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NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN: URSODIOL

DRUG IDENTIFICATION

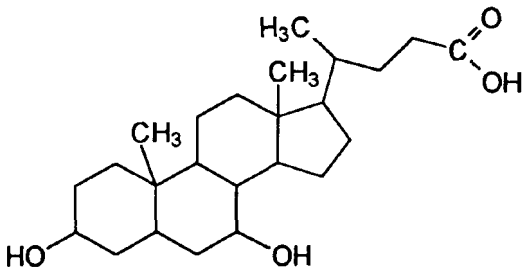
CAS Registry No.: 128-13-2

CAS Name (9CI): 3α,7β-Dihydroxy-(3α,5128β)-cholan-24-oic Acid

Synonyms: Actigall® (Active Ingredient)
Deursil® (Active Ingredient)
Ursodeoxycholic Acid (UDCA)

Molecular Wt.: 392.6

Structure:



EXECUTIVE SUMMARY

Ursodiol is a minor bile acid found in trace amounts in human and rat bile. It is marketed as Actigall® (Ciba-Geigy, Summit, NJ) for gallbladder stone dissolution [1]. Ursodiol has also been used clinically to treat patients with a variety of liver diseases including chronic hepatitis, primary biliary cirrhosis, and cholestasis [*e.g.*, 2,3].

Bile acids are amphiphilic molecules synthesized in the liver from cholesterol. They play an important role, through formation of micelles, in solubilizing cholesterol in bile and in digestion and absorption of lipids in the small intestine. Enterohepatic circulation effectively conserves 95% of the bile acids within the body. However, approximately 5% of primary bile acids are lost into the large bowel during every cycle and undergo extensive degradation (deconjugation and dehydroxylation) by anaerobic flora, leading to the formation of unconjugated secondary bile acids. Specific bile acids, including primary bile acids cholic acid (CHOL) and chenodeoxycholic acid (CDCA), and the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA), have been implicated in the promotion of colon carcinogenesis [4]. For example, blood levels of DCA in men have been related to the incidence of colorectal adenomas [5] and colon cancer patients are reported to have higher levels of DCA and LCA in fecal water [6]. The mechanisms presumably are cytotoxicity and compensatory cellular proliferation, which may be mediated by indirect or direct activation of protein kinase C (PKC) [4,5,7–18]. Induction of mitotic aneuploidy as demonstrated in yeast may also play a role in tumor promoting activity of bile acids [19].

In contrast to the harmful effects of other bile acids [*e.g.*, 20,21], ursodiol inhibits 7α-dehydroxylase in colonic bacteria, resulting in significantly lower formation of DCA from primary bile acids

CHOL and CDCA [22–24]. In addition, ursodiol increased hepatic glutathione-*S*-transferase (GST) activity in mice [25] and stabilized erythrocyte and hepatocyte cell membranes against the cytotoxic effects of DCA and CDCA *in vitro* [20,21]. Further, studies carried out in patients with primary biliary cirrhosis demonstrated the immunomodulatory properties of ursodiol. For example, immunosuppression induced by other endogenous bile acids was reduced by replacing them with ursodiol, and ursodiol also reduced hepatic expression of human leukocyte antigens (HLA class I), which have been implicated as targets for cell-mediated immune damage [26–29].

No preclinical efficacy studies have been sponsored by the NCI, Chemoprevention Branch. In the published literature, the chemopreventive efficacy of ursodiol in AOM-induced colon carcinogenesis has been demonstrated in two identical rat studies [30,31].

The pharmacological and toxicological properties of ursodiol have been reviewed [32]. In humans, maximum ursodiol plasma concentration is reached approximately 60 minutes after ingestion, with another peak recorded at three hours. Ursodiol is rapidly conjugated with glycine and taurine in the liver and excreted into the bile; it is concentrated in the gallbladder and expelled into the duodenum where a large proportion is reabsorbed. In colon, free ursodiol or the conjugated products undergo microbial transformation, forming either LCA or 7-ketolitholic acid. Any remaining free ursodiol, LCA, and 7-ketolitholic acid are poorly soluble in fecal water and therefore excreted in feces.

No preclinical toxicity studies have been sponsored by the Chemoprevention Branch. Preclinical toxicity data reported by the manufacturer for FDA approval of ursodiol (Actigall®, Ciba-Geigy Co., Summit, NJ) as a treatment for the dissolution of gallstones included two-year carcinogenicity studies in CD-1 mice and Sprague-Dawley rats at doses of 50, 250, and 1,000 mg/kg diet (mouse: *ca.* 0.01, 0.08, 0.33 mmol/kg-bw/day; rat: *ca.* 0.006, 0.03, 0.13 mmol/kg-bw/day), reproductive studies in rats and rabbits with doses up to approximately 200 times the human therapeutic dose, and an Ames *Salmonella* mutagenicity test [1]. The only toxic effect observed was a significant increase in the incidence of pheochromocytomas in the adrenal medulla of males and females in the rat carcinogenicity studies, a common condition in that species. Therefore, no MTD has been established for rats; in mice the MTD is ≥1,000 mg/kg diet (*ca.* 0.33 mmol/kg-bw/day).

A number of large-scale clinical trials are ongoing or have been completed with ursodiol in support of the approved use for treatment of gallbladder stones. Ursodiol is considered to be a relatively safe drug [*e.g.*, 33–37]. The majority of the reported adverse reactions to Actigall® were either gastrointestinal or dermatological; overall, they were minor and of low frequency. The most common reaction reported was diarrhea, which occurred in <1–6% of subjects exposed to recommended doses of 8–10 mg/kg-bw/day (0.02–0.025 mmol/kg-bw/day); other minor gastrointestinal reactions included dyspepsia, vomiting, constipation, and flatulence. Examples of minor dermatological complaints included pruritus, urticaria, rash, and dry skin [5].

Ursodiol is marketed as Actigall® by Ciba-Geigy (Summit, NJ) in the form of 300 mg capsules [1]. Bulk drug is also available from Sigma, Aldrich and TCI America [38–40]. Other sources of formulated ursodiol include Destolit (Lepetit), Deursil® (Gipharmex, Milan, Italy), Ursacol (Zambon Group S.P.A., Vincenza, Italy), URSO (Tokyo Tanabe Co., Ltd., Tokyo, Japan), and enteric-coated ursodiol (Erregierre S.P.A., Bergamo, Italy). Based on an agreement with the manufacturer of Actigall®, Ciba-Geigy will supply the drug and placebo for Chemoprevention Branch-sponsored trials.

The primary target organ for clinical development of ursodiol is the colon. The NCI, Chemoprevention Branch is sponsoring a Phase I/IIa (Drs. David Alberts and David Earnest, University of Arizona) single- and multidose study (three weeks) in asymptomatic (healthy, normal) subjects and individuals at increased risk for colon cancer. In the second phase of the study (three weeks), patients with total colectomies and either an ileostomy or an ileoanal anastomosis will be administered ursodiol doses selected from the first phase of the study. In these studies, single and postprandial blood pharmacokinetics, safety, and efficacy (reduction in fecal and ileostomy fluid DCA concentration) will be assessed. Longer term (three months) studies are also planned to assess safety, adenomatous polyp recurrence, bile acid concentration in blood and feces, and rectal mucosal proliferation rates measured by PCNA. Intermediate biomarkers and drug effect measurements which have been modulated by ursodiol in animal models include PKC [31,41], GST

[25], and MHC antigen expression [42]. Based on these limited data, it may be of interest to examine the effects of ursodiol on rectal PKC and rectal and lymphocyte GST activity in Phase I and Phase II randomized, controlled clinical trials. Additionally, based on recently published data [4,43], the effects of ursodiol on colon mucosal ODC and rectal PGE_2 in asymptomatic (healthy, normal) and high-risk colon cancer populations may be evaluated.

PRECLINICAL EFFICACY STUDIES

No preclinical efficacy studies using ursodiol have been funded by the Chemoprevention Branch. Two parallel studies to examine the efficacy of ursodiol against AOM-induced colon carcinogenesis in F344 rats were carried out by Earnest *et al.* at University of Arizona and University of Chicago [30,31]. Administration of 0.4% ursodiol (*ca.* 0.5 mmol/kg-bw/day) in the diet significantly reduced the incidence (53%) and total number (41%) of colon tumors. Ten (22%) ursodiol-treated animals had tumors compared with 20 (47%) AOM control animals. The total number of tumors was also significantly reduced from 22 in the control group to 13 in the ursodiol-treated group ($p<0.05$). In contrast, 0.2% ursodiol (*ca.* 0.25 mmol/kg-bw/day) insignificantly increased the incidence (2%), multiplicity (18%), and total number (32%) of tumors. The quality of these results was increased by the fact that similar results were obtained at both locations.

PRECLINICAL SAFETY STUDIES

No preclinical safety studies have been funded by the Chemoprevention Branch; the following data are compiled from the literature. Preclinical toxicity data generated by the manufacturer (Ciba-Geigy Co., Summit, NJ) for FDA approval of ursodiol (Actigall®) included two-year carcinogenicity studies in CD-1 mice and Sprague-Dawley rats, reproductive studies in rats and rabbits, and an Ames *Salmonella* mutagenicity test [1]. The only toxic effect observed was a significant dose-related increase in the incidence of pheochromocytomas in the adrenal medulla of males and females in the rat carcinogenicity studies, a common condition in that species. Therefore, no MTD has been established for rats; in mice the MTD is ≥1,000 mg/kg diet (*ca.* 0.33 mmol/kg-bw/day).

ADME: Bile acid metabolism is highly species-specific. Compared with man, bile acid synthesis and metabolism are significantly different in other species such as the rat. In rats, amidation with taurine is the major conjugation pathway, CHOL and 6β-hydroxylated metabolites are the major bile acids, and CDCA is of negligible quantitative importance [44].

The pharmacokinetic profile and metabolism of ursodiol was studied in Sprague-Dawley rats [44, 45] and hamsters [46]. Following oral administration of 20 mg [^{3}H]-ursodiol (*ca.* 0.17 mmol/kg-bw) to rats, serum levels were low, with a C_{max} (0.108– 0.056% of administered dose) between one and three hours after ingestion. Conjugation with taurine (80%) peaked after one hour and with glycine (15%) after two hours. Bile samples revealed that 35% of the administered dose was secreted within the first six hours, with the highest rates in the first two hours. Only 1.5% of the administered dose was excreted in the urine, mostly in the first 12 hours. In feces, 15–20% of the administered dose was excreted in the first 24 hours; the major metabolite in feces was LCA (80%) [45].

In another recent study in rats, 3α,7β-dihydroxy-5β-chol-22-en-24-oic acid (Δ^{22}-UDCA) was identified as the major metabolite in the plasma, bile, intestinal contents, and liver tissue after intravenous infusion (1 ml/hr for two hours) or administration of ursodiol in diet (0.4%, *ca.* 0.5 mmol/kg-bw/day; 1%, ca. 1.3 mmol/kg-bw/day, 10 days) [44]. It should be noted that this metabolite has not been found in human studies; however, the dose administered in human studies (10–15 mg/kg-bw/day, or 0.025–0.038 mmol/kg-bw/day) was significantly lower than that used in the rat study [e.g., 1].

In hamsters, 0.5 mg of radiolabelled CDCA and ursodiol were injected simultaneously into the jejunal loop, and the taurine and glycine conjugation products were measured in collected bile samples. Both radiolabelled products were recovered in the bile within one hour. CDCA appeared to be more efficiently conjugated with glycine than ursodiol; the relative proportions of glycine conjugate, taurine conjugate, and unconjugated form of ursodiol were 57.3%, 36.5%, and 6.2%, respectively [46].

The formation of LCA from CDCA and ursodiol was compared in rhesus monkeys and patients with asymptomatic gallstones. Formation of LCA from CDCA and ursodiol was similar in both *in vivo* and *in vitro* experiments [47]. In the *in vitro* studies, conversion of both bile acids to [^{14}C]LCA at 12 hours was approximately 90%; the rate of formation of

LCA was similar in the *in vivo* studies.

The $t_{1/2}$ of exogenously administered ursodiol has been estimated at 2.5 and 3.75 days in mice following oral and iv exposure, respectively, and at two days in rhesus monkeys after oral administration [32].

Safety: Ursodiol is virtually non-toxic with an oral LD_{50} of 7,500 mg/kg-bw (*ca.* 19.1 mmol/kg-bw) in mice and >5,000 mg/kg-bw (*ca.* 12.7 mmol/kg-bw) in rats [1]. Two-year carcinogenicity studies in CD-1 mice and Sprague-Dawley rats were performed by the manufacturer (Ciba-Geigy Co., Summit, NJ) at doses of 50, 250, and 1,000 mg/kg diet. No evidence of tumorigenicity was found in mice; therefore, based on these data, the MTD in mice is ≥1,000 mg/kg diet (*ca.* 0.33 mmol/kg-bw/day). In rats, there was a dose-related, significant increase in pheochromocytomas in the adrenal medulla of males and females; the significance of this effect is not known [1], since this condition is common in this species. Ursodiol was not mutagenic in the Ames *Salmonella* mutagenicity test [1], but positive in a Chinese hamster ovary (CHO) clastogenicity assay [48].

Reproductive studies in rats and rabbits with doses up to approximately 200 times the human therapeutic dose showed no effect on fertility or the fetus [1].

CLINICAL SAFETY: PHASE I STUDIES

Safety: Ursodiol was approved by the FDA in 1987 [49] for use in the treatment of gallstones under the brand name Actigall®. It is relatively safe and well-tolerated. After chronic use up to one year in length, only mild gastrointestinal (GI) and dermatological adverse reactions have been reported at recommended doses of 8–10 mg/kg-bw/day (0.02–0.025 mmol/kg-bw/day) [1]. Ursodiol has been used safely in Japan for more than 100 years.

The main adverse reaction is diarrhea, ranging in incidence from <1% to 6% depending on conditions of the study [1]. Two ongoing double-blind, placebo-controlled studies in the US have found minor dermatological side effects such as pruritus, urticaria, rash, dry skin, sweating, and hair thinning. Besides diarrhea, other GI disorders have been noted, among them vomiting, dyspepsia, metallic taste, abdominal pain, cholecystitis, constipation, stomatitis, and flatulence. In addition, general symptoms of headache, fatigue, anxiety, depression, sleep disorder, arthralgia, myalgia, back pain, cough, and rhinitis have been noted [1]. The frequency of these reactions is not known, since these double-blinded studies have not been completed.

During the clinical trials for approval of Actigall®, four women were accidentally exposed to the drug in the first trimester of their pregnancy. No adverse effects to fetuses or newborn babies were reported [1]. The safety of Actigall® in nursing mothers and children has not been established.

A single- and multidose Phase I trial of ursodiol is being sponsored by the NCI, Chemoprevention Branch. The funded study is divided into three parts, designated IA, IB, and II; Task II is described under CLINICAL EFFICACY: PHASE II/III STUDIES, below. Task IA will attempt to identify the lowest dose of commercially available ursodiol which will reduce the proportion of DCA in the aqueous phase of stool by 40% or more in 18 asymptomatic (healthy, normal) subjects after three weeks of daily (300, 600, 900 mg) ursodiol therapy. Single-dose and steady-state pharmacokinetics will be assessed; since saturation levels of ursodiol are not reached until after approximately two weeks of daily administration, a postprandial pharmacokinetics study will be carried out after three weeks (21 days) of therapy. The lowest dose level which reduces DCA by 40% or more will be used in Task IB.

The pharmacokinetic and toxicity profile measurements in Task IB will be identical to Task IA. However, under this task, DCA concentration will be measured after three weeks of therapy in ileostomy fluid of six patients with total colectomies and either an ileostomy or an ileoanal anastomosis. Pharmacokinetics values will be compared and contrasted between Tasks IA and IB. Adverse events will also be observed and quantitated. Based on these data, doses will be selected for Task II (see CLINICAL EFFICACY: PHASE II/III STUDIES, below).

Drug Effect Measurement: Increases in the concentration of bile acids in blood and feces have been related to colon polyp development [5,6]; therefore, these are appropriate initial measurements to explore possible ursodiol activity against colon cancer. Recently published data have shown significant increases in hepatic GST activity in mice after three weeks of ursodiol (URSO, Tokyo Tanabe Co., Ltd., Tokyo, Japan) at dietary doses of 0.3% (*ca.* 0.98 mmol/kg-bw/day) and 0.5% (*ca.* 1.6 mmol/kg-bw/day) [25]. In this study, increased hepatic GST activity correlated to a higher survival rate by reducing the toxic effects of 1,2-dichloro-4-nitrobenzene, a substrate for this enzyme. Based on these limited

data, it may be of interest to evaluate GST activity in colon and lymphocytes in a clinical trial.

Because of the extensive enterohepatic circulation, ursodiol levels in plasma and serum are not indicative of colon bioavailability, but they are useful in determining patient compliance and relative bioavailability. If the first-pass hepatic clearance is constant, there is a "spill-over" into peripheral circulation; therefore, the AUC may be used to compare bioavailability.

ADME: In humans, *ca.* 90% of a therapeutic dose of ursodiol (8–10 mg/kg-bw/day, tid) is absorbed from the small intestine and proximal colon after oral ingestion. From there, ursodiol enters the portal vein and is extracted from the portal blood by the liver (the first-pass effect) where it is conjugated with either taurine or glycine and excreted into the bile. Ursodiol in bile is concentrated in the gallbladder, and expelled into the duodenum where a large proportion is reabsorbed. There is also some overflow of absorbed ursodiol from enterohepatic into systemic circulation; 0.01% of the initial dose is excreted in the urine [1,50]. After the conjugates and/or free ursodiol in the bile reach the colon, there is some deconjugation and degradation by the bacterial flora, which either oxidize or reduce ursodiol at the 7-carbon to give either 7-ketolithocholic acid or LCA [1]. Any remaining free ursodiol, 7-ketolithocholic acid, and LCA are poorly soluble in fecal water, resulting in fecal excretion of the major portion [1].

Several urinary metabolites of ursodiol have also been identified. One study carried out in ten healthy subjects demonstrated that *N*-acetylglucosamine conjugates are major urinary metabolites (50%) of ursodiol (Ursofalk, Herbert Falk GmbH, Freiburg, Germany) administered at 750 mg/day (*ca.* 0.03 mmol/kg-bw/day) for ten days [51]. Other urinary metabolites tentatively identified after administration of the same dose for 2–3 weeks to seven gallstone patients include 3α,7β,22-trihydroxycholan-24-oic acid, 3α,5α,7β-trihydroxycholan-24-oic acid, 1β,3α,7β-trihydroxycholan-24-oic acid, and 3α,6α, 7β-trihydroxycholan-24-oic acid, which account for 10–15% of ursodiol in urine [52].

In a single-dose bioavailability study, four men and three women were administered a capsule containing 500 mg [^{14}C]-ursodiol (Roussel Uclaf Laboratories, Romainville, France). After 40 minutes, ursodiol appeared in the plasma and peaked between 60 and 80 minutes post-dose. Maximum plasma levels were *ca.* 11.3 μM for this time period. An average of 28% was recovered from the feces in the first 72 hours; maximum fecal excretion occurred on the third day (17–21%). The major bile acid present was LCA [50].

In a multidose study, patients with chronic hepatitis C infection were treated with daily doses of 150, 600, and 900 mg ursodiol (*ca.* 0.0063, 0.024, 0.036 mmol/kg-bw/day) (URSO, Tokyo Tanabe Co., Ltd., Tokyo, Japan) for 16 weeks [53]. The maximal concentrations of total serum ursodiol (free, glycine and taurine conjugates) were 5.6 (week 16), 21.4 (week 16), and 14.7 μM (week 12) for 150, 600, and 900 mg/day doses, respectively. These levels were significantly higher than that for the control group (0.3 M at week 16).

CLINICAL EFFICACY: PHASE II/III STUDIES

No Phase II chemoprevention clinical trials have been carried out by the NCI, Chemoprevention Branch; however, a Phase II study to evaluate the effects of ursodiol on colorectal adenoma recurrence is under consideration.

Task II of the funded Phase I study will attempt to obtain preliminary data on effects of ursodiol on bile acid concentration in blood and feces and rectal mucosal proliferation. This is a double-blind, placebo-controlled randomized trial in 22 patients at increased risk for colon cancer (at least one adenomatous polyp removed within the previous ten years). Ursodiol and placebo will be administered for three months. Two ursodiol doses will be used, the one selected in Task 1A (described above under CLINICAL SAFETY: PHASE I STUDIES) and 50% of this dose. Safety, adenomatous polyp recurrence, bile acid concentration in blood and feces, and rectal mucosal proliferation rates measured by PCNA will be assessed. Total duration of the study is estimated to be 18 months.

PHARMACODYNAMICS

The recommended dosage of Actigall® for optimal solubilization of gallstones is 8–10 mg/kg-bw/day, tid (0.02–0.025 mmol/kg-bw/day) for periods not greater than 24 months. Doses of 5–20 mg/kg-bw/day (0.013–0.05 mmol/kg-bw/day) have been well-tolerated for periods of 6–78 months in clinical trials carried out with ursodiol [1]. The MTD in mice (≥1,000 mg/kg diet, or *ca.* 0.33 mmol/kg-bw/day) is approximately 10-fold higher than the therapeutic dose in humans (10 mg/kg-bw/day, or 0.025

mmol/kg-bw/day), an acceptable margin of safety.

In the preclinical chemoprevention studies, a dose of 0.4% ursodiol in the diet (*ca.* 0.5 mmol/kg-bw/day) was effective in decreasing the incidence of AOM-induced colon tumors in rats [30]. This dose is approximately 20-fold higher than that used in human subjects for gallstone solubilization (0.025 mmol/kg-bw/day). Additionally, in experimental animals, Kurtz *et al.* found a *ca.* 50% reduction in the percent of DCA in the colonic wall and lumen contents of rats fed 90 mg/kg-bw/day (0.23 mmol/kg-bw/day), corresponding to 15 mg/kg-bw/day (0.038 mmol/kg-bw/day) in humans, corrected for body surface [22]. In a clinical study, the same ursodiol dose (Tokyo Tanabe Co., Ltd., Tokyo, Japan) for 5–6 weeks reduced the percent of duodenal bile acid DCA from 21.7% to 10%, a >50% reduction [54]. Based on these data, 8–10 mg/kg-bw/day (0.02–0.025 mmol/kg-bw/day for the average human) should be well-tolerated and safe for a long-term chemoprevention study. In the Phase I clinical trial sponsored by the Chemoprevention Branch, the safest and most efficacious dose for the second phase of the study will be selected from administration of 300, 600, and 900 mg/day (*ca.* 0.012, 0.024, 0.036 mmol/kg-bw/day) for three weeks.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

Blood levels of DCA in men have been related to the incidence of colorectal adenomas [5]; therefore, monitoring changes in concentrations of bile acids in blood and feces (particularly DCA) are most suitable drug effect measurements [5]. Based on recent publications, it may be of interest to examine the effects of ursodiol on GST activity, possibly in lymphocytes or colon, since oral administration of this agent was shown to increase survival of mice by increasing hepatic GST activity [25].

Safety Issues

Ursodiol has very low toxicity and is well tolerated at doses used in the treatment of gallstones and other liver diseases; however, LCA is a toxic metabolite formed by anaerobic bacterial degradation of ursodiol conjugates in the colon. In humans, unabsorbed LCA, largely insoluble in fecal water, is excreted in the feces. Absorbed LCA is efficiently detoxified in the human liver by sulfation, a factor which may put those with impaired liver function at increased risk. Although no reports of liver toxicity were found, impaired liver function could result in adverse reactions [1]. Patients should have SGOT and SGPT measured before beginning therapy and monitored during trials, with special attention/more frequent monitoring in anyone with current or previous liver related symptoms.

Pharmacodynamics Issues

The primary target tissue for clinical development of ursodiol is the colon; another possibility may be the liver, since ursodiol has been used in treating chronic hepatitis C infection [53]. The primary chemopreventive mechanism of ursodiol is believed to be reducing the concentration of cytotoxic secondary bile acid DCA in the colon mucosa or feces. Clinical trials of ursodiol in gallstone treatment found that a dose of 8–10 mg/kg-bw/day (0.02–0.025 mmol/kg-bw/day) was safe and effective [1]. Additionally, in clinical trials for treatment of primary biliary cirrhosis, doses of 13–15 mg/kg-bw/day (0.033–0.038 mmol/kg-bw/day) were considered safe and well-tolerated. Further, administration of ursodiol at 15 mg/kg-bw/day (0.038 mmol/kg-bw/day) to subjects for 5–6 weeks reduced the percent of duodenal bile acid DCA by >50% [54]. Therefore, the dose-range finding Phase I study sponsored by the Chemoprevention Branch will be carried out in asymptomatic (healthy, normal) subjects at doses of 300, 600, 900 mg/day (*ca.* 0.012, 0.024, 0.036 mmol/kg-bw/day) for three weeks; the selected doses are well within those considered safe.

Regulatory Issues

Ursodiol was approved by the FDA in 1987 for treating gallstones in two or three divided doses of 8–10 mg/kg-bw/day (0.02–0.025 mmol/kg-bw/day) for up to 24 months. Only minor adverse reactions have been reported in treating this condition. Clinical trials performed in support of ursodiol registration for this purpose, and those carried out in patients with primary biliary cirrhosis, gave doses ranging from 5–20 mg/kg-bw/day (0.013–0.05 mmol/kg-bw/day) to approximately 1,400 subjects for periods between six and 78 months [*e.g.*, 1,34, 55–57], apparently without major side effects. There are no other regulatory issues concerning initiation of the Phase I study at the proposed doses.

Intermediate Biomarker Issues

It is of interest to evaluate the effects of ursodiol on colon cell proliferation. Therefore, the proposed endpoint under Task II of the Phase I study is rectal mucosal proliferation measured by PCNA following three months of ursodiol treatment in subjects at high risk for colon cancer. Additionally, bile acids (*e.g.*, DCA) can release prostaglandin E_2 (PGE_2) from colonic tissue and enhance release of arachidonate and subsequently the synthesis of PGE_2. This mechanism has been implicated in increased cellular proliferation [4]. Therefore, it may be of interest to examine the effects of ursodiol on rectal PGE_2 activity in this population. There are other proliferation-related biomarkers which have been investigated thus far in the rat colon and their relevance has yet to be assessed. These include modulation of PKC isoforms ($PKC\alpha$, $PKC\beta_{II}$, $PKC\zeta$) in colon mucosa which alters intracellular signal transduction [31, 41], and up-regulation of MHC antigen expression in rat colonic epithelial cells [42]; expression of these antigens is reduced during human colon carcinogenesis. Additionally, although not demonstrated with ursodiol in animal models, modulation of colon mucosal ODC activity may be examined [43]. This biomarker associated with proliferation is induced by CHOL; ODC is inhibited by other potential colon chemopreventive agents, such as calcium carbonate [43].

For the future Phase II trial, based on results from the Phase I study, other endpoints such as the appearance of colorectal adenomatous polyps, as well as other histologic biomarkers including nuclear size, shape, texture, and ploidy as determined by quantitative computer-assisted image analysis in rectal epithelial cells, may be evaluated.

Supply and Formulation Issues

Ursodiol is currently marketed by Ciba-Geigy (Summit, NJ) as Actigall® in 300 mg capsules for gallbladder stone dissolution, a treatment which may require up to 24 months of dosing [1]. Bulk drug is also available from Sigma, Aldrich and TCI America [38–40]. Other sources of ursodiol include Destolit (Lepetit) [32], Deursil® (Gipharmex, Milan, Italy) [58], Ursacol (Zambon Group S.P.A., Vincenza, Italy) [32], URSO (Tokyo Tanabe Co., Ltd., Tokyo, Japan) [53], and enteric-coated ursodiol (Erregierre S.P.A., Bergamo, Italy) [59]. Based on an agreement with Ciba-Geigy, the manufacturer will supply ursodiol and placebo for the Chemoprevention Branch-sponsored clinical trials.

Recently, a new formulation of ursodiol was developed by Erregierre S.P.A. (Bergamo, Italy). The new 450 mg enteric-coated ursodiol formulation appears to be better absorbed than the commercially available formulation (30–40% of the administered dose absorbed), since it is pH-dependent and is released only at a pH≥6.5 [59].

Clinical Studies Issues

There appears to be ample justification for proceeding with an ursodiol clinical chemoprevention trial in colon. The two-year carcinogenesis studies in rats and mice, the mutagenesis and reproductive toxicity studies, in addition to clinical trials performed to support the registration of ursodiol as a treatment for gallstones and other therapies, provide a wealth of information on the safety of this agent, while evidence of significant inhibition of AOM-induced colon cancer in rats was provided by Earnest *et al.* [30]. The case for ursodiol as a chemopreventive agent is supported by its ability to reduce the concentration of DCA, a known cancer promotor, in the rat colonic wall and lumen contents [15,22].

Because LCA is increased in bile and feces following ursodiol treatment, subjects with impaired liver function should be excluded, although two reports (Roussel Uclaf Laboratories, Romainville, France and Sanofi-Winthrop, Italy) demonstrated the beneficial effects of ursodiol treatment on liver enzymes in patients with chronic hepatitis and biliary cirrhosis [2,50].

Based on published studies [60–62], fecal bile acid concentration and absorption of secondary bile acids such as DCA from the large bowel is age-dependent. This factor needs to be considered in designing Phase I or II clinical protocols. Specifically, higher input of DCA from the large bowel into the enterohepatic circulation was found in elderly patients (mean age 67 years) compared with younger patients (mean age 22 years) [62]. Although the pool size and synthesis rate of CHOL were similar, the DCA pool size was higher in the older groups suggesting that active ileal absorption of conjugated bile acids is decreased in this population. Similar results were demonstrated in patients over 60 (mean 67 years) and those below 60 years of age (mean 37 years)[60]. Additionally, fecal secondary bile acid (*e.g.*, LCA, DCA, isoDCA) concentration was higher in elderly (mean age 67 years) compared with younger age groups (mean age 22

years). Fecal concentrations of primary bile acids (*e.g.*, CHOL, CDCA) and ursodiol were similar among these groups.

Ursodiol has also been reported to modulate cholesterol levels [reviewed in 63]. In several clinical trials evaluating the efficacy of ursodiol against primary biliary cirrhosis, for example [34–37], and in healthy subjects with or without hyperlipidemia [54,64], ursodiol treatment for two weeks at 1 g/day (*ca.* 0.04 mmol/kg-bw/day), or 5–6 weeks at 15 mg/kg-bw/day (0.038 mmol/kg-bw/day) for up to two years at daily doses of 13–15 mg/kg-bw (0.033–0.038 mmol/kg-bw/day), resulted in significant decreases in cholesterol levels. The mechanism of action of ursodiol in this regard, which is not considered to be by inhibition of HMG CoA reductase activity, has recently been reviewed [*e.g.*, 65, 66]. It appears that lowered cholesterol levels—in particular, low-density lipoprotein (LDL)—is related to ursodiol's interference with the enterohepatic circulation of normal bile acids, especially when administered at high doses. This property may also be explained by an increase in LDL receptor interaction and therefore uptake by ursodiol [67]. These effects may be relevant to evaluate in future chemoprevention studies, possibly in combination with such agents as aspirin.

The NCI, Chemoprevention Branch has funded a Phase I/IIa Pharmacokinetics, safety and efficacy study in the colon. Based on the results of this study, a Phase II trial to evaluate the effects of ursodiol on colorectal adenoma recurrence may be sponsored.

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Table I. Clinical Trials of Ursodiol Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase I (Safety and ADME)					
NO1-CN-55122 Phase I Single and Multiple-Dose Safety and Pharmacokinetic Clinical Study of Chemopreventive Agent, Ursodiol (Drs. David S. Alberts and David Earnest, University of Arizona) 6/95–12/96 IND 50,236	---	Task 1A: Normal, healthy subjects, Task 1B: Total colectomy and either an ileostomy or ileoanal anastomosis, Task II: High risk for colon cancer Task 1A: 18 subjects Task 1B: 6 patients Task II: 22 patients	Task 1A: Single- and multidose (3 wk): 300, 600, 900 mg/day tid Tasks 1B and II: To be determined	Tasks 1A, 1B and II: Efficacy: Bile acid concentrations in feces and fluid from the distal small intestine (ileum) Single and postprandial pharmacokinetics and safety Task II: Efficacy: PCNA	Study in progress

URSODIOL DEVELOPMENT STATUS

Task Name	1995	1996	1997	1998
CLINICAL, PHASE I (PHASE IIa)				