5.1 Summary

The bioavailability /or efficiency of a new chemical entity can be improved by understanding its pharmacokinetic and pharmacodynamic characteristics. This thesis describes approaches that have been applied to improve bioavailability / efficiency of new antihyperlipidemic candidate DHP. The studies also helped in gaining in-depth knowledge of its pharmacokinetic and lipid lowering activity.

The Chapter 1 gives a brief introduction of the new drug discovery and development and describes the importance of pharmacokinetic and drug delivery in developmental phase. Drug discovery is a long process taking around 10-15 years and an investment of nearly $800 million/drug molecule (www.tuffs.edu, 2007). Given the historical success rate, ~40% of the discovery compounds goes from preclinical testing to humans and only 10% of those making it into market (Sri Venkatesh and Robert, 2000). One of the main reason for such failure was poor biopharmaceutical properties (39%) resulted in unfavorable pharmacokinetic properties (Chaturvedi et al., 2001). Thus preventing efficient use of NCE despite good in-vitro activity and less toxicity. Increasing efficiency of NCE in the development stage helps in understanding true potential of the compound and can reduce attrition in clinical trial.

This chapter includes current and future trends in the area of antihyperlipidemic therapy. Antihyperlipidimic drugs have gained considerable attention because of their tremendous potential to prevent cardiovascular diseases by retarding the accelerated arteriosclerosis and its complications. However, even the most successful class of lipid lowering drugs like statins, are also not free from adverse effects or potential drug-drug interactions with concurrently administered drugs. Thus, the quest for a novel drug candidate with high lipid lowering properties as well as minimal or negligible adverse effects is still on. The candidate drug 16-dehydropregnenolone (DHP) was developed by Central Drug Research Institute (CDRI), Lucknow in this context and is currently being developed as a potential and safe drug for lowering plasma LDL cholesterol levels. DHP increases HDL levels, inhibits platelet aggregation and decreases cholesterol biosynthesis. Earlier pre-clinical pharmacokinetic reports on DHP indicated extremely poor oral bioavailability. This leads to higher dose administration. Thus, it is necessary to investigate its pharmacokinetic and enhance its bioavailability to improve the therapeutic efficacy.
With the objective to improve the efficacy of DHP, the following three approaches were investigated:

1. **Rationale selection of route of administration:**
   Drug should reach the target site at sufficient rate and concentration for pharmacological action. Often in the developmental phase information about the target site, pharmacokinetic and mode of action are ill defined. Thus the administered route should efficiently delivery to the site of action for desire activity, reduce metabolism/elimination and reduce access to non target site for its better safety. Detail explanation was discussed in Chapter -2.

2. **Formulation to modulate biopharmaceutical properties:**
   The poor bioavailability of DHP may be due to its low aqueous solubility. This hypothesis was evaluated by comparing conventional formulation with aqueous soluble DHP hydroxypropyl-β-cyclodextrin complex. Study details were discussed in chapter -2.

3. **Drug-Drug interaction:**
   Co-administration of drug might improve therapy by modulating its pharmacokinetic and pharmacodynamic of drug. The study was described in chapter 3 and 4. Chapter 3 deals with the preliminary DHP interaction with other lipid lowering drug. Selection of co-administered antihyperlipidemic drug was based on their mode of action. Combination of drugs of different mode of action might improve overall lipid lowering activity. Interaction study was carried out with atorvastatin, ezetimibe and fenofibrate.

   Chapter 4 deals with interaction of DHP with other agents with the objective to improve its bioavailability and understanding its metabolic interaction. Interaction was carried out with piperine and ketoconazole. Piperine was known to enhance bioavailability of many drugs by virtue of inhibition of metabolism. Interaction with ketoconazole was evaluated to understand the metabolic pathway of DHP.

   Pharmacokinetic and pharmacodynamic obtained by these approaches forms the basis to understand the true potential and design strategy for utilization of drug candidates.

   The second chapter deals with first and second approaches as described above. Oral, intravenous, intramuscular and transdermal drug delivery system was evaluated. During the study, no adverse effect or death was observed. Oral route was most preferred route to evaluate NCE unless other wise specified. Initial in-vivo studies are carried out using suspension as formulation to avoid any interference of excipients such as surfactant etc.
We presume two reasons for its poor oral bioavailability, it may be due to its poor aqueous solubility and extensive first pass metabolism. Latter was evaluated by comparing PK by perenteral route of administration. Aqueous solubility of DHP was increased by complexing with cyclodextrin. Phase solubility and molecular modeling of \( \alpha \)-CD, \( \beta \)-CD HP-\( \beta \)-CD and \( \gamma \)-CD with DHP was evaluated to select the best suited cyclodextrin for our study. These cyclodextrin exhibits different phase solubility profile indicating different modes of complexation. As suggested by phase solubility and docking study DHP being bulky molecule it cannot enter the cavity of \( \alpha \)-CD. Thus no solubility enhancement was observed. \( \beta \)-CD exhibits \( A_p \) type phase solubility indication stable 1:2 DHP:\( \beta \)-CD complex. This was further confirmed by molecular modeling. HP-\( \beta \)-CD exhibits \( A_L \) phase solubility curve with highest \( K_{1:1} \) association constant. Phase solubility curve and molecular modeling suggests formation of 1:1 DHP:HP-\( \beta \)-CD complex. Stability of complex was enhanced due to stabilization of D-ring carbonyl group by hydrogen bond interaction with 2-hydroxypropyl of HP-\( \beta \)-CD. 

\( \gamma \)-CD exhibits \( B_S \) phase solubility profile where initial solubility increment was similar to HP-\( \beta \)-CD, thereafter further increment in \( \gamma \)-CD concentration did not increase solubility. DHP penetrates completely into \( \gamma \)-CD due to its large internal cavity. Larger internal cavity may be reason for low non-covalent interaction that resulted in low \( K_{1:1} \) (70.64 M\(^{-1}\)). DHP being bulky group displaces most of internal water molecules and –OH of A-ring and carbonyl group of D-ring interact with the rim –OH group. Thus decreasing its overall surrounding polar interaction and decrease in overall aqueous solubility of complex (\( B_S \) phase solubility profile). A molecular modeling show A-ring may be most preferred site of cyclodextrin penetration, where as solubility was greatly enhanced by penetration of D-ring carbonyl group. Solubility increment by cyclodextrin was in the order of \( \alpha \)-CD < \( \beta \)-CD < \( \gamma \)-CD < HP-\( \beta \)-CD. HP-\( \beta \)-CD was selected for final formulation due to high solubilizing capacity and low \( K_{1:1} \), ensuring instantaneous release of DHP.

Using triton induce hyperlipidemic model, the optimum oral dose by suspension was found to be 72 – 100 mg/kg. Further increasing dose did not increase lipid lowering activity significantly and activity at 36 mg/kg dose was undesirable low (below 30% reduction). DHP administered as suspension shows non-linear PK due to saturation of absorption. This was confirmed by saturation of \( C_{\text{max}} \), AUC, ratio of AUCp/AUCm and lipid lowering activity.
Administering aqueous soluble DHP-HP-β-CD significantly enhances oral bioavailability by four fold. Thus aqueous solubility was rate limiting factor in oral absorption of DHP. Although bioavailability was enhanced by ~4% compared to suspension, pharmacokinetic and lipid lowering activity at 18 mg/kg (4 fold dose reduction) was not equivalent to 72 mg/kg suspension. Linear extrapolation of dose was not possible due to the non-linear pharmacokinetic observed from 18 mg/kg to 72 mg/kg. Non-linear positive deviation of ratio of AUCp/AUCm vs dose confirms saturation of metabolism due to enhanced bioavailability. Pharmacodynamic and lipid lowering activity at 25 mg/kg was equivalent to that of suspension 72 mg/kg thus 1/3rd dose reduction was possible due to enhanced efficiency of drug delivery.

The observed multiple peaks in the plasma concentration profile of DHP and M1 by oral route was due to enterohepatic recirculation as confirmed by similar appearance in IV profile. IV administration at a dose of 1 to 10 mg/kg reduces clearance by two fold. M1 formation was low as compared to oral administration, despite being high systemic availability. Systemic clearance after an intravenous administration reflects hepatic elimination and that metabolism of systemically available drug by intestinal enzymes localized in enterocytes is negligible (Holtbecker et al., 1997). This confirms that GI was main site for M1 formation which was by-passed by perenteral administration.

Intramuscular administration was used to avoid first-pass metabolism and prolong systemic availability. DHP by IM route (10 mg/kg) resulted in high systemic availability with prolong half-life. Volume of distribution was high indicating rapid distribution to peripheral compartment. Despite high systemic availability of DHP, significantly low amount of M1 was observed indicating significant reduction in metabolism of DHP by IM administration. Reducing dose from 10 mg/kg to 5 mg/kg significantly reduces cholesterol, triglyceride and phospholipids. No significant increase in lipid lowering activity was observed on further increasing IM dose. Thus 10 mg/kg was optimum IM dose. Despite being high systemic availability of DHP by IM administration (10 mg/kg), lipid lowering activity was not significantly different from oral 25 mg/kg as DHP-HP-β-CD and suspension 72 mg/kg. Variability among the corresponding lipid lowering parameters was higher by IM route than oral DHP (DHP-HP-β-CD). High volume of distribution by IM route resulted in distribution to non target organ, thus this route may not be efficient route for DHP lipid lowering activity. Transdermal route of administration was used to by-pass first pass metabolism and prolong drug action by

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systemic delivery of drug through skin. Low systemic availability of DHP through skin was observed. Thus transdermal drug delivery system may not be the route of choice.

Acceptability of route depends upon efficient delivery of drug to its site of action and minimum to non target sites, however during drug development phase target sites are not well defined. Although oral route has low systemic availability, lipid lowering activity was comparable at approximately same IM dose range. It can be concluded that liver and intestine may be its target organ. From the literature it was evident that DHP was selective inhibitor of BAR/FXR receptor (Wu et al., 2002), which is present in intestine and in Liver (Caron et al., 2006). Thus it can be concluded that oral route of administration was ideal for lipid lowering activity.

Further Studies may be required on designing suitable oral formulation such as sustained release formulation etc, to improve efficiency and prolong antihyperlipidemic activity of DHP.

Chapter 3 deals with preliminary DHP interaction with atorvastatin, ezetimibe and fenofibrate. Drug concomitantly administered may alter absorption, distribution, metabolism and excretion, which may increase or decrease availability of individual drug. The outcome may be beneficial or harmful if the interaction causes an increase in adverse effects. Beneficial effects results from co-administration of drugs that shows synergistic activity or increase the availability of drug. In general co-administration of drugs of different mode of action may have favorable pharmacodynamic interaction due to additive or synergistic action. DHP inhibit BAR/FXR receptor (Pellicciari et al., 2006), a nuclear hormone receptor responsible for bile acid transportation and synthesis. DHP has different mode of action as compared to statins, ezetimibe and fibrates, thus its combination may complement lipid lowering activity by targeting multiple lipid pathway. The present study evaluates the interaction potential of DHP with other lipid lowering drug to predict potential combinations.

Except for atorvastatin, no drug-drug interaction was observed. Increased plasma levels of DHP upon co-administration of atorvastatin indicate DHP and atorvastatin was metabolized by same pathway i.e. by CYP3A isoform. Cholesterol and phospholipid lowering was significantly enhanced at combination dose as compared to individual dose. The enhanced lipid lowering at low dose of DHP administered in combination with
atorvastatin was may be due to targeting complementary pathway (HMG-CoA inhibition and BAR/FXR receptor).

Mode of action of DHP being different from ezetimibe and fibrates, combination with DHP might improve lipid lowering profile. No significant pharmacokinetic interaction of DHP was observed with these drugs, which indicates the possibility of its co-administration with high degree of safety. Further efficacy and safety evaluation are needed for prediction of optimum dose combination for clinical use.

Chapter 4 describes pharmacokinetic interaction of DHP with piperine (a known bioavailability enhancer) and ketoconazole. The aim of the study was to understand DHP metabolic pathway and explore the possibility of increasing bioavailability by co-administration with piperine.

DHP was metabolized by CYP3A isoform as indicated by interaction study with atorvastatin (chapter 3). Inhibition of CYP3A isoform decreases M1 (metabolite) formation and increases DHP plasma level. Thus CYP3A isoform metabolized DHP to M1. Interaction with ketoconazole (potent CYP3A isoform and UDP-glucuronosyl transferase) also decreased M1 formation as compared to control. However inhibition of UDP-glucuronosyltransferase by ketoconazole significantly reduces DHP plasma level as observed. Piperine a known inhibitor of intestinal UDP-glucuronosyltransferase and non specific CYP inhibitor significantly reduces AUC of DHP. Significant suppression of multiple peaks was observed upon co-administration of piperine and ketoconazole. Thus it can be concluded that inhibition of UDP-glucuronosyltransferase (Phase II enzyme) significantly reduces systemic availability of DHP by inhibiting enterohepatic recirculation. Interaction of piperine increase plasma level of M1, which may be due to reduced further metabolism to Phase II metabolite. As piperine and ketoconazole decreases the bioavailability of DHP, co-administration of DHP with piperine and ketoconazole was not recommended. Further in-vitro study is required to understand metabolic pathway of DHP.

The study deals with the holistic approaches to understand and improve bioavailability of DHP, an antihyperlipidemic agent by optimizing route of administration, formulation and drug-drug interaction. Apart from these, understanding of basic pharmacokinetic and lipid lowering profile was studied.