Bioavailability Study of Indomethacin Self-nanemulsifying Oral Formulation in Rats

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Abstract: In the present study, a new self-nanoemulsified drug delivery formulation (SNEDDF) of indomethacin (IND) was tested in rats for its in vivo performance. Pharmacokinetic studies of IND SNEDDF was carried out after oral administration of the prepared formulation (capsules) and compared with that of orally administered IND oily solution (filled in capsule). From the pharmacokinetic parameters, it was found that IND SNEDD capsules have improved bioavailability over IND oily solution capsules. The values of AUC and Cmax for IND SNEDD capsules were 792.17 ± 68.44 ng.hr/ml and 130.45 ± 4.84 ng/ml respectively as compared to 151.50 ± 33.14 ng.hr/ml and 23.83 ± 3.66 ng/ml for IND oily solution capsules. The study showed that SNEDDF could be used not only to enhance the bioavailability of lipophilic drugs but also to decrease the regular doses of these drugs, which may lead to eliminate or reduce their side effects as well.

Key words: Indomethacin, Nano-emulsion, Plasma concentration and Bioavailability.

INTRODUCTION

Oral drug delivery has been a major route of drug administration for the treatment of many diseases. However, oral delivery of 50% of drugs is hampered because of the high lipophilicity of the drug itself. Every 4 out of 10 new candidate drugs have poor water solubility, and the oral delivery of such drugs is frequently associated with implications of low bioavailability, high intra and intersubject variability, and lack of dose proportionality (Taha et al., 2007). For this type of drugs, SEDDFs represent a possible alternative. SEDDFs are isotropic mixtures of natural or synthetic oil, surfactant(s) with or without a co-surfactant. Upon mild agitation followed by dilution in organic media, such as gastrointestinal fluids, these systems can form fine oil-in-water emulsions (Charman et al., 1992). Self-emulsifying formulations spread readily in the gastrointestinal tract, and the digestive motility of the stomach and the intestine provide agitation necessary for emulsifying (Shah et al., 1994). However, studies have shown that the self-emulsification process is specific to the nature of the oil/surfactant pair, surfactant concentration, oil/surfactant ratio and temperature at which self-emulsification occurs (Gershanik and Benita, 2000). Numerous bioavailability studies (Gershanik and Benita, 2000; Hone et al., 2006) carried out in animals and humans suggested that hydrophobic drugs are better absorbed when administered in self-dispersing lipid formulations. On the other hand, Charman et al. (1992) reported that SEDDF improved the reproducibility of the plasma profile in terms of the maximum plasma concentration (Cmax) and the time to reach the maximum concentration (Tmax) of the lipophilic compound WIN 54954, but there was no significant difference in the absolute bioavailability from the SEDDFs. On the other hand, many publications have established the usefulness of SNEDDF in enhancement the bioavailability of lipophilic drugs (Taha et al., 2007). IND is a lipophilic non-steroidal anti-inflammatory drug commonly used to reduce fever, pain, stiffness, and swelling (Inoue et al., 1994). IND is the drug of choice for the closure of a patent ductus arteriosus in neonates by virtue of its vasoconstrictive action in the tissues of the ductus (Al Zaabi et al., 2006). Because IND inhibits both COX 1 and COX 2, it inhibits the production of prostaglandins in the stomach and intestines which maintain the mucous lining of the gastrointestinal tract (Yokota et al., 2005). IND, therefore, like other nonselective COX inhibitors, can cause peptic ulcers (Pongiz et al., 2007). The ulcers can result in serious bleeding and/or perforation requiring hospitalization of the patient. Some even die from these complications (Risty et al., 2007). Also, IND is extensively bound to plasma...
proteins, and has wide intersubject variability in the plasma concentrations, half life and therapeutic response in premature neonates (Al Zaabi et al., 2006). The objectives of this study was to emphasize the useful of SNEDDF not only to improve the bioavailability of IND but also to eliminate or reduce its side effects in long term therapy, in other words, minimizing its regular dose.

MATERIALS AND METHODS

Materials:
IND, ibuprofen, acetonitrile, methanol, castor oil and orthophosphoric acid were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Cremophor RH 40 was obtained from BASF Corp. (Mount Olive. N.J., USA). Capmul MCM-C8 was obtained from Abitec Corp. (Jamesville, Wisc., USA). Hard gelatin capsules size 9 were supplied by Shionogi Qualicaps (Whitest, N.C., USA). All chemicals were used as received.

Preparation of dosing:
Two dosage forms of IND were used: Formulation 1 (F1): was IND SNEDD, which consists of a mixture of IND (0.9 mg), castor oil (3.6 mg) as solvent for IND, Cremophor RH 40 (3.6 mg) as surfactant, and Capmul MCM C8 (28.8 mg) as co-surfactant. IND was dissolved in castor oil and then the other ingredients were mixed to the oily solution using a magnetic stirrer until a clear solution was obtained, and then filled into two of size 9 hard gelatin capsules. It is worthy to mention that castor oil has been chosen in the preparation of IND SNEDDF because of its ability to dissolve the drug. Formulation 2 (F2): was composed of 0.9 mg of IND dissolved in 10 mg of castor oil and then filled in size 9 hard gelatin capsules.

Animals and Dosing Procedures:
Male Sprague Dawley rats (Charles River Laboratories, Charlotte, N.C., USA) weighing 300 - 312 g were divided into two groups corresponding to the two prepared formulations; each was comprised of 6 rats. Rats of group 1 received a single oral dose of F1 (two capsules at once) and group 2 a single oral dose of F2. The amount of IND in each formulation was adjusted to contain 3 mg/kg body weight. On the day of the experiment, animals were anesthetized by an intramuscular injection of a mixture of xylazine (20 mg/ml) and ketamine (100 mg/ml) solution in a ratio of 1:2. The initial dose was 0.3 ml/300 g body weight. Anesthesia was maintained with additional doses of the anesthetic solution as needed during the experiments. Blood samples (about 0.3 ml) were collected from the tail vein just before (0 h) and after dosing: 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 h in a heparinized microcentrifuge tube, stored on ice until the plasma was separated by centrifugation (1,320 g for 5 min). The plasma was stored at -20°C until further analysis. All studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Chromatographic Equipment and Conditions:
A waters isocratic liquid chromatography system (Waters, Boston, Mass., USA) consisting of an automatic sample injector, included a model 6000A Solvent Delivery system, an autoinjector and A model 450 Wavelength Detector. All analyses were conducted at 20 ± 3 °C. Separations were performed on a Hypersil ODS C18 column (5 µm particles, 125 mm × 4 mm i.d., Agilent Technologies). The mobile phase of methanol, water and orthophosphoric acid (0.5%, v/v) in a ratio of 70:29.5:0.1 v/v was membrane filtered (Millipore, 0.45 µm pore size) and degassed under negative atmospheric pressure, then pumped at 1.5 ml/min. The eluent was monitored at 220 nm, the predetermined maximum wavelength for IND in the mobile phase.

Sample Preparation:
The method of sample preparation was a modification of Al Za’abi et al., (2006). Briefly, 100 µL of plasma was pipetted into a 1.5 mL polypropylene centrifuge tube to which was added 250 µL of working internal standard (IS) solution (ibuprofen in ethanol, 250 µg/ml). The tube was vortex mixed for 30 seconds and then centrifuged (5000 × g, for 2 min). The supernatant was carefully transferred to a 10 ml pyrex glass tube and the contents were evaporated to dryness at room temperature under a gentle nitrogen stream. The residue was dissolved in mobile phase (0.5 ml), and a 100 µl aliquot was injected.

Statistical Data Analysis:
Statistical data analysis was performed using the Student t test and ANOVA with p < 0.05 as the minimal level of significance.
RESULTS AND DISCUSSION

The observed retention times for IND and IS were 7 and 4 min, respectively. The pharmacokinetic parameters and the plasma concentration time profiles are shown in Table 1 and Figure 1, respectively. IND in F1 showed rapid absorption as indicated from T\textsubscript{\text{max}} results. The observed T\textsubscript{\text{max}} value was 1.5 h for F1 compared to 2 h for F2. The mean absorption rate constants (± SD) were 1.10 ± 0.02 and 0.77 ± 0.04 h\textsuperscript{-1} for F1, and F2, respectively. With respect to C\textsubscript{\text{max}}, F1 had higher value (130.45 ± 4.84) compared to that of F2 (23.83 ± 3.66). The difference between the groups, with regard to these parameters, was found to be significant at p < 0.05. The area under the plasma concentration time curve (AUC ± SD) was found to be 792.17 ± 68.44 and 151.50 ± 33.14 ng.h/ml in F1 and F2, respectively. Again, the difference between the two groups was found to be significant at p < 0.05. The calculated relative bioavailability of F1 relative to F2 based on AUC was 522.88%. The results of AUC and C\textsubscript{\text{max}} are presented in Figures 2 and 3, respectively.

Discussion:

The most important determinant of product performance is the in vivo bioavailability evaluation. It has been recognized that SNEDDF enhanced the bioavailability of several preparations in humans and animals with an increase in its in vitro drug release characteristics (Taha et al., 2007; Gao et al., 2003; Andrysek, 2003). However, in some reports, the bioavailability has not increased even though the in vitro dissolution rate or emulsification rate has increased (Lyon et al., 2001; Kommuru et al., 1999). Although there are some reports about anaphylactic reactions to Cremophor, these reactions were observed during intravenous administration, but not during oral administration, based on cytotoxicity studies for oral SNEDDFs containing Cremophor (Nazzal et al., 2002; Palamakula and Khan, 2004). The preliminary studies indicated that the prepared IND SNEDDF has improved emulsification and in vitro drug release characteristics. Therefore, it was of interest to perform the in vivo bioavailability study of this formulation and compare the results with F2 as control. From the results, it is obvious that there was an increase in the rate and extent of drug absorption from IND SNEDDF compared to F2 (Table 1, Fig. 1). SNEDDF consist mainly of an oily solution of the drug mixed with a blend of surfactant and co-surfactant. The system readily emulsifies the oily solution of the drug into nanometer size droplets (F1 formulation has droplet size of 30 nm and turbidity of 1.2 NTU) when exposed to water or gastrointestinal media. Therefore, it is conceivable that a SNEDF improves the solubility of the drug in GIT fluids which in turn provide a much greater chance of absorption. From the results of bioavailability, it is obvious that the newly developed IND SNEDD formulation improved the bioavailability five fold compared to that of F2. It is interesting to observe that the enhancement of bioavailability was correlated with the in vitro release study. The results of in vitro dissolution showed that after 30 min, the cumulative percent of drug released from F1, which was 93%. On the other hand, we could not perform a dissolution experiment for F2 due to phase separation because of lack of surfactants. This correlation indicates that dissolution is the rate limiting step in absorption and bioavailability of IND from this SNEDDF.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>F1</th>
<th>F2</th>
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<tbody>
<tr>
<td>T\textsubscript{\text{max}} (h)</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>C\textsubscript{\text{max}} (ng/ml)</td>
<td>130.45 ± 4.84</td>
<td>23.83 ± 3.66</td>
</tr>
<tr>
<td>K\textsubscript{el} (h\textsuperscript{-1})</td>
<td>0.77 ± 0.04</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>T\textsubscript{1/2,el} (h)</td>
<td>3.99 ± 0.38</td>
<td>3.30 ± 0.72</td>
</tr>
<tr>
<td>AUC\textsubscript{\text{max}} (ng.h/ml)</td>
<td>792.17 ± 68.44</td>
<td>151.50 ± 33.14</td>
</tr>
</tbody>
</table>

Conclusion:

IND SNEDDF filled capsules showed a significant increase in the rate and extent of drug absorption and in the bioavailability compared to the capsule filled with an oily solution of IND. Based on this finding, SNEDDF may be one of the promising approaches to enhance the absorption of IND in human volunteers and to overcome the formulation difficulties of lipophilic drugs. Also the study showed that the newly developed formulation enhanced the bioavailability of IND by about 5 fold comparing to that of IND oily solution capsules, which suggesting reduction in the regular dose of this drug to potentially eliminate its side effect.
Fig. 1: Mean plasma concentration (± SD) time profiles of IND formulations after single oral dose administration

Fig. 2: Area under the curve (± SD) of IND formulations after single oral dose administration

Fig. 3: Plasma maximum concentration (± SD) of IND formulations after single oral dose administration

REFERENCES


