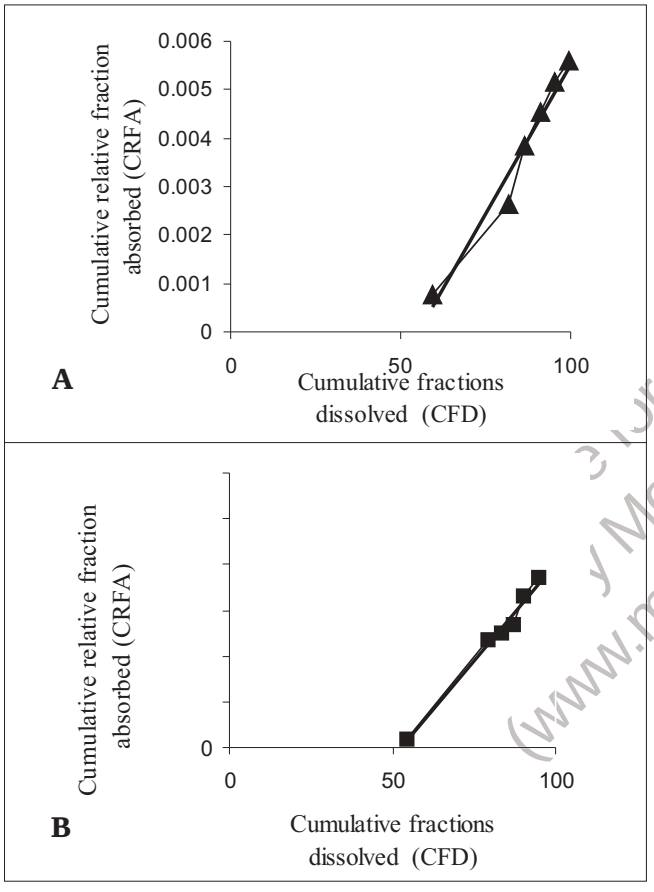


**Fig. 2: *In vitro* and *in vivo* correlation for 2 (a) product A and 2 (b) product B**
**Correlation between the cumulative fractions dissolved (CFD) *in vitro*, cumulative relative fraction absorbed (CRFA) *in vivo* up to 8 hours for product A (-▲-) and product B(- ■-)**

cumulative relative fraction absorbed (CRFA) *in vivo* up to 8 h to obtain *in vitro-in vivo* correlation.

On the basis of FDA recommendation[20], the two products A and B can be considered bioequivalent. No untoward effects were observed by any of the subjects after the administration of either product. Thus, the two formulations can be considered similar, because all the subjects very well tolerated them. These findings clearly shown that the absence of high peak concentrations (>5000 ng/ml), which are very often associated with adverse effects[5], which was reported due to accumulation effect. The products A and B included in this study were found to be bioequivalent.

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