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Short Communication

In Situ Intestinal Absorption of Ursodeoxycholic acid from Solid Dispersions and β-cyclodextrin Complexes in Rats

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ABSTRACT

Ursodeoxycholic acid (UDCA) is a water insoluble drug used for the dissolution of cholesterol gallstones because it reduces the cholesterol saturation of bile along with the treatment of other liver diseases, such as primary biliary cirrhosis, chronic hepatitis and biliary pains. However *in vivo* studies have shown that intestinal absorption and consequently the bioavailability of the drug are generally poor and erratic both among different subjects, and within the same subject. More than 50% is lost in the stool after a single oral dose of 300 mg. Aqueous solubility of UDCA was enhanced by preparing its solid dispersions using polyvinyl pyrrolidone (PVP) as water soluble carrier and cyclodextrin complexes with β-cyclodextrin. Absorption studies using *in-situ* rat gut technique exhibited greater rate of intestinal absorption with solid dispersions of UDCA when compared with β-cyclodextrin. The intestinal absorption followed the first order rate kinetics. Statistical correlation of *in vitro* drug dissolution and *in vitro* drug absorption indicates a positive correlation. This increased absorption may be due to the solubilisation and improved wetting of UDCA in PVP rich micro-environment.

INTRODUCTION

A method is reported for studying gastrointestinal drug absorption from isolated gut segments of the anesthetized rat *in situ*. The experimental technique is simple and utilizes readily available laboratory equipment. The results are closely reproducible and yield absorption rates which are realistic in terms of the known absorption behavior of drugs in humans and intact animals [1].

Solid dispersion is a unique approach. The concept of solid dispersion was introduced by Sekiguchi and Obi. In solid dispersion method the drug is dispersed in extremely fine state in an inert water-soluble carrier in solid state [2]. Polyvinylpyrrolidone (PVP) has been used extensively for the enhancement of solubility and dissolution rate of low solubility drugs [3]. PVP-coprecipitate of water insoluble drugs is formed by dissolving both components in a common solvent and subsequently removing the solvent [4].

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Cyclodextrins and their derivatives play an important role in formulation development due to their effect on solubility, dissolution rate, chemical stability and absorption of drugs [5]. Though cyclodextrins have been investigated widely during the last two decades, their commercial application in pharmaceutical formulation was started only in recent years with drugs such as piroxicam and nimesulide [6].

Ursodeoxycholic acid (UDCA) (Figure 1) is a white, odorless, crystalline powder with a bitter taste. Chemically it is 3α, 7β-dihydroxy-5-cholan-24-oic acid. It is a water insoluble drug used as a drug for the dissolution of cholesterol gallstones because it reduces the cholesterol saturation of bile [7-9]. The use of UDCA for the treatment of other liver diseases, such as primary biliary cirrhosis, chronic hepatitis and biliary pains has also been demonstrated. However *in vivo* studies have shown that intestinal absorption and consequently the bioavailability of the drug are generally poor and erratic both among different subjects, and within the same subject. More than

50% is lost in the stool after a single oral dose of 300 mg [10-12].

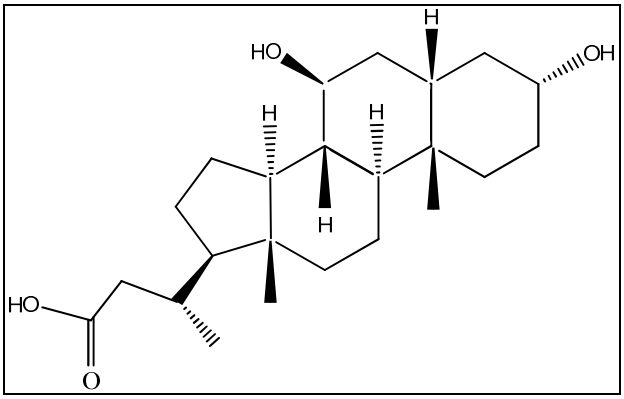


Figure 1: Ursodeoxycholic acid

UDCA is insoluble in water and therefore attempt has been made to develop its solid dispersions using PVP as water soluble carrier and cyclodextrin complexes with β-cyclodextrin (β-CD) with an aim to improve its extent and rate of dissolution and to carry out its absorption studies using *in-situ* rat gut technique.

MATERIALS AND METHODS

Ursodeoxycholic acid (Lab. Chem. Sdn. Bhd., Malaysia), β-cyclodextrin (Cerestar, USA Inc.Hammond Indiana) of commercial purity grade were used. All other chemicals used were of analytical reagent grade.

Preparation of Solid Dispersions of Ursodeoxycholic acid

Quench cooling was used for the preparation of solid dispersions of Ursodeoxycholic acid (UDCA). Weighed quantities of polyvinyl pyrrolidone (PVP) and UDCA in different ratios such as 1:0.5, 1:1 and 1:2 by weight basis were thoroughly mixed and melted on hot plate with constant stirring to obtain a uniform melt and subsequent quench cooling of the melt over crushed ice. The quench-cooled product was removed from the stainless steel plate, powdered and kept in a desiccator for two days. The powder was ground, passed through sieve #100 and stored in closed airtight container [3].

Preparation of Molecular Inclusion Complexes of Ursodeoxycholic acid

Solid inclusion complexes of UDCA with β-CD were prepared in 1:1 and 1:2 molar ratios using kneading method [5, 13]. Accurately weighed quantity of β-CD was taken in a glass mortar; water was added slowly and mixed to obtain a homogeneous paste. Weighed quantity of UDCA was added slowly by grinding. The mixture was ground for one hour. During this process appropriate quantity of water was added to maintain suitable consistency. The obtained solid mass was further dried under vaccum to a constant weight at room temperature and pulverized, sieved through mesh # 100 and stored in desicator [14].

In Situ Rat Gut Technique

The extent of absorption of UDCA from selected solid dispersions and β-CD molecular inclusion complex which had shown good *in-vitro* results were determined in the rat intestine. The experiments were carried out as per the guidelines of Animal Ethics Committee. Six rats of either sex weighing between 200-250g were fasted for 2 days prior to experiment. Rats were anaesthetized by administering pentobarbital (60 mg/kg, *i.p*) and placed on a heated pad to keep normal body temperature. Small intestine of the animals was exposed by a midline abdominal incision. The duodenal and ileal ends of the intestine were cut while keeping the blood supply to intestine intact. Two L-shaped glass cannulae were inserted, and secured by ligation with silk suture in the small slits at the duodenal and ileal ends of the small intestine which was returned to the abdominal cavity to maintain its integrity. Four-centimeter segments of Tygon tubing were attached to the exposed ends of both cannulae and a 30ml hypodermic syringe was fitted with a three way stopcock (Figure 2).

Perfusion fluid (anhydrous disodium hydrogen phosphate- 40mM; sodium dihydrogen phosphate- 26mM and sodium chloride 119mM) at 37°C was passed slowly through the duodenal cannula and passed out through the ileal cannula until all the intestinal contents were expelled out from the intestine. Air was pumped through the syringe to expel the perfusion fluid from the gut. The drug solution (10 ml) was immediately introduced into the intestine by means of the syringe.

An aliquot of 0.1ml of solution was withdrawn at 0, 15, 30, 45, 60, 75, 90 minutes from the time of administration of the drug solution. To ensure uniform drug solution concentration throughout the gut segment, aliquots were removed from the two syringes alternatively. Finally, the animal was euthanitized with a cardiac injection of saturated solution of potassium chloride. After making suitable dilutions, absorbance was measured and the amount of drug present in the sample solution was calculated from the regression equation [15-17].

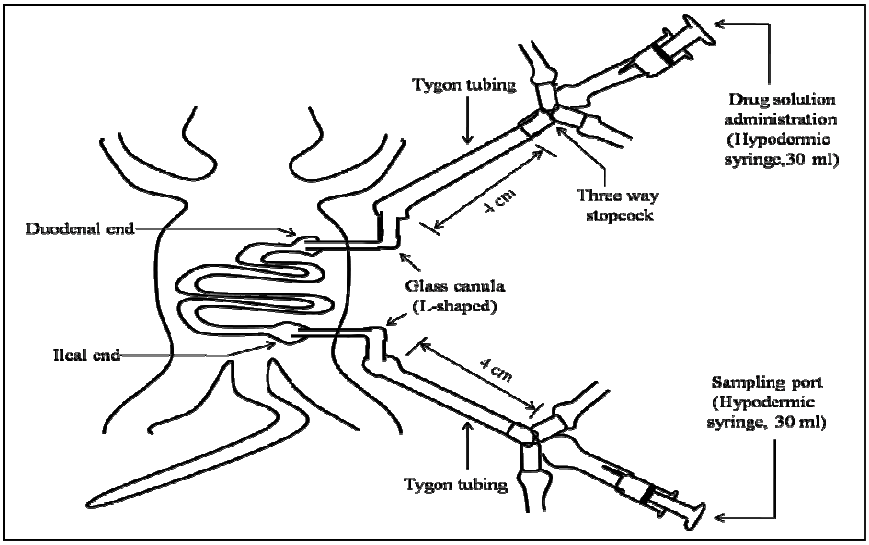
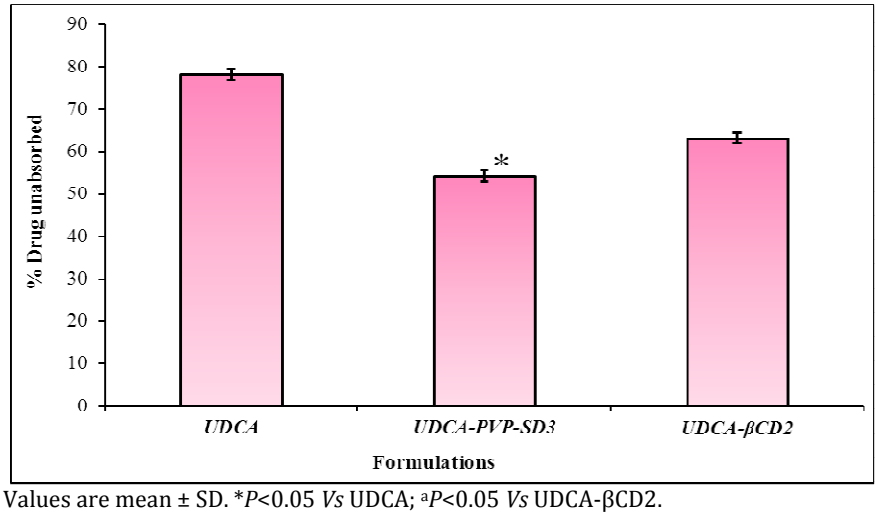


Figure 2: Arrangement for carrying out in situ rat gut technique [15].

Table 1: Comparison of intestinal absorption of selected formulations of ursodeoxycholic acid using in situ rat gut technique

Time (min)	*Percent ursodeoxycholic acid unabsorbed*			*Log percent ursodeoxycholic acid unabsorbed*		
	UDCA	*UDCA-PVP-SD3*	*UDCA-βCD2*	*UDCA*	*UDCA-PVP-SD3*	*UDCA-βCD2*
0	100(0.33)	100(0.43)	100(0.19)	2.000	2.000	2.000
15	97.24(0.71)	97.13(0.23)	98.12(0.71)	1.988	1.987	1.992
30	96.14(0.83)	89.12*,a(0.64)	91.13*(0.86)	1.983	1.950	1.960
45	87.31(0.75)	82.14*,a(0.51)	85.12*(0.98)	1.941	1.915	1.930
60	83.12(0.81)	74.16*,a(0.45)	80.13*(0.87)	1.920	1.870	1.904
75	80.17(0.85)	65.12*,a(0.71)	74.13*(1.04)	1.904	1.814	1.870
90	78.16(1.32)	54.31*,a(1.31)	63.21*(1.23)	1.893	1.735	1.801

UDCA: ursodeoxycholic acid; βCD: β-cyclodextrin; PVP: polyvinyl pyrrolidone; SD: solid dispersion. Values in parenthesis indicates the standard deviation (n = 6). *P<0.05 *Vs* UDCA; [a]P<0.05 *Vs* UDCA-βCD2.



Values are mean ± SD. *P<0.05 *Vs* UDCA; [a]P<0.05 *Vs* UDCA-βCD2.

Figure 3: Comparative analysis of intestinal absorption of ursodeoxycholic acid from selected formulations.

Table 2: Comparison of orders of intestinal absorption (in situ rat gut technique) of selected formulations of ursodeoxycholic acid

Formulation	Correlation coefficient (R^2)	
	Zero order	First order
UDCA	0.9593	0.9617
UDCA-PVP-SD3	0.9562	0.9829
UDCA-βCD2	0.9528	0.9755

UDCA: ursodeoxycholic acid; βCD: β-cyclodextrin; PVP: polyvinyl pyrrolidone; SD: solid dispersion.

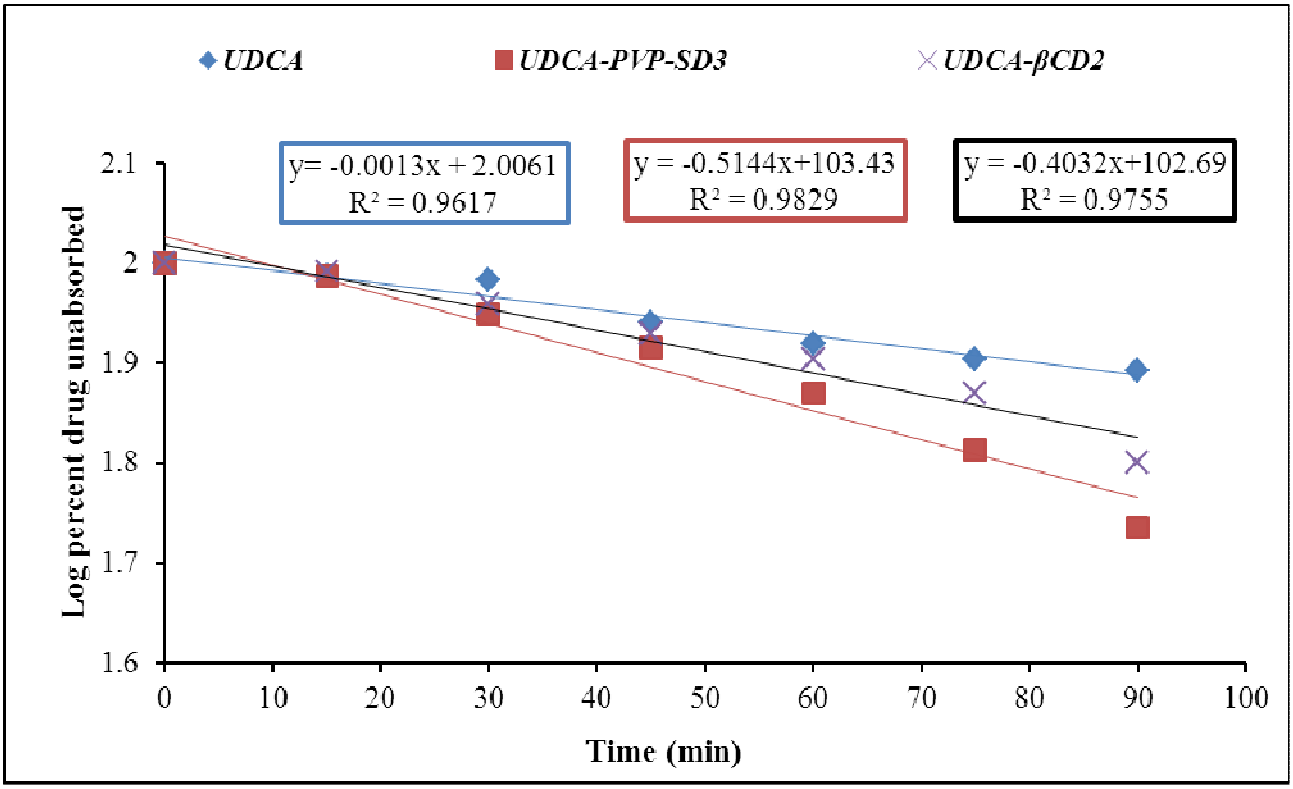


Figure 4: Kinetics plot of selected formulations of ursodeoxycholic acid.

RESULTS AND DISCUSSION

The intestinal absorption of UDCA from these formulations demonstrated the following order: SDs > β-CD molecular inclusion complex > UDCA (Table 1; Figure 3). The absorption order of UDCA from these formulations corresponds to its *in vitro* release pattern. A statistically significant difference was observed in the rate of absorption of UDCA from UDCA-PVP-SD3 (1:2) when compared with UDCA-βCD2 (1:2 M) and pure drug as well (P<0.05). This increased absorption may be due to the solubilisation and improved wetting of UDCA in PVP rich micro-environment [3].

The correlation coefficient (R^2) values and the equations best describing the kinetics of drug absorption are given in Table 2 and Figure 4. The release of UDCA from all these formulations was found to follow first order release kinetics, since value of R^2 for first order was higher in comparison to zero order. The present findings were in agreement with the previous reports by Rawat and Jain [18].

CONCLUSION

The maximum intestinal absorption of ursodeoxycholic acid using *in-situ* rat gut technique was observed from the solid dispersions with polyvinyl pyrrolidone as compared to β-cyclodextrin complexes followed the first order rate kinetics. The solid dispersions of ursodeoxycholic acid with PVP lends an ample credence for better therapeutic efficacy.

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