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Ursodeoxycholic acid: Effects of formulation on in vitro dissolution

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Abstract

A new rapid-dissolving granule formulation of ursodeoxycholic acid has been developed which achieves an increased ursodeoxycholic acid solubility in vitro. Granules were prepared with excipients designed to accelerate the disintegration rate and improve the wetting of ursodeoxycholic acid and therefore solubility in vivo. The granules contained polyvinylpyrrolidone, lactose and croscarmallose sodium together with ursodeoxycholic acid (100 or 250 mg) in size '0' hard gelatin capsules and their dissolution characteristics were assessed, at pH 7.2, using an in vitro dissolution method based on the USP XXII (apparatus 2). Detection of dissolved ursodeoxycholic acid was achieved with a specific enzyme assay based on 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50). The 100 mg rapid-dissolving granule formulation was found to release at least 90% of the ursodeoxycholic acid into solution at 15 min, increasing to 100% after 60 min, while the 250 mg rapid-dissolving granule formulation was found to release 76 and 86% of the ursodeoxycholic acid at 15 and 60 min, respectively. A dissolution study carried out using 250 mg capsules containing unformulated ursodeoxycholic acid showed that physical form greatly affected solubility. The sodium salt of ursodeoxycholic acid was soluble in dissolution media, 97% after 15 min, whereas the pharmaceutically approved free acid reached only 20% dissolution in the crystalline form and 66% dissolution in the micronised form, increasing to 38 and 83%, respectively, after 60 min. A comparative dissolution study, with volume corrections to dissolution media to take account of potency, was carried out using two commercial preparations of ursodeoxycholic acid, Destolit® and Actigall®. These preparations were found to release 45.8 and 27.5% ursodeoxycholic acid at 15 min increasing to 89 and 39% at 60 min, respectively, and were therefore all potentially less effective than the 250 mg rapid-dissolving granule formulation in vivo. The medical implications of variable ursodeoxycholic acid solubility achieved with different formulations are discussed.

Key words: Ursodeoxycholic acid; Gallstone dissolution; Actigall®; Destolit®

1. Introduction

Ursodeoxycholic acid is a recognised and well tolerated treatment for the dissolution of radiolucent gallstones, formed predominantly from cholesterol (Bouchier, 1980; Bachrach and Hofmann, 1982a,b). Ursodeoxycholic acid is a bile acid with only poor membrane solubilising capacity (Barnwell et al., 1983; Coleman, 1987; Heuman et al., 1991a,b), therefore, gallstone dissolution results from the interaction of multiple mecha-

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nisms; biliary cholesterol secretion is reduced without affecting the synthesis of endogenous bile acids, and ursodeoxycholic acid can also induce the formation of cholesterol-phospholipid liquid crystals on solid cholesterol (gallstone) surfaces. In addition to the treatment of cholesterol gallstones, ursodeoxycholic acid is being increasingly studied as a potential treatment for a number of serious liver disorders, for example, chronic active hepatitis, primary biliary cirrhosis and chronic cholestasis resulting from conditions such as cystic fibrosis (Cotting et al., 1990; Crosignani et al., 1990; Podda et al., 1990; Poupon et al., 1991; Rolandi et al., 1991; Fried et al., 1992; Galabert et al., 1992). Ursodeoxycholic acid may also be useful in the treatment of dyspeptic symptoms and biliary pain (Frigerio, 1979; DelVecchio-Blanco et al., 1982; Salvioli et al., 1983; Portincasa et al., 1993).

Ursodeoxycholic acid, when used in long-term therapy, becomes the major bile acid in the human enterohepatic circulation, typically making up around 40% of total bile acids. This change in bile acid composition is believed to be the result of a general expansion in the size of the bile acid pool without greatly affecting the synthesis or enterohepatic recirculation of endogenous bile acids (Beuers et al., 1992; Fischer et al., 1993; Mazzella et al., 1993).

Studies have shown that the bioavailability of ursodeoxycholic acid in vivo is poor and erratic, both within the same subject and between subjects (Parquet et al., 1985), and is generally believed to result from the poor aqueous solubility of ursodeoxycholic acid at physiological pH (Igimi and Carey, 1980; Moroi et al., 1992). The present study reports the development of an improved rapid-dissolving granule formulation of ursodeoxycholic acid and an in vitro dissolution method to assess its performance. Comparative dissolution studies were carried out using the rapid-dissolving granule formulation and several commercial preparations of ursodeoxycholic acid. The potential in vivo implications of these results are discussed.

2. Materials and methods

2.1. Materials

Destolit® 150 mg tablets (Merrell Dow, U.K.) and Actigall® 300 mg capsules (Summit Pharmaceuticals, Division of Ciba-Geigy Corp., U.S.A.) were obtained from commercial sources. Lactose E.P. (Pharmatose 450 mesh), croscarmellose sodium U.S.P. NF (Ac-Di-Sol), and polyvinyl pyrrolidone (Plasdone K29–32) were obtained from DMV International (Diss, U.K.), Honeywill & Stein Ltd (Sutton, U.K.) and ISP (Great Britain) Co. Ltd (Manchester, U.K.), respectively. Micronised and non-micronised ursodeoxycholic acid Fr.P. (free acid and sodium salt) was supplied by Alfa Chemicals Ltd (Preston, U.K.). The bile acid concentration in dissolution media was determined by an enzymatic method, based on 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50) using a modified application of the bile acid test kit supplied by Sigma Chemicals Ltd (Poole, U.K.). Size '0' white opaque hard gelatin capsules (Licaps®) were supplied by Capsugel (Pontypool, U.K.). All other reagents were of a suitable grade and supplied by Sigma Chemicals Ltd (Poole, U.K.), BDH Chemicals Ltd (Poole, U.K.) and Fisons Scientific Equipment (Loughborough, U.K.).

2.2. Dissolution studies

In order to evaluate the dissolution behaviour of ursodeoxycholic acid formulations, a test method was devised based on the USP XXII (apparatus 2) dissolution test for tablets and capsules. The dissolution media consisted of 1:5 dilutions of a range of standard USP XXII phosphate buffers in distilled water, equilibrated to 37°C, with pH ranging from 6.4 to 8.0. For assessment of rapid-dissolving granule formulations (100 and 250 mg) 750 ml of dissolution media was used with a paddle rotation speed of 100 rpm. The capsules were placed in the dissolution vessels with a wire sinker around the body of each capsule. At each time point a 5 ml aliquot of the

dissolution media was removed for analysis and replaced with a fresh 5 ml aliquot of dissolution media. Samples were taken over a 60 min period. The samples were centrifuged at $1 \times 10^4 \times g \cdot \min$ to remove any suspended undissolved material and then analysed for their ursodeoxycholic acid content using a bile acid enzymatic assay kit (Sigma Chemicals Ltd) after 5-fold dilution in methanol:water (5:1) (v/v) against a standard calibration curve.

Dissolution studies were carried out using the commercial ursodeoxycholic acid formulations described above, with raw drug substance and the 100 and 250 mg rapid-dissolving granule formulation of ursodeoxycholic acid. For the comparative dissolution studies the volume of dissolution media was adjusted to ensure that matched concentrations of drug were present in the tests.

2.3. Manufacture of dosage forms

The rapid-dissolving granule formulation of ursodeoxycholic acid was made at two strengths, 100 and 250 mg/capsule, using a wet granulation process. An intimate mixture of the following ingredients: ursodeoxycholic acid (micronised), lactose and croscarmellose sodium was formed. The ingredients were mixed thoroughly before being screened through a 500 μm aperture sieve screen. A 10% w/v aqueous solution of polyvinyl pyrrolidone was prepared by addition to purified water with stirring. The solution was then added slowly to the screened dry mixture and mixed until a suitable wet mass was obtained. The wet mass was then screened through a 1.7 mm sieve screen and the resultant mass transferred to a Uni-Glatt® fluidised bed drier and dried at 60°C until a moisture content of less than 2% w/w was achieved. The dried mass was subsequently screened through a 1.0 mm aperture sieve screen and then filled to a potency of 100 or 250 mg into size 0 hard gelatin capsules.

2.4. Raw drug substance dissolution testing

In the case of raw drug substance, 250 mg of ursodeoxycholic acid was filled into size 0 hard gelatin capsules before performing the dissolution test described above.

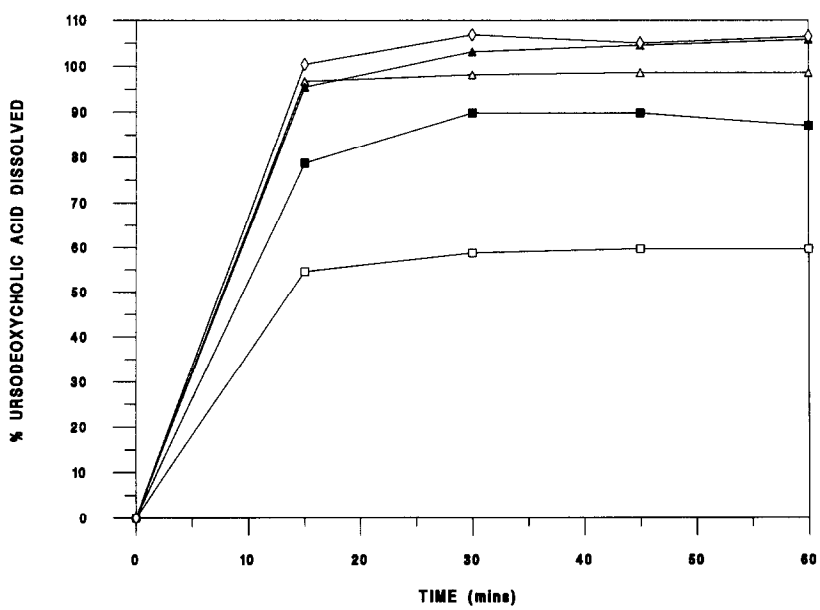

Fig. 1. Dissolution profiles of the 100 mg rapid-dissolving granule formulation of ursodeoxycholic acid at pH 6.4 (□), pH 6.8 (■), pH 7.2 (△), pH 7.6 (▲) and pH 8.0 (◇).

3. Results

3.1. Effect of pH on the dissolution of the 100 mg rapid-dissolving granule formulation of ursodeoxycholic acid

Dissolution testing of the 100 mg rapid-dissolving granule formulation was carried out in 1:5 dilutions of standard USP XXII phosphate buffers adjusted to pH 6.4, 6.8, 7.2, 7.6, 8.0 and also in distilled water. The results in Fig. 1 show that at pH 7.2, 7.6 and 8.0 the 100 mg rapid-dissolving granule formulation released more than 90% of the ursodeoxycholic acid into solution within 15 min and achieved almost complete dissolution by the end of the 60 min study period. At pH 6.8 dissolution of ursodeoxycholic acid reached around 80% after 15 min, increasing to 90% after 30 min, a level maintained until the end of the 60 min dissolution study. At pH 6.4, close to the $pK_a = 5.8$ for ursodeoxycholic acid (Igimi and Carey, 1980; Moroi et al., 1992), approx. 55% of the ursodeoxycholic acid was found to be dissolved in the dissolution media after 15 min, a level which was maintained for the remainder of the study. The concentration of ursodeoxycholic acid dissolved in 750 ml of dissolution media using the 100 mg rapid-dissolving granule formulation was about 192 μM at pH 7.2. Performing the same dissolution study using the rapid-dissolving granule formulation in distilled water resulted in less than 10% ursodeoxycholic acid dissolution during the 60 min study period. As a result of this preliminary study pH 7.2 was taken as the reference pH for further dissolution studies.

3.2. Comparison of the dissolution characteristics of 100 and 250 mg rapid-dissolving granule formulations of ursodeoxycholic acid

The results in Table 1 compare the dissolution characteristics of 100 and 250 mg rapid-dissolving granule formulations of ursodeoxycholic acid. At 15 min the 250 mg rapid-dissolving granule had released 76% of the ursodeoxycholic acid from the formulation compared with 97% for the 100 mg rapid-dissolving granule. After 60 min these values had increased to 86 and 99% ursodeoxycholic acid release for the 250 and 100 mg rapid-dissolving granule formulations, respectively. The concentration of ursodeoxycholic acid dissolved in 750 ml of pH 7.2 dissolution media, assuming 86% solubilisation at 60 min, was approx. 413 μM using the 250 mg rapid-dissolving granule formulation.

Table 1
Comparison of 250 and 100 mg rapid-dissolving granule formulation dissolution

Time (min)	% ursodeoxycholic acid released	
	100 mg capsules	250 mg capsules
0	0	0
5	ND	49.2
10	ND	68.5
15	96.7	75.7
30	98.1	81.3
45	98.6	85.1
60	98.6	86.0

Values are means of six individual capsule dissolution determinations. The bile acid content of each dissolution sample was determined in duplicate or triplicate. In each case the coefficient of variance was less than 3% for samples collected after 10 min. N.D. signifies bile acid concentration not determined.

3.3. Effect of physical form on the dissolution characteristics of ursodeoxycholic acid

A dissolution study was carried out using capsules containing 250 mg of unformulated ursodeoxycholic acid in different physical forms. Ursodeoxycholic acid from batch A was pharmaceutical grade material (Fr.P) in a crystalline form (not more than 30% greater than 250 μm). Ursodeoxycholic acid from batch B (used in the preparation of the rapid-dissolving granule formulations) was a pharmaceutical grade material (Fr.P.) in a micronised form (particle size; 100% < 10 μm, 95% < 5 μm). Batch C was a non-pharmaceutical grade sodium salt of ursodeoxycholic acid. The dissolution characteristics of these three materials are presented in Table 2. The results show that standard crystalline ursodeoxycholic acid from batch A has very poor dissolution characteristics compared with the micronised material in batch B. After 15 min only

Table 2
Effect of physical form on ursodeoxycholic acid dissolution

Time (min)	% ursodeoxycholic acid released		
	Batch A (crystalline)	Batch B (micronised)	Batch C (sodium salt)
0	0	0	0
5	7	19	38
10	13	53	83
15	20	66	91
30	28	78	92
45	35	81	93
60	38	83	93

Values are means of six individual capsule dissolution studies. The bile acid content of each dissolution sample was determined in duplicate or triplicate. In each case the coefficient of variance was less than 3% for samples collected after 10 min.

20% of the crystalline ursodeoxycholic acid from batch A was dissolved compared with 66% for the micronised batch B material. The amount of batch B material dissolved at the end of the dissolution study was more than 2-fold greater than for batch A material, 83% compared with 38%. The sodium salt of ursodeoxycholic acid from batch C was, however, found to have superior dissolution characteristics to either batch A or B with in excess of 90% dissolution taking place after 15 min.

3.4. Comparison of the dissolution characteristics of ursodeoxycholic acid preparations

A study was carried out to compare the dissolution characteristics of the following ursodeoxycholic acid preparations; Actigall® 300 mg capsules, Destolit® 150 mg tablets and the 250 mg rapid-dissolving granule formulation. To provide a suitable comparison, the volume of dissolution media was adjusted to 900 ml for Actigall® capsules and 450 ml for Destolit® tablets. The results in Fig. 2 show a comparison of the dissolution profiles obtained from the different ursodeoxycholic acid formulations. The lowest levels of ursodeoxycholic acid dissolution, 27% after 15 min increasing to 39% at 60 min, were obtained with Actigall®. Dissolution studies with Actigall® were accompanied by the appearance of large amounts of undissolved granular material at the bottom of the dissolution vessel.

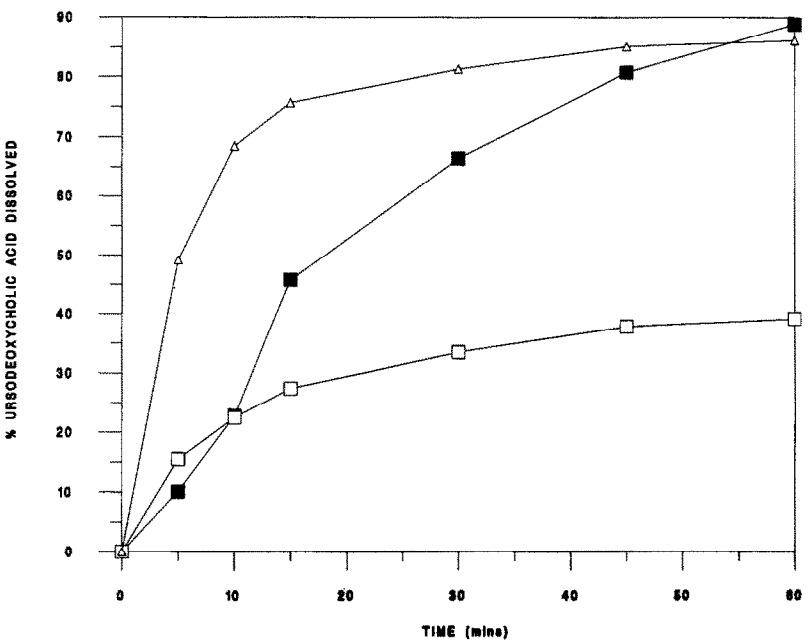

Fig. 2. Dissolution profiles of ursodeoxycholic acid formulations at pH 7.2; Actigall® (□), Destolit® (■), and 250 mg rapid-dissolving granule formulation (△).

Destolit® tablets gave the most efficient dissolution characteristics of the commercial preparations studied, with 46 and 88% ursodeoxycholic acid dissolution occurring at 15 and 60 min, respectively. Destolit® tablets were initially slow to disintegrate, however, the large granular material initially released subsequently dissolved without residual material remaining in the dissolution vessel after 60 min. In comparison to the commercial preparations the 250 mg rapid-dissolving granule formulation resulted in the most efficient dissolution of ursodeoxycholic acid, i.e., 76% at 15 min and 86% at 60 min. After capsule opening the granules rapidly dispersed and then dissolved in the dissolution media forming a homogeneous solution without accumulation of undissolved material at the bottom of the dissolution vessel.

4. Discussion

The poor aqueous solubility of ursodeoxycholic acid has been extensively investigated (Igimi and Carey, 1980; Moroi et al., 1992). Poor solubility of ursodeoxycholic acid in gastrointestinal fluid has been interpreted as the explanation for poor and highly variable in vivo bioavailability by Parquet et al. (1985). The present study used an in vitro dissolution method to investigate the performance of several commercial preparations of ursodeoxycholic acid; also studied were the dissolution profiles of unformulated ursodeoxycholic acid in various forms and a novel rapid-dissolving granule formulation designed to improve the solubility of ursodeoxycholic acid in vivo. Both of the commercial preparations tested, Actigall® and Destolit®, showed slow dissolution characteristics during the first 15 min of the dissolution study. After 60 min only 27.5% of the ursodeoxycholic acid was released into solution from the Actigall® formulation, a value considerably below that achieved with unformulated crystalline ursodeoxycholic acid from batch A (cf. Fig. 2 with Table 2). The dissolution profile of Destolit® was considerably better than that of Actigall® and quite closely resembled the dissolution profile obtained with unformulated micronised ursodeoxycholic acid (cf. Fig. 2 with Table 2). In comparison to the commercial preparations tested, the 250 mg rapid-dissolving granule formulation of ursodeoxycholic acid resulted in the dissolution of at least 65% more bile acid at 15 min than Destolit®, the most effective commercial preparation, and nearly 3-fold more bile acid than the least effective formulation Actigall® (Fig. 2). After 60 min the amount of ursodeoxycholic acid dissolved in the dissolution media was similar for Destolit® and the 250 mg rapid-dissolving granule, levels which were at least 2-fold greater than the least effective formulation, Actigall® (Fig. 2). The 250 mg rapid-dissolving granule formulation was the only formulation able to overcome the initially slow dissolution characteristics of unformulated micronised ursodeoxycholic acid while resulting in similar or enhanced final levels of dissolution after 60 min (cf. Table 2 and Fig. 2). This rapid and enhanced dissolution of ursodeoxycholic acid was probably achieved by the increased 'wetting effect' bestowed on the rapid-dissolving granule formulation by a soluble filler, lactose, and the rapid disintegrating effect brought about by the inclusion of croscarmellose sodium in the formulation. Croscarmellose sodium acts as a water wicking/swelling disintegrant, allowing rapid water ingress into the granules promoting their subsequent swelling and disintegration. In comparison, the formulation with the poorest performance, Actigall®, is reported to contain excipients relatively insoluble in water; silicon dioxide, magnesium stearate, and starch (Physicians Desk Reference, 1993).

The likely consequences of using an ursodeoxycholic acid formulation with poor dissolution characteristics in vivo are low and erratic bioavailability, with 50% or more of the drug not being absorbed from the gastrointestinal tract (Parquet et al., 1985). Although the dissolution of cholesterol gallstones with ursodeoxycholic acid requires long-term treatment involving modifications to the composition of bile acids contained in the human bile acid pool, sub-optimal dissolution of ursodeoxycholic acid from current commercial formulations will mean that these modifications to the bile acid pool, resulting in gallstone dissolution, will take longer to achieve. Furthermore, inefficient ursodeoxycholic acid delivery during

long-term therapy may result in a shift from the ideal bile acid pool composition for maximum effective gallstone dissolution. Perhaps most importantly, however, is the undesirability for large amounts of residual bile acids, from poorly dissolving formulations, to be present in the large intestine because of the potential for increased risk of colorectal cancer (Reddy and Wynder, 1973; Wynder and Reddy, 1974; Reddy et al., 1977; Takano et al., 1984).

In conclusion, the rapid-dissolving granule formulation described in the present study has the potential to improve greatly the delivery of ursodeoxycholic acid in vivo by increasing solubility in gastrointestinal fluid. The rapid and almost complete dissolution of more than 2-fold more ursodeoxycholic acid, compared with some commercial preparations, raises the possibility of reducing the administered dose by 50% for the same therapeutic efficacy in gallstone dissolution. It is believed that the rapid-dissolving granule formulation of ursodeoxycholic acid will prevent the accumulation of bile acids in the large intestine during gallstone dissolution therapy, particularly where optimum dissolution characteristics of the formulation are maintained by the application of an appropriate pH sensitive enteric-coating material. The development of more efficient formulations for ursodeoxycholic acid may increase the likelihood of its expanded clinical use, for the treatment of hepatobiliary disorders in addition to the medical dissolution of gallstones.

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