INVESTIGATION OF SOLUBILITY ENHANCEMENT OF PRASUGREL HYDROCHLORIDE: NANOSUSPENSIONS AND CYCLODEXTRIN INCLUSION COMPLEXES

Devara R. K., Reddipogu P., Kumar S., Rambabu B., Jithan A.* and Habibuddin M.

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ABSTRACT

The objective of this study was to investigate nanosuspensions, hydroxypropyl-β-cyclodextrin (HPβCD) complexes and SLS powders for enhancing the solubility and dissolution rate of Prasugrel HCl (PHCl) so as to reduce the fluctuations in its oral bioavailability. PHCl nanosuspensions were prepared using evaporative precipitation method. HPβCD inclusion complexes of PHCl were prepared using physical mixture, co-evaporation and kneading methods. Powders of the pure drug with different SLS amounts were prepared. The formulations were characterized using techniques such as powder x-ray diffractometry, scanning electron microscopy, *in vitro* dissolution and *in vivo* absorption in rats. To further aid in the betterment of development of nevirapine nanosuspension, *in vitro in vivo* correlation (IVIVC) was established using deconvolution technique. Nanosuspensions and HPβCD inclusion complexes of PHCl were successfully prepared. The dissolution rate and oral absorption of PHCl in the form of nanosuspensions was significantly higher than that of HPβCD complexes, SLS powders as well as pure drug. All the techniques investigated in this study can be used to enhance dissolution rate and oral absorption of prasugrel HCl and thus can reduce the fluctuations in its oral bioavailability. Nanosuspensions demonstrated to be better and superior technique when compared to other techniques investigated in enhancing oral bioavailability of PHCl. IVIVC that could aid in further formulation development of PHCl nanosuspension was successfully developed using a deconvolution approach.

Keywords: cyclodextrin complexes, dissolution rate, nanosuspension, prasugrel, SLS

INTRODUCTION

Prasugrel hydrochloride (PHCl) is a novel member of a third generation thienopyridine class of anti-platelet agents[1]. It is a prodrug. Formulation strategy for the hydrochloride salt of prasugrel is focused on developing an immediate-release tablet for oral administration. A reaction between PHCl and an excipient was observed late in the development stages during manufacture and storage. This reaction leads to a partial and irreversible formation of prasugrel free base in the tablets. Analysing the samples used for clinical phase 3 study indicated that salt-to base formation of at least up to 70% had no clinical impact and a requirement has been included in the finished product specification[2]. Its base form had significantly lower bioavailability compared to salt form and its bioavailability is tremendously influenced by the changes in the pH[3]. Because of these reasons, PHCl rather than its base form was selected for final development and marketing.

Solubility determination results and permeability and metabolism information suggest that PHCl is a BCS class II compound[4]. Dissolution is affected by pH and decreases with increasing pH. The bioavailability of PHCl exceeds 79%. The peak plasma concentration of the active metabolite occurs 30 minutes after dosing. The parent compound is not detectable in the plasma. Although the oral bioavailability is generally high, it could be variable because of its poor solubility[5]. Its aqueous solubility is $2.37e^{-03}$ g/L. At low pH values from pH 1 to pH 4, it is slightly soluble and above this pH value, it is insoluble[6]. As PHCl is a poorly soluble compound, many problems are encountered in its systemic delivery and achievement of therapeutic action. One of the critical problems associated with

***For correspondence**
Mother Teresa College of Pharmacy
NFC Nagar, RR Dist, 501301, Andhra Pradesh, India
E-mail: aukunjv@gmail.com

poorly soluble drugs is too low bioavailability and/or erratic absorption. The oral bioavailability/erratic absorption depends on several factors including aqueous solubility, drug permeability, dissolution rate and first pass metabolism. As a formulator, the problem of poor solublility, especially for BCS class II compounds, can be successfully solved by enhancing aqueous solubility and dissolution rate.

Various approaches can be attempted to enhance the solubility of poorly soluble PHCl. Different techniques are used to improve the solubility, thereby increasing bioavailability of poorly soluble drugs and reducing erratic absorption[7]. The approaches include aqueous pH shifted solutions (provided the drug has ionizable groups), micronization, solubilization using co-solvents, solid dispersions, oily solutions and salt formation. These techniques for solubility enhancement have certain limitations. Excepting for pH shifted solutions, large amounts of excipients are needed to improve the solubility of the drugs using other techniques. A classical solubility approach commonly used is micronization[8]. In micronization, a coarse drug powder is milled to an ultrafine powder with a mean size being typically in the range of 1-10 μm. The solubility of the drugs is enhanced by increase in the surface area. However, new drugs such as PHCl are so poorly soluble that micronization technique cannot solve the solubility issue. The various other techniques used to enhance the solubility and thereby bioavailability includes microemulsions, nanoemulsions, liposomes and cyclodextrins. Excepting for addition of cyclodextrins, the other techniques are not practically possible because these formulations are poorly stable and also their manufacture is difficult[9]. Because of these reasons, there is an urgent need to find an efficient way of enhancing solubility and dissolution rate of poorly soluble drugs such as PHCl. Recent addition to this list includes nanotechnology based nanosuspensions[10]. Nanotechnology can be used to solve the problems associated with various other approaches described. It is also a practically feasible methodology. Currently nine nanosuspension based formulations are available in US market and all of these formulations are approved by USFDA. One aim of this study was to prepare PHCl nanosuspensions, cyclodextrin inclusion complexes, SLS drug powders, characterize and evaluate the same for enhancement of dissolution rate and *in vivo* absorption in rats. The second aim was to develop IVIVC for the PHCl nanosuspensions that could aid in the further formulation development of nanosuspensions.

A series of PHCl nanosuspensions were developed altering the two factors: surfactant concentrations and solvent/antisolvent ratio. The oral absorption of PHCl from these formulations was evaluated after a single dose to the rats. Further optimization development of a nano formulation involving alterations in formulation development, preparation process, equipment, batch sizes would require additional animal or human studies. These refinements would delay product development of a nanosuspension. It would be desirable to develop a relationship, referred to as an IVIVC, to predict *in vivo* absorption of any refined formulation from *in vitro* dissolution test as a surrogate for animal/human testing. Establishment of an *in vitro* dissolution test as a surrogate for animal testing/human studies was explored in this study to support PHCl nanosuspension formulation development. Such a development for nanosuspensions was proposed and investigated for the first time.

MATERIALS AND METHODS

Prasugrel and HPβ-cyclodextrin were obtained as a gift sample from Suven Nishtaa Pharma, Hyderabad. Ethanol, Tween 80® and Sodium lauryl sulphate were obtained from S D Fine Chemicals Limited. All the other ingredients used were of analytical grade.

Preparation and Optimization of PHCl Nanosuspensions

PHCl nanosuspensions were prepared by evaporative precipitation method[11]. Ethanol was used as a solvent and Tween 80® was used as a surfactant to stabilize the nanosuspension formulation. The drug was dissolved in the solvent (ethanol). The syringe was filled with the prepared solution and injected drop

Table I: Composition and Particle Size of Nanosuspensions

Ingredient/ Formulation	F1	F2	F3	F4	F5
PHCl (mg)	100	100	100	100	100
Distilled water (mL)	100	100	100	100	100
Ethanol (mL)	15	10	20	20	20
Tween 80 (mL)	0.2	0.2	0.1	0.3	0.2
Particle size (mcm)	0.79	0.70	1.52	0.94	1.05
Solvent/antisolvent ratio	1:6.67	1:10	1:5	1:5	1:5

wise into the anti solvent (water containing Tween 80®) with constant stirring on a magnetic stirrer. Vacuum was applied while stirring till the solvent evaporates to form the product. Finally the product was filtered and centrifuged at 7000 rpm for 10 min. In order to optimize the nanosuspension formulations prepared, several nanosuspensions were prepared based on different compositions (Table I). The two factors, surfactant concentration and solvent-antisolvent ratio, were investigated at different levels to study their effect on particle size. Several previous studies indicated that these two factors signficantly affect the particle size of the fabricated nanosuspensions[12].

Preparation of HPβCD Inclusion Complexes of PHCl

PHCl HPβCD inclusion complexes were prepared by three methods. These methods are physical mixing, kneading method and co-evaporation method. In physical mixing, the drug PHCl and cyclodextrin was taken in equal ratios. They were mixed thoroughly by trituration. The obtained mixture was passed through sieves to obtain desired particle size. By this inclusion complex was obtained. In case of kneading method, required quantities of cyclodextrin and distilled water were mixed together in motor and pestle to obtain a homogenous paste. The drug was added to the paste. While grinding the paste, small amount of ethanol was added to assist the dissolution of drug. The paste was triturated for 2 hrs and dried in the oven at 45-50°C for 24 hrs. The dried complex was pulverized and then sieved to obtain the final product. In coevaporation method, the drug was dissolved in alcohol and the cyclodextrin was dissolved in distilled water. Drug solution was added to the aqueous solution. Mixture was evaporated at 37°C. Dried mass was passed through sieves to get desired size particles. By this inclusion complex was obtained. In all the three techniques, seive # 120 was used.

Preparation of PHCl-SLS Powders

PHCl SLS powders were prepared by the addition of SLS in different amounts to the drug, pulverized and mixed thoroughly. SLS was used in the ratio of 0.1%, 0.2% and 1% in the mixtures, the remaining being the drug.

Characterization of Various Formulations of PHCl

The mean particle size of nanosuspensions was determined using optical microscope. In this method size of 200 particles was determined by using stage micro meter. The average article size was determined. In order to examine the particle surface morphology and shape, Scanning Electron Microscopy (SEM) was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with gold layer 20 nm thick. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 20 kV. The drug crystalline state in the nanosuspension sample was evaluated by Powder X-Ray Diffraction (PXRD) analysis. X-ray spectra were recorded with X'Pert-PRO multipurpose X-Ray diffractometer (PAN analytical, Tokyo, Japan) using Ni-filtered, Cu K radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4°/min over a 2θ range of 5° to 40°. The samples were grinded using a mortar and pestle, placed into the cavity of an aluminum sample holder and packed smoothly using a glass slide. *In vitro* dissolution studies of samples were carried out using USP apparatus II paddle method by dispersed powder technique. Accurately weighed nanosuspension samples containing 50 mg of drug

were added to 500 mL of buffer media at 37 ± 0.5°C and stirred at 75 rpm. An aliquot of 10mL was withdrawn at different time intervals. An equal volume of fresh dissolution medium was immediately replaced. The samples were assayed spectrophotometrically at 254 nm. Citrate-phosphate buffer pH 4.0 was used as dissolution medium.This method is based on USFDA recommendations for Prasugrel HCl tablets. The dissolution of nanosuspensions was compared with that of dissolution of equivalent amount of the pure drug.

The PHCl HPβCD complexes were characterized using the following: Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and *in vitro* dissolution studies. The FTIR spectra of drug, cyclodextrin, complexes prepared with physical mixture, kneading method and co-evaporation method were obtained. About 5 mg of sample was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 Psi for 3 minutes. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the IR spectrum was recorded from 4000 cm^{-1} to 625 cm^{-1} in a scan time of 12 minutes. Thermal properties of the powder samples were investigated with a differential scanning calorimeter. Approximately 10 mg of sample was analyzed in an open aluminum pan and heated at scanning rate of 10°C/min between 0°C and 400°C. Magnesia was used as a standard reference material. *In vitro* dissolution studies of samples were carried out using USP apparatus II paddle method. Accurately weighed complexes containing 50 mg of the drug were added to 500 mL of buffer media at 37 ± 0.5°C and stirred at 75 rpm. For PHCl SLS powders, *in vitro* dissolution studies of samples were carried out using USP apparatus II paddle method by dispersed powder technique. The powder mixture containing 50 mg of the drug was used. The experiment was carried at 37 ± 0.5°C and stirred at 75 rpm. The SLS was added in the drug at amounts of 0.1%, 0.2%, 1% and used. Dissolution studies were performed as previously described for the other two methods.

In vivo Pharmacokinetic Studies in Rats

All the animal studies were conducted as per the guidelines of CPCSEA, India. The protocol was approved by Institutional Animal Ethics Committee of Geetanjali College of Pharmacy, Hyderabad (IAEC No. 1648/PO/a/12/CPCSEA). Wistar rats (weighted 180-220 g) were used as experimental animals. Twenty four rats were randomly divided into four groups with six rats in each group. Prior to the experimentation the rats were fasted for 12 h with free access to water. The next day, PHCl nanosuspensions (F2), PHCl cyclodextrin complexes prepared using kneading method and PHCl with 1% SLS, PHCl oral suspension prepared using sodium CMC as suspending agent were given to the four groups of rats via the oral route. All the formulations contained 50 mg of drug. About 0.5 mL blood samples were collected via the orbit vein at 0.125, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hr after administration. The collected blood samples were placed in heparinized tube and then separated immediately by centrifugation at 3000 rpm for 10 min and stored at -20ºC prior to the analysis. The plasma samples were then extracted and the prasugrel was analyzed using a LCMS method as described earlier[13]. The method was validated over a concentration range of 25-10000 ng/mL. The peak areas of all the metabolites was summed and assumed to be the active drug in the plasma that was absorbed. The plasma concentration time profile for all the batches was determined and plotted using excel.

Establishment of IVIVC for Nanosuspensions

The plasma data and the *in vitro* release data obtained using F2 formulation was used in the development of IVIVC. A Level A IVIVC, point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate was studied. Mean *in vitro* dissolution and mean *in vivo* concentration time data were used to build a correlation. The procedure of developing an IVIVC consisted of following steps: calculation of cumulative *in vitro* percentage dissolved, calculation of cumulative *in vivo* absorbed from concentration-time data by deconvolution, and modeling the relationship between cumulative *in vivo* absorbed

and cumulative *in vitro* absorbed. Cumulative *in vivo* drug absorbed was determined using a method as previously described [14].

RESULTS

Nanosuspensions of PHCl (F1-F5) were successfully prepared using evaporative precipitation technique. Several batches of nanosuspsensions by altering various parameters were prepared at the initial step. The yield and the particle size were used to draw final conclusions for further studies. Subsequently, it was identified that the surfactant levels as well as solvent:antisolvent ratio were found to have a significant influence on the size. Different parameters were thus altered so as to obtain the smallest particle size of the nanosuspensions. The different batches that were prepared for studying the influence of selected parameters is shown in Table I. Increase in the concentration of surfactant reduced the particle size. The particle size was also reduced as the solvent/ antisolvent ratio was decreased. Based on the process development studies, formulation which had the lowest particle size was considered to be the optimized batch. F2 was considered to be the optimized batch. Further studies were conducted using this batch. Particle size, surface morphology and shape were visualized using SEM (Fig 1). Particles were spherical in shape. From XRPD graphs, we can conclude that the crystallanity of the drug was changed in the nano suspensions (Fig 2). The peaks obtained for pure drug was very clear and sharp, the intensity of the peaks was very high when compared to peaks of PHCl nanosuspensions. The peak intensity reduction indicates the reduction in the crystallization nature of the drug. *In vitro* drug release from the PHCl and PHCl nanosuspensions in Citrate-phosphate buffer pH 4 was performed by the dissolution experiment using USP dissolution apparatus II. In dissolution studies, it was observed that the dissolution was higher for nanosuspensions compared to pure drug (Fig. 3). At the end of 1 hr, 90% of drug was released with nanosuspensions, while this release was only 45% with pure drug.

DSC studies were conducted with drug cyclodextrin complexes. From the overlay of DSC thermograms, it has been observed that prasugrel is in crystalline nature (Fig 4). It exhibited a broad exotherm at 134.4°C and peak onset temperature was found at 117.82°C. For cyclodextrin the DSC thermogram exhibits the peak at 150.29°C and peak onset temperature at 107.83°C. This peak was the characteristic peak for cyclodextrins. The thermograms of the complexes prepared using all the three techniques demonstrated characteristic peaks of both PHCl and cyclodextrins. However, the intensity of drug peaks was lower compared to pure drug. In co-evaporation method an additional sharp endotherm peak was found at 197.78°C. This could be because of conversion of PHCl to its base form during the process of complexation. From all the DSC peaks we can conclude that the inclusion complex was formed between the PHCl and HPβCD. Second evidence is obtained from FTIR results (spectrums not shown). Pure HPβCD has characteristic IR peaks at 3421 cm^{-1} (H-S moiety), 2436 cm^{-1} (pyridine group), 1757.21cm^{-1}(C=O), 1689.70cm^{-1}(C=O), 1492.95cm^{-1} (C-C aromatic stretch), 1232.55cm^{-1}(C-O), 758 cm^{-1} (C-H aromatic ring). Pure HPβCD has characteristic peaks at 3421.83cm^{-1}, 2931.90 cm^{-1}, 1508.38cm^{-1}, 1082.210 cm^{-1}. In physical mixture, kneading and co- evaporation IR graphs both drug and cyclodextrin peaks were seen, however, peak intensities were reduced indicating an interaction between two compounds. The results indicate a possible complex formation because of the disappearance of pyrrolidine group in IR spectra. Stretching bands at 1000-1200 cm^{-1} indicates the presence of ether group(C-O). The intensity of this peak appeared stronger for co-evaporation and kneading methods. Stretching region of hydroxyl group (O-H) appeared at the range of 3600-3200cm^{-1}. Carbonyl group (C=O) appeared at the range of 1650-1620 cm^{-1}. Inclusion complex bands formed by co-evaporation and kneading methods were shifted to higher wave number indicating the formation of complexes. FTIR results suggest the formation of inclusion complexes of PHCl with cyclodextrins with all the three techniques. *In vitro* drug release from the prasugrel and prasugrel-cyclodextrin complexes in Citrate-phosphate buffer pH 4 was performed by the dissolution experiment using USP dissolution

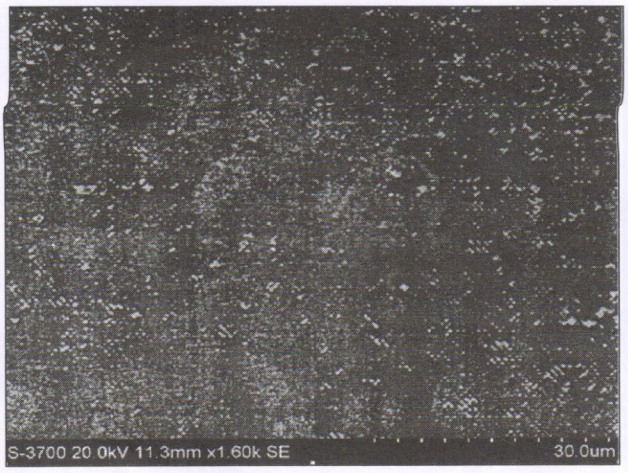

Fig. 1: SEM Pictures of Prasugrel Nanosuspensions

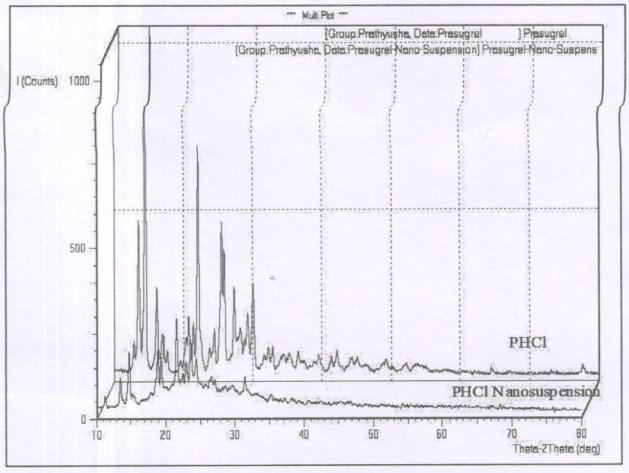

Fig. 2: XRD graph of PHCl and PHCl nanosuspensions

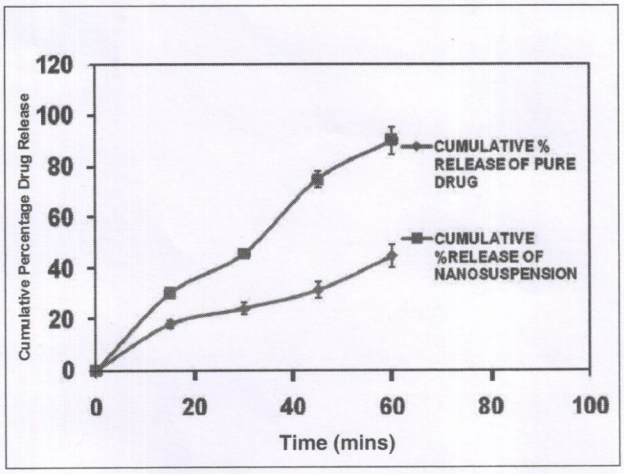

Fig. 3: Cumulative % drug release of PHCl nanosuspensions

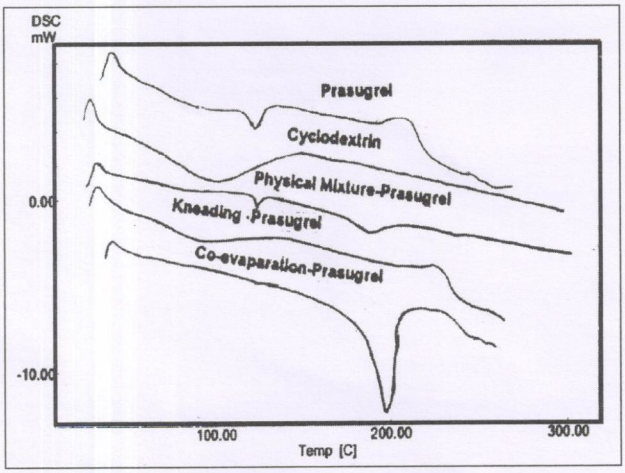

Fig. 4:DSC studies of drug-HPβCD complexes

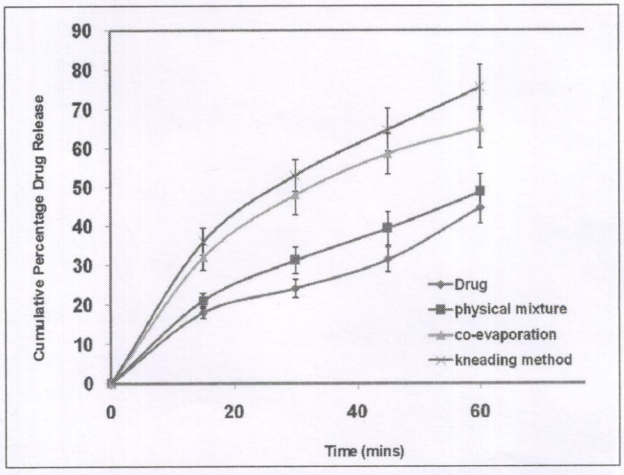

Fig. 5: Dissolution profile of PHCl HPβCD inclusion complexes

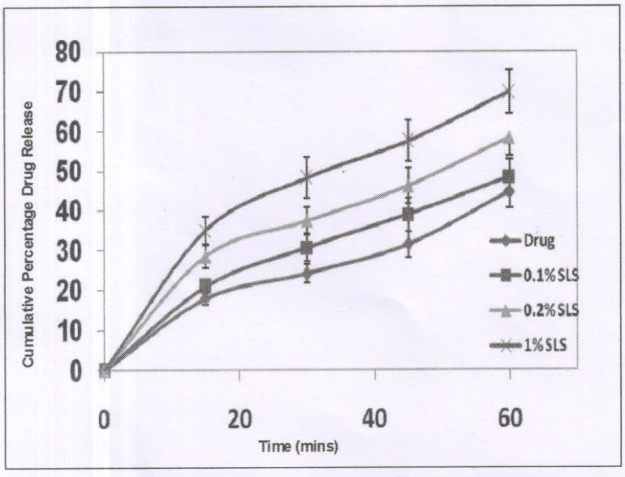

Fig. 6: Dissolution profile of drug/SLS complexes

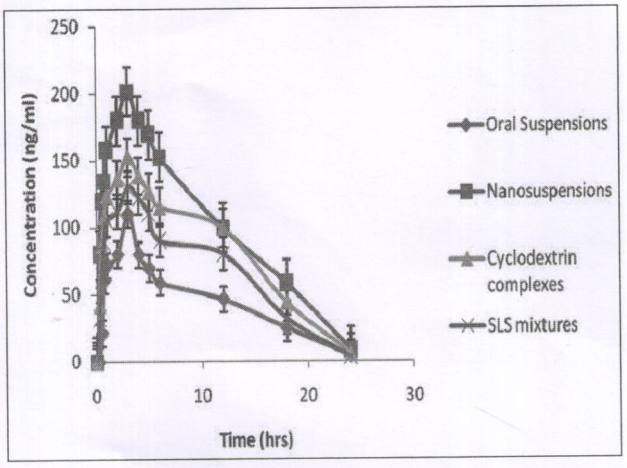

Fig. 7: *In vivo* Absorption of Prasugrel from various novel formulations

apparatus. This is a USFDA recommended method for PHCl. When three methods of preparation of inclusion complexes were compared, the drug release was more in kneading method (Fig. 5). Cumulative% drug release was more for inclusion complexes when compared to the pure drug. The % drug release from complexes prepared using kneading method was 75% at the end of 1 hr while the pure drug demonstrated only 45% drug release. By this dissolution experiment, we can conclude that cyclodextrin complexes enhanced the dissolution of PHCl. The inclusion of SLS with drug in the powder form demonstrated similar results in the dissolution. As the percentage of SLS increased, there was an increase in the dissolution of the drug. The % drug release was 70% using 1% SLS at the end of 1 hr while the pure drug demonstrated only 45% drug release (Fig. 6).

The *in vitro* data was conveniently supported using the *in vivo* data. The *in vivo* data obtained for various formulations is shown in Fig 7. As we observe, the C_{max}, t_{max} and AUC were higher for nanosuspensions, followed by cyclodextrin complexes, SLS mixture and by pure drug (drug administered as NaCMC suspension). This clearly suggests that all the techniques employed here were not only successful in enhancing dissolution rate of PHCl but also resulted in enhanced oral absorption. The feasibility of developing a Level A IVIVC for nanosuspensions was evaluated by plotting fraction dissolved *in vitro* with respect to fraction absorbed *in vivo*. A 100% IVIVC was achieved in our study.

Statistics

All experiments were done six times and the data were expressed as mean ± STDEV and Tukey's post hoc test was done to analyze significance of difference between different groups using the statistical analysis software package SPSS (Version 16.0, IBM, USA).

DISCUSSION

Prasugrel is an inhibitor of platelet activation and aggregation. This is achieved through the irreversible binding of its active metabolite R-138727 to the P2Y12 class of adenosine diphosphate (ADP) receptors on platelets[4]. The innovator product contains the hydrochloride salt of prasugrel. It is reported that the hydrochloride is partly converted to prasugrel base during storage of the tablets. Differences in Cmax were observed if the innovator product was co-administered with proton pump inhibitors depending of the salt-to-base conversion rate of prasugrel[3]. Thus, further development in the formulation is envisaged. In a study involving percutaneous coronary intervention (PCI), it was found that periprocedural myocardial infarction and other events occur within first hour after the procedure. In these cases, rapid treatment is essential. The drug of choice for such conditions is PHCl and therefore a rapid and complete absorption of this drug after oral administration is required for effective treatment[15]. Similarly, it is true for other diseases where PHCl is used. Thus, the solution of enhancement in solubility and dissolution rate of PHCl has clinical relevance.

Solubility and dissolution rate can be enhanced using a variety of approaches. Lot of research has been conducted over several decades to enhance the solubility and dissolution rate of poorly soluble drugs. Several techniques have been developed. Of these techniques, some of the techniques have achieved clinical reality. These include the use of cyclodextrins, co-administration with surfactants

and nanosuspensions. In this study, all of these techniques were investigated to improve the solubility and dissolution rate of PHCl.

The first technique that was investigated for PHCl was nanosuspensions. Nanosuspensions can solve the problem of solubility and improve the bioavailability and therby reducing the fluctuations in the bioavailability. For instance, Liversidge and Cundy demonstrated the enhancement of bioavailability of poorly soluble danazol and reduced the fluctuations in the bioavailability[16]. A dramatic improvement of oral bioavailability of danazol was achieved with oral nanosuspension formulation. Danazol nanosuspensions led to an absolute bioavailability of 82.3% where as the marketed danazol suspension danocrine demonstrated 5.2% bioavailability. In addition, danazol nanosuspension resulted in a reduction in intersubject variability and fed/fasted ratio of danazol. In this study the one objective was to prepare nanosuspensions of PHCl and then demonstrate the enhancement in solubility compared to pure drug suspension. There are currently several nanosuspension formulations in the market. They offer many advantages compared to the other techniques of enhancing the solubility. For instance, the saturation solubility can be enhanced thereby Cmax of the drug in the plasma can be enhanced after oral administration[17]. Although their production is sometimes difficult, currently many methods have been developed so as to surpass this problem. Currently used methods include wet milling, homogenization, emulsification solvent evaporation and supercritical fluid method[18]. Of these wet milling and homogenization have lead to clinical realization of these products into the market.

The other techniques that can be used in the laboratory to produce these products are precipitation techniques, solvent-antisolvent precipitation technique. For instance, Sucker and co-workers used a precipitation technique to produce nanosuspensions by dissolving the drug in a solvent and adding this to a non-solvent that cause a precipitation of the drug[19]. In this study, we used a similar technique. Ethanol was used as a solvent and water was used as an antisolvent. Precipitation was achieved by evaporating the solvent using vacuum while constantly stirring the product. Fine nanosuspensions were obtained with this technique. Diffferent factors influenced their formation. The concentration of the surfactant and the solvent:antisolvent ratio has an influence on the particle size of the nanosuspensions. Optical microscopy was used in the determination of their particle size. The particle size was further verified using SEM. The surface morphology was also determined using SEM. The particles appeared to be spherical. XRPD studies were conducted to characterize the solid state of the particles. In order to investigate the physical nature of the encapsulated drug, the powder X-ray diffraction technique (XRPD) was used. XRPD is used for analysis of a variety of transformations during pharmaceutical processing and storage such as: polymorphic transformations; alterations in crystallanity, changes in state and degree of hydration[20]. The reduction in the peaks indicated that the crystallinity was reduced upon formation of nanosuspensions. Such results were previously achieved. Literature indicates that PHCl may give rise to a variety of polymorphs having distinct crystal structures and physical properties like melting point, thermal behaviours (e.g. measured by thermogravimetric analysis - "TGA", or differential scanning calorimetry - "DSC"), x-ray diffraction pattern, e.g., powder x-ray diffraction (PXRD), infrared absorption fingerprint (IR), and solid state NMR spectrum[21]. One or more of these techniques may be used to distinguish different polymorphic forms of a compound.

Discovering new polymorphic forms and solvates of a pharmaceutical product can provide materials having desirable processing properties, such as ease of handling, ease of processing, storage stability, ease of purification or as desirable intermediate crystal forms that facilitate conversion to other polymorphic forms. New polymorphic forms and solvates of a pharmaceutically useful compound or salts thereof can also provide an opportunity to improve the performance characteristics of a pharmaceutical

product. This also serves to enlarge the repertoire of materials that a formulation scientist has available for formulation optimization, for example by providing a product with different properties, e.g., better processing or handling characteristics, improved dissolution profile, or improved shelf-life. For at least these reasons, there is a need in the art for new polymorphic forms of Prasugrel hydrochloride. However, the aim of this study was to investigate nanosuspension rather than polymorphic forms to enhance the solubility and thereby bioavalability. As indicated in the literature, nanosuspensions are successful in enhancing bioavailability of many poorly soluble compounds[22]. The size suggests that the particles obtained are of nano size and thus it can be concluded that nanosuspensions are formed. Further, the crystallinity is reduced as indicated by the XRPD studies and that could be because of formation of amorphous nanosuspensions. The dissolution rate was enhanced with nanosuspension suggesting their application in the enhancement in the solubility of PHCl. As the objective of this study was already met, we did not further investigate amorphous nanosuspensions for PHCl. In our next studies, we would be aiming to develop stable amorphous nanosuspensions or develop crystalline nanosuspensions for PHCl.

The second technique that was investigated for enhancement in the dissolution rate of PHCl was the use of cyclodextrins. Cyclodextrins were first described in 1891 by Villiers[23]. The first pharmaceutical product containing cyclodextrin was marketed since 1976 by a Japanese Company. Slowly cyclodextrins were incorporated in formulations for further improvements in several countries. Especially in USA their pharmaceutical application started in 1997. Currently more than 30-40 drugs are marketed as cyclodextrin inclusion complexes. Cyclodextrins form inclusion complexes in which water molecules located within the lipophilic central cavity are replaced with a lipophilic guest molecule or a lipophilic moiety on for instance a drug molecule. However, hydroxyl groups on outer surface of cyclodextrins can form hydrogen bonds and form water soluble complexes with lipophilic water insoluble compounds. Thus, cyclodextrins can form inclusion and noninclusion complexes with various drugs enhancing their pharmaceutical properties. This was one technique we investigated in this study to enhance the solubility and dissolution rate of PHCl. In the next studies, PHCl inclusion complexes were investigated. Evidence indicating that the preparation methods resulted in the interaction with PHCl and HPβCD was obtained. A loss of crystallinity was observed with the drug-cyclodextrin mixtures. Second, a molecular interaction between the drugs and the cyclodextrins in the solid state was observed. Similar explanation was previously given for other drugs[24]. Further the dissolution rate of PHCl was enhanced with the use of HPβCD. The third technique that was used in the enhancement of dissolution rate of PHCl was the inclusion of SLS in the tablet formulation. Such an application for SLS for other drugs is previously known. In this study, it was found that SLS can enhance the solubility of PHCl upon inclusion in a tablet formulation. The enhancement in dissolution was best for nanosuspensions compared to other two methods.

Simple *in vitro* drug release studies may not indicate the *in vivo* performance of the formulation. For this reason, we also conducted *in vivo* studies in rats. The results clearly indicated that all the techniques investigated in this study resulted in enhancement in bioavailability of PHCl when compared to that of a simple oral suspension. This could result in rapid onset of action with this drug of whose action is some times required within fraction of seconds after its administration orally. Thus, formulations assessed in this study are definitely helpful to the patients. Further, of all the formulations tested nanoformulations in the form of nanosuspensions appear to be better. In this regard, we would further delve more into the formulation aspects. As a reason, we also developed an IVIVC that could further aid in the formulation development of PHCl. A 100% IVIVC was obtained in our study using our experimental design. Several linear models were evaluated to explain the relationship between fraction absorbed *in vivo* estimated by numberical deconvolution and fraction dissolved *in vitro* obtained experimentally. A deconvolution model with time-scaling factor best explained the relationship. A 100% correlation was obtained with such a methodology. Thus, the methods of developing IVIVC for PHCl nanosuspensions in

this study are valid and can be conveniently used in further formulation development.

CONCLUSIONS

In conclusion, nanosuspensions of PHCl are better compared to HPβCD complexes which are better than SLS powders in enhancing the solubility of PHCl. Thus, nanosuspensions of PHCl can be used to enhance the bioavailability as well as to reduce the fluctuations in the bioavailability.

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