B. Pharmacokinetics of UDCA

1. Physicochemical properties

- UDCA, of all dihydroxy-BAs, is poorly soluble in its protonated form. The aqueous solubility is ca. 9 µmol/l (ca. one third that of CDCA). The low aqueous solubility is explained largely by the stability of the crystal lattice [P.F. Lindley and M.C. Carey, J. Cryst. Spectrom. Res. 17:231-249 (1987)], which, in turn, is indicated by the melting point of the UDCA crystal [(203°C); S.H. Yalkowsky, J. Pharm. Sci. 70:971-973 (1981)].

- CMC, hydrophobicity, pKa, CMpH

BAs are amphipathic molecules and in water their anions self-associate to form aggregates that have generally been termed micelles. Such aggregation occurs stepwise, but nonetheless occurs over a moderately narrow aqueous concentration range. A concentration within this range is termed the critical micellization concentration (CMC). The CMC values of most natural BAs that predominate in bile, at the Na^+ concentration prevailing in body fluids (0.15M), range from 3 to 10 mM. The CMC of UDCA[24] has been reported to be about 7 mM, about twice that of CDCA. The conjugates of UDCA have a CMC value slightly lower than that of unconjugated UDCA.

BAs are surface active.[25] UDCA is less surface active than CDCA but has a hydrophobicity [lipophile-hydrophile balance] that is similar to that of CDCA. The passive permeation of UDCA across lipid bilayers is nearly as rapid as that of CDCA but the UDCA anion is extremely hydrophilic, having less affinity for the hydrophobic stationary phase than the cholate anion.

- BAs are carboxylic acids that may be considered derivatives of isopentanoic acid. The pKa of all unconjugated BAs is ca. 5.1 [A. Fini et al., J. Solution Chem. 14:595-603 (1985)]. The pKa increases when BAs aggregate in micelles because of the charge density at the surface of the micelle. The pKa of GLY conjugated BAs is ca. 3.9, whereas that of TAU conjugated BAs is <2.

- The solubility of any weak acid increases exponentially with pH, because the anion is water soluble. There is a narrow pH over which the solubility increases markedly, and this pH range is termed the critical micellization pH (CMpH). For UDCA, the CMpH is close to pH8. Accordingly, dissolution of UDCA in the proximal jejunum, where intraaluminal pH is <7, can only occur by solubilization of mixed micelles of other conjugated BAs. The CMpH of the GLY conjugate of UDCA is ca. 6, and that of the TAU conjugate much lower.

- The final physicochemical property of BAs to be considered is their extraordinary ability to solubilize in mixed micellar form polar lipid classes, such as phophatidylcholines (PCS), monoacyl glycerols, or mixed fatty acids (FAs) and soaps.[26]

- BAs are surface active and must bind to membranes. Such binding must create a spreading force, and in time, the outer cellular hemileaflet becomes unstable. Presumably, it buds off, forms a mixed micelle, and the vesicle once again becomes stable.

- UDCA seems to have a lower affinity for vesicles than CA or CDCA.

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[24] Neither the size nor the molecular arrangement of UDCA [redacted] has been defined. Micelles of some of the common natural conjugated BAs are considered to be helical in arrangement [A.R. Campanelli et al., J. Incl. Phenomena & Molec. Recogn. Chem. 10:367-377 (1991)].

[25] Bile acids are surface active. The affinity of the bile acid molecule for the air/water interface is indicated by the effect on surface tension; this can be quantified by the slope of the line in which the surface tension is plotted against the logarithm of the concentration.

[26] This ability is evidenced in bile where lipid is present as mixed bile acid-phosphatidylcholine-cholesterol micelles and in chyme, where lipid is present as bile acid-monoglyceride-fatty acid (partially ionized) micelles. The ability of bile acids to solubilize such lipids (often called swelling amphiphiles) can be depicted using triangular coordinates to show the phase equilibria of the ternary system of water-bile acid-swelling amphiphile. In such a diagram, there is a large micellar zone. If the bile acid is replaced by a typical anionic detergent such as dodecyl sulfate, the micellar area is much smaller.

- The affinity of UDCA for membranes is sufficiently low that the monomeric concentration can reach the CMC before vesicle disruption occurs. At this point, simple micelle formation occurs, and the vesicle remains intact.

- The lack of strong membrane affinity exhibited by UDCA molecules explains the appearance of a transient mesomorphic phase when cholesterol crystals are incubated with a vesicular dispersion containing conjugated UDCA and phosphatidylcholine. The latter absorbs to the cholesterol and forms a liquid crystalline phase on the surface.

- The low solubilizing capacity of UDCA micelles may also explain why the cholesterol crystal appearance time (also called nucleation time) is much longer when gallbladder bile is enriched with UDCA conjugates as a result of UDCA ingestion in cholesterol gallstone patients.

2. <u>Existing Tools to Study Bioavailability of BAs, Including UDCA - Opportunities and Constraints</u>

- After ingestion, BAs produce an enrichment of both bile and serum BAs.

- Sampling of bile gives solid representation of the average biliary composition [No concentration]. Bile in the duodenal aspirate is a good representation of the gallbladder bile, but concentration is variable as it depends on the dilution of the bile sample. It is important to take fasting samples, after contraction of the gallbladder with CCK.

- 1 ml of bile is sufficient to do BA analysis. In the bile all BAs are conjugated. One should report different percentages of the BAs.

- It is important to consider when to sample and whether one needs baseline BA composition. The FDA position is that, to properly describe the bioavailability of ingested UDCA, one needs to assess both, rate and extent of absorption of the BA.

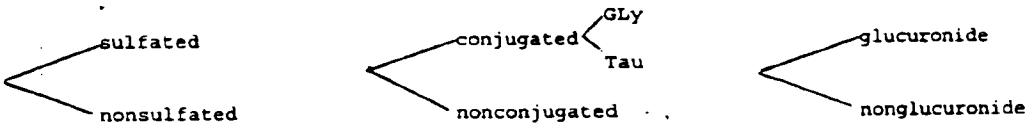
- Serum BAs need to be analyzed as a function of time, for 24h, with emphasis on the first 6 and the last 6 hours after intake. Taken under fasting conditions, these data would be useful to explore correlations between serum and bile (as was done in the Mayo Clinic trial).

  - For serum BA determinations, use of capillary GC is needed. But this technique takes several months to standardize and is available only at a few US centers.

  - Serum BAs need to be determined before and after hepatic uptake. It is worth noting that there is never complete clearance: early after oral administration some BA in the serum is still unconjugated. There will be total conjugation as time goes on.

  - The effects of administering UDCA in various regimens: q.i.d. vs t.i.d. vs b.i.d. vs once-a-day are considered in the subsequent subsection.

  - To completely assess the BA picture, one needs to do: UDCA, CDCA, CA, DCA, LCA and others, such as isomers of LCA, 7-Keto-LCA, etc. For every BA, one needs to do:



In the serum, both concentration and composition should be reported.

3. Additional Considerations (Regarding Available Tools to Study Bioavailability of BAs)

Following duodenal intubation and balloon obstruction to aspirate a sample of duodenal contents, the non-used sample is reinfused so as not to deplete BA pool. But the dilatation is painful to the patient and non-physiologic because is accompanied by a release of g.i. hormones. Collection of bile in bile fistula patients (T-tube post-cholecystectomy) is useful to determine biliary excretion of drugs and/or metabolites. But it is also non-physiologic because the interruption of the EPHBC induces an increased elaboration of BAs by the liver [reported by Erickson (1957)]. The sampling of portal vein blood can only be done in decompensated cirrhotics with portal hypertension under the effects of anesthesia [it is therefore non-physiologic]. Bile can be sampled from the gallbladder in patients scheduled to undergo gallstone dissolution with locally acting agents (MTBE, EP) via a transhepatic catheter. But this procedure is available only at a few centers. In addition, the subjects are usually relatively sick patients who cannot tolerate or do not want surgery.

A good approach to study BA bioavailability is the balance method. But sampling of the stools is cumbersome and may be inaccurate. In addition, endogenous BA excretion is confounding; even today (1996), some colonic products have not been properly identified. There have been some reports using labeled oral + labeled i.v. BAs. But this can be done at highly specialized centers only. Also, there is no assurance that the radioactive BA is on the identical physical form as the non-radioactive BA, the bioavailability of which is being tested.

With all the constraints mentioned above, it is not surprising that the information on the bioavailability and absorption of UDCA is incomplete.

4. Bioavailability, Absorption and Metabolism of UDCA

a. Fate in the stomach

- UDCA is ingested as tablets containing the protonated acid.
- Ingested UDCA remains insoluble in the stomach.
- Fate of tablet depends on the rate of dispersion relative to the rate of gastric emptying.
- If ingested with a meal, the tablets of the dispersed BA are emptied exponentially.
- If ingested during fasting, discharge into the intestine would depend on how soon an interdigestive myoelectric complex occurred; these powerful contractions empty the stomach of all solid material and occur about once an hour.

b. Fate in the small intestine

- UDCA must be solubilized in BA micelles and titrated by pancreatic bicarbonate.
- It would not be solubilized in appreciable amounts unless the bulk pH is close to 7, i.e. about 1 pH unit below its CMpH.
- It would not be well solubilized in the duodenum where the pH is 6 to 7.
- Dissolution would probably occur in the mid-jejunum and in the more distal small intestine.
- Intestinal absorption of UDCA tablets requires dispersion of the particles, dissolution of the particles into monomers and micelles, diffusion through the aqueous boundary layer, passage across the apical membrane of the enterocyte, movement through the enterocyte, and exit across the basolateral membrane of the enterocyte. Paracellular transport of unconjugated UDCA is not expected to occur to a great extent in adult humans because of the size of the BA molecule.

c. Bioavailability studies in man

- There are few studies available.
- Whether blood levels alone are enough in comparative bioavailability studies is controversial. Urinary excretion is much too low and shows no linear relationship with regards to blood levels and dose.
- Using ileostomy patients [A. Stiehl et al. Gastroenterology 94:1201-1206 (1988)] and biliary fistula patients [S. Walker et al., Gastroenterology 102:810-815 (1992)] it has been convincingly shown that UDCA is incompletely absorbed (unabsorbed UDCA is recovered in the ileostomy effluent). At pH 8, UDCA is absorbed either by alkalinization of the medium or by the already noted process of solubilization through micelles.
- Early studies of bile acid composition suggested that UDCA was absorbed in direct proportion to dose. For the most part, these studies used the sodium salt of UDCA or a solution of sodium UDCA. Absorption was inferred from the plasma levels since fractional hepatic extraction of BAs is believed to remain relatively constant throughout the day. The first pass extraction of UDCA in humans is known to be about 50% [S. Ewerth, Gastroenterology 88:126-133 (1985)].
- More contemporary studies have cast doubt on the assumption that UDCA is absorbed rapidly and completely when ingested in gelatin capsules of the protonated acid. In fact it seems that the absorption of UDCA is erratic and prolonged, especially when compared to that of CDCA. Since UDCA is rapidly absorbed from a solution of the sodium salt, dissolution of the protonated acid is likely to be the rate-limiting step.
- Despite these problems in the intestinal absorption of UDCA, there are multiple publications indicating that when UDCA is ingested chronically in doses of 8-12 mg/Kg/day, enrichment of UDCA in biliary BAs is predictable and averages 30 to 60%. Such enrichment occurs in patients with chronic liver disease[27], in gallstone patients and in patients with hyperlipidemia.
- At the dose of 15 mg/Kg/day, a biliary enrichment of 70% has been reported [A.W.M. Huijbregts et al., Neth. J. Med. 24:108-113 (1981)].
- There also appears to be an upper limit in the attainable levels of biliary UDCA. A study by M.C. Bateson et al. [Gut 21:305-310 (1980)] showed very similar values for all BAs following 6-month treatment at doses of 750 and 1000 mg/day. These data indicated that UDCA concentrations had risen sharply initially and then reached a plateau.
- The available information allows the conclusion that after administration of UDCA, enrichment of bile in UDCA (30 to 60%) is less than enrichment of bile in CDCA after oral dosage with CDCA (70 to 80%).

NOTE: Even scantier is the information on biliary enrichment under steady conditions following administration of UDCA as a single daily dose or in divided doses (q.i.d. vs t.i.d. vs b.i.d.). The assessment of data in the present NDA emphasizes information after administration of UDCA in divided doses as used in the Mayo Clinic Trial: with each meal and bedtime snack.

d. Mechanism and rate of absorption

1) Absorbed passively through the intestine.

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[27] [A. Crosignani et al., Hepatology 13:339-344 (1991); M. Podda et al.., Dig, Dis, Sci, 34(Suppl 12):59S-65S (1989); A.K. Batta et al., Amer. J. Gastroenterol., 88:691-700 (1993); S. Fischer et al., Eur. J. Clin. Invest., 23:28-36 (1993); P. Mazzella et al., Dig. Dis. Sci. 38:896-902 (1993); M.A. Lacerda et al., Gastroenterology 104:A933 (1993); A. Crosignani et al., Hepatology 14:1000-1007 (1991)]

2) Rate of BA absorption is inversely proportional to the number of H bonds and correlates directly with the partition coefficient into solvents such as n-octanol or ethyl acetate.

- For CDCA: No experimental data available.

e. Enterocyte biotransformation does not occur.

f. Transport from the intestine

1) Absorbed entirely via the portal route.

2) In serum, 96 to 99% of the BA is bound to protein (albumin).

3) Whether UDCA is present in serum LPS (as shown for cholate) is unknown.

g. Hepatic uptake

1) First pass clearance has been estimated indirectly from AUC values and directly by blood sampling.

- According to S. Ewerth et al. [Gastroenterology 88:126-133 (1985)], first pass extraction of UDCA in humans is ca. 50%.

2) Has high intrinsic hepatic clearance, which removal is "flow limited": The greater the hepatic blood flow, the greater will be the mass of BA cleared by the liver. With meals, hepatic blood flow ↑ and the mass of BA cleared by the liver ↑. The fractional extraction, however, is considered to remain constant.

3) UDCA which enters the hepatocyte is conjugated with Gly or Tau to form N-acyl conjugates (amidates).

4) Conjugation involves the formation of the Coenzyme A derivative followed by acylation with Gly or Tau.

5) Conjugation is thought to be complete, i.e. all of the UDCA entering the hepatocyte is fully conjugated with Gly or Tau before canalicular secretion.

6) The conjugating ENZ is considered to prefer Tau to Gly. However, in health, conjugation occurs mostly with Gly because the hepatocyte pool of Tau is rate limiting and "unsaturated" in this amino acid. The degree of saturation with Tau ↑ when Tau is ingested in a meal.

h. Metabolism of conjugates of UDCA

1) UDC-Gly and UDC-Tau undergo a cycle of intestinal absorption, passage to the liver, and resecretion into bile.

2) The ileal active transport system may have less affinity for UDC conjugates than CDC conjugates.

i. Fate of unabsorbed UDC conjugates in the intestine

1) In the steady state, absorption of newly ingested UDCA must be balanced by non-absorption, i.e. loss of their conjugates.

2) Deconjugation

- During EPHB Cycling, there is likely to be deconjugation in the distal small intestine before complete reabsorption occurs from the terminal ileum.

- When UDC-Tau is adm. to pts. there is extensive deconjugation and reconjugation (mostly with Gly).

- UDC conjugates that escape absorption in the terminal ileum pass into the colon either as such or as the unconjugated UDCA.
- In the colon, any conjugates will be deconjugated. Fecal UDCA is fully deconjugated.
- Absorption of UDCA from the colon has not been measured.

3) 7-dehydrogenation

- By bacteria in the distal small int. and colon.
- The 7-OXO BA formed may a) be absorbed, pass to the liver and resecreted in bile as CDCA or b) remain in the intestine.

4) Dehydroxylation

The most important bacterial biotransformation of UDCA is 7-dehydroxylation to LCA. UDCA is completely converted to LCA before its elimination from the body.

5) Epimerization

Bacteria rapidly convert UDC to CDC and vice versa.

6) Desaturation without dehydroxylation/side chain degradation

(Little importance)

7) Fate of LCA

(See above)

5. Comparative Bioavailability Data (Table 3)

NOTE: Only a short summary is given here. Data on the full report are being evaluated by the Biopharm Division.

- A single-dose, crossover study was conducted by Dr. C.N. Williams comparing the four preparations of UDCA listed below.

UDCA Formulations Tested

Drug	Dosage Form	Strength (mg)	Dose	Comment
URSO	tablet	250	2 x 250 mg	[redacted]
Actigall	capsule	300	2 x 300 mg	Ciba-Geigy
URSO	tablet	250	2 x 250 mg	[redacted]
[redacted]	capsule	200	3 x 200 mg	[redacted]

- Levels of UDCA were determined in the bile (nasoduodenal tube) and serum samples collected over 0 to 6h periods following oral administration of test medications to 24 healthy volunteers. BA data were analyzed using descriptive statistics. Mean AUC serum levels (0 to 6h) were analyzed using a paired t-test approach. [Bile and serum levels were mathematically corrected for dose on the basis that the AUCs are directly proportional to dose].

1. 3

UDCA Formulations: Comparative Bioavailability Data

I. Biliary Bile Acids

	Mean Biliary BAs (μmol/ml) 4h[a]	5h	6h	Mean Biliary UDCA (μmol/ml) 4h	5h	6h
URSO [redacted]	4.3[b]	4.4	5.1	0.5	0.4	0.5
Actigall (Ciba)	4.9	3.2	3.3	0.5	0.3	0.2
URSO [redacted]	3.4	2.6	3.0	0.4	0.2	0.2
Ursolvan	3.0	3.9	2.4	0.3	0.4	0.2

a) Time after oral adm. of the UDCA formulation.
b) Rounded figures. SD Mean has been deleted, for ease of presentation.

Drug	n	Mean Biliary bile acids (μmol/ml) TOTAL	URSO	% URSO/Total
URSO [redacted]	3	27.3	3.6	13.4
Actigall (Ciba)	2	22.8	2.8	12.3
URSO [redacted]	3	45.3	4.5	9.6
Ursolvan	3	44.4	3.1	8.6

II. Serum Bile Acids (Adjusted to 500 mg)

Two-tailed paired t-test			
Product	n	Bile Acids Mean AUC (SD) Total	URSO
URSO [redacted]	24	87.9	61.6
Actigall (Ciba)	24	66.3	47.4
URSO [redacted]	24	93.3	68.0
Ursolvan	24	78.4	56.8

Two-tailed probabilities		
Comparison	Total	URSO
URSO [redacted] *vs* Actigall	0.000	0.005
URSO [redacted] *vs* URSO (RR)	N.S.	N.S.
URSO [redacted] *vs* Ursolvan	N.S.	N.S.
Actigall *vs* URSO	0.000	0.000
Actigall *vs* Ursolvan	0.020	0.043
URSO [redacted] *vs* Ursolvan	0.008	0.066

VII. PHARMACODYNAMIC EFFECTS-SUMMARY

NOTE: The bulk of the material that follows was taken from the excellent and comprehensive review by A.F. Hofmann on the subject matter [Scand. J. Gastroenterol. 29(Suppl.204):1-15 (1994)] with updates when applicable.

A. Effects on Cholesterol and BA Metabolism

- Because UDCA decreases biliary lipid secretion of CHOL, it must have an effect on CHOL metabolism.
  - A number of studies have concluded that ingestion of UDCA in doses of 15 mg/Kg/day does not suppress HMG CoA reductase in humans in contrast to the well-documented suppression of HMG CoA reductase induced by similar doses of CDCA.
  - In the steady state, ↓ CHOL secretion into bile can only result from ↓ input into the exchangeable pool, ↑ conversion to bile acids, or both.
- It seems likely that UDCA invariably causes ↓ CHOL absorption.
  - Several measurements of CHOL absorption during UDCA therapy imply that fractional absorption of CHOL does not change or decreases.
  - As biliary CHOL secretion is ↓ during UDCA therapy, unchanged fractional absorption indicates that the actual mass of CHOL absorbed daily from the intestine falls.
- Animal studies have also confirmed a powerful effect of UDC conjugates on CHOL absorption.[28]
- Part of the difficulty in elucidating the mechanism by which UDCA inhibits intestinal CHOL absorption results from continuing ignorance as to the mechanism of CHOL absorption.[29]
- UDCA administration ↑ the conversion of CHOL to BAs in patients with cholestatic liver disease. The effect has been observed in all studies and is also true in healthy subjects, as well as in patients with hyperlipidemia.
  - During UDCA administration, the pool size of the primary conjugated BAs becomes smaller, and the fractional turnover rate increases. The product of these, BA synthesis, increases.[30]
- In animal studies, UDC conjugates do not suppress cholesterol 7-hydroxylase. Nonetheless, UDC conjugates are likely to have a weak suppressive effect on primary BA biosynthesis. Since bile acid secretion during UDCA administration does not change, and since UDCA, at steady state, comprises 40-50% of the circulating bile acid pool, the return of non-UDCA BAs to the liver falls by 40-50%. Were such a decrease in the return of BAs to the liver

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[28] In intestinal perfusion studies in rats, the replacement of cholyltaurine by UDC-taurine abolishes CHOL absorption from an emulsion of triolein containing dissolved CHOL. If mixed micelles of FA and CHOL are prepared using taurine conjugated BAs, CHOL absorption, but not FA absorption, is less if UDC-taurine is used as compared to CDC-taurine and cholyltaurine. The mechanism responsible for the decreased efficiency of CHOL absorption when UDC-taurine is the solubilizing agent has not been clarified.

[29] Possibilities to explain the decreased uptake of CHOL include the greater rigidity of the interior of the simple micelle of UDC-taurine as compared to simple micelles of other taurine conjugated BAs, as well as the reluctance of UDC conjugates to form mixed micelles with lipolytic products. The monomeric activity of CHOL in model systems simulating bile can be measured, but values for CHOL monomeric activity in systems simulating small intestinal content have not been reported.

[30] However, in patients with radiolucent gallstones, two studies have indicated that primary BA biosynthesis is ↓ during UDCA administration. The differing effects of UDCA on bile acid biosynthesis in gallstone disease compared to healthy controls is noteworthy and unexplained.

to be induced by ileal dysfunction or bile acid sequestrant administration, it is likely that BA biosynthesis would increase two to sixfold, whereas with UDCA administration bile acid biosynthesis increases less than two-fold. The modest increase in bile acid biosynthesis indicates that UDCA does not function as a "null" BA, but rather as a BA with a weak suppressive effect on BA biosynthesis in humans.

- UDCA desaturates bile and induces CHOL gallstone dissolution with an efficacy that does not differ greatly from that of CDCA, although in the experience of some authorities its dissolution efficacy is actually inferior to that of CDCA. Desaturation efficacy is believed to be >90%, and resistance to desaturation by optimal dose UDCA has not been reported. Dissolution efficacy [redacted] depending on stone type. The far lower dissolution efficacy as compared to desaturation efficacy is presumed to result from non-CHOL surfaces on gallstones that prevent their dissolution. Mixtures of CDCA and UDCA were claimed to be superior in dissolution efficacy to UDCA alone. However, a controlled study of the combination versus monotherapy with UDCA for the dissolution of post-shockwave lithotripsy fragments showed no difference between the two regimens.

B. Effects on Membranes

- When incubated with isolated hepatocytes, UDC conjugates are much less cytotoxic than conjugates of CDCA or DCA.
  - The lack of cytotoxicity of UDCA conjugates (compared to those of these dihydroxyconjugates) is also observed with erythrocytes, mast cells, and cultured enterocytes.
- The lack of cytotoxicity of UDCA or its conjugates has two explanations.
  - First, UDCA is less surface active, so that for a given aqueous phase concentration a smaller surface concentration is present.
  - Second, and probably more importantly, when UDCA or its conjugates are present at concentrations above the CMC and thus present in micellar form, there is little solubilization of membrane lipids by the UDCA micelles. With other BAs, there is solubilization of the outer leaflet into mixed micelles.
- One hypothesis to explain BA cytotoxicity is that BA molecules adsorb to the outer hemileaflet of the lipid bilayer and rest between the charged heads of the PLs, forcing them apart. When the surface concentration of BAs becomes sufficiently great, the outer hemileaflet becomes unstable and buds off, forming a vesicle rich in PLs. If additional BA is available, the vesicle becomes transformed to a mixed micelle. The outer hemileaflet is restored by PC molecules flipping from the inner hemileaflet to the outer hemileaflet, catalyzed by one or more membrane "flippases".
- Despite conjugates of UDCA having a lower surface activity than those of other dihydroxy-bile acids, UDC conjugates induce vigorous PL secretion by the rodent or canine liver. Therefore, there must be a difference mechanistically between membrane solubilization causing cell injury and membrane solubilization causing biliary PL secretion. Perhaps [says Alan Hofmann] if UDCA was incubated with cells at concentrations similar to those estimated to be present in the canaliculus (20-40 mM), some membrane solubilization would occur. A second possibility is that membrane PLs in the canalicular membrane are much more labile.

C. Effects on the Biliary Epithelium

- When assessed histochemically, γ-GT is located predominantly in the biliary ductular epithelium.
  - The consistent decrease in plasma γ-GT levels in cholestatic patients ingesting UDCA suggests that there is decreased ductule cell injury during UDCA therapy. The mechanism of this effect, if true, is also obscure.

- In the patient with PBC [or PSC], it is possible that there is a small flux of conjugated BAs across the biliary ductular epithelium, presumably by a carrier-mediated mechanism. Were this to be the case, it is possible that enrichment of the circulating BAs in UDC conjugates would improve biliary ductule epithelial cell function by lowering the intracellular concentration of toxic conjugated dihydroxy-bile acids by the same manner proposed for the hepatocyte [see E. and F., below].

  The evidence that nor-UDCA induces hypercholeresis in humans suggests that it might be possible to synthesize a UDCA derivative that would be secreted into bile in unconjugated form and undergo continuous cholehepatic shunting through the biliary ductular epithelium. Such shunting might decrease the concentration of toxic BAs in the biliary ductular epithelium.

D. Effects on the Immune System

- A controlled study conducted by Poupon et al. (locus cited) unexpectedly showed that some of the immunological markers of PBC improved with UDCA suggesting that bile acids and/or UDCA interfere with immune regulation in this disease.

- In a study undertaken to evaluate the effects of UDCA therapy on hepatic expression of class I and II HLA, 12 untreated patients with PBC were compared with 8 patients treated for at least one year with 13 to 15 mg/Kg/day of UDCA. The control group consisted of 8 patients without hepatobiliary disease. The beneficial effect observed in the UDCA group could have resulted from an improvement in the cholestasis or direct effect on hepatocytes.

E. Effects on the Normal Hepatocyte

- Conjugates of UDCA induce bile flow in a manner similar to that of any other natural conjugated BA. When UDC-taurine is given at a rate exceeding the T_{max} of canalicular secretion, bile flow plateaus rather than falls as it does with other taurine conjugated BAs, such as cholyltaurine and CDC-taurine. As previously mentioned, in rodents, when given at a rate exceeding the conjugation capacity of the hepatocytes, UDCA is secreted in unconjugated form, undergoes cholehepatic shunting, and induces hypercholeresis.

- As previously noted, there is no evidence to date that when UDCA is administered in the usual doses (8-10 mg/Kg/day) it induces hypercholeresis in healthy volunteers, in gallstone patients, or in patients with cholestatic liver disease. Criteria for hypercholeresis include an apparent choleretic activity >20 gml/μmol, the presence of the unconjugated BA in bile, and an enrichment in biliary bicarbonate. Therefore, its absence in patients ingesting UDCA implies that hepatic conjugation capacity exceeds the load of unconjugated UDCA presented to the liver. The load is not only the UDCA that is ingested orally, but unconjugated UDCA formed from the conjugated UDCA that is secreted into the intestine during digestion. In health, the conjugation requirement resulting from the load of unconjugated BAs to the liver resulting from deconjugation during digestion of secreted bile acids may exceed the conjugation requirement resulting from de novo biosynthesis.

F. Effects on the Hepatocyte in Cholestatic Liver Disease

- As previously mentioned, Leuschner and his colleagues showed that administration of UDCA to patients with chronic liver disease improved their liver tests, confirming older Japanese studies that had been ignored by the Western world.

- As also mentioned, Poupon and his colleagues reported that UDCA administration caused a marked improvement in laboratory tests and in symptoms in patients with PBC.

  - This open study stimulated the development of a number of double-blind prospective trials concerned with defining the safety and efficacy of UDCA in PBC, in PSC, in cholestasis of pregnancy, in pediatric cholestatic liver disease, and a variety of other cholestatic conditions.

- The question is the mechanism by which UDCA improves liver tests. If abnormal liver tests indicate hepatocyte injury, whereas improved tests during UDCA therapy indicate decreased liver cell injury, the question is how UDCA achieves this desired effect.

    - In animal studies, if UDCA is co-administered with a cholestatic BA, UDCA prevents the cholestasis and ↓ the hepatic retention of the cholestatic BA.

    - Thus, if this argument can be applied to cholestatic disease in humans, UDCA decreases the concentration of bile acids within the hepatocyte.

    - If this reasonable assumption is true, the mechanism by which UDCA stimulates canalicular secretion of BAs is completely unclear. UDCA might displace the toxic dihydroxy conjugates from intracellular binding proteins. Were this to occur, there could be ↑ canalicular secretion as well as ↓ injury to intracellular organelles.

    - UDCA might stimulate canalicular secretion by causing 'trans' stimulation of the canalicular transporter, but such trans stimulation is not considered to occur with ATP-dependent transporters.

    - UDCA administration might actually increase the level of canalicular transporters, either by increasing their biosynthesis (or decreasing their turnover rate) or by increasing the fraction of the carrier present in the membrane and decreasing the fraction present in vesicles in the cytosol [J.L. Boyer et al., Hepatology 18:A107 (1993)].

- Hepatic exocrine function, that is, the V_{max} for canalicular secretion of BAs or an organic anion such as ceftriaxone or conjugated BSP is not usually measured, in part because such experiments might be hazardous. Rather, it is inferred from the plasma level of conjugated BIL. An indirect measurement of canalicular transport function for BAs suggests that it is increased modestly by UDCA administration. Stiehl has also found that UDCA increases canalicular bile acid secretion during UDCA administration in PBC patients.

VIII. REVIEWER'S SUMMARY/CONCLUSIONS FROM PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS

For medications intended to treat PBC the site of action (target organ) is the liver. UDCA is ingested as tablets containing the protonated acid. Ingested UDCA remains insoluble in the stomach and the fate of the tablet depends on the rate of dispersion relative to the rate of gastric emptying. In the intestine, UDCA must be solubilized in BA micelles and titrated by pancreatic bicarbonate. Dissolution probably occurs in the mid-jejunum and in the more distal small intestine. Following dissolution of the particles into monomers and micelles, the following takes place: diffusion through the aqueous boundary layer, passage across the apical membrane of the enterocyte, movement through the enterocyte and exit across the basolateral membrane of the enterocyte. UDCA is absorbed entirely via the portal view route and in the serum, it circulates bound to albumin (96 to 99%). All the existing methods to assess bioavailability of UDCA give results that must be interpreted with caution. But despite these problems, there are multiple publications indicating that orally administered UDCA is inefficiently (incompletely) absorbed. When UDCA is ingested chronically in doses of 8 to 12 mg/Kg/day, enrichment of UDCA in biliary BAs, even in patients with chronic liver disease, averages 30 to 60%. At the dose of 15 mg/Kg/day, a biliary enrichment of 70% has been reported. The first pass extraction of UDCA in man

is ca. 50%. The mass of BA cleared by the liver is proportional to the hepatic blood flow. UDCA which enters the hepatocyte is conjugated (amidated) with glycine or taurine. This way, the absorbed UDCA has entered the entero-portal-biliary circulation and this is essential for this enterohepatic drug to exert its beneficial effect in PBC.

To properly describe the bioavailability of UDCA it is important to measure both the rate as well as the extent of the absorption of this bile acid. UDC-Gly and UDC-Tau undergo a cycle of intestinal absorption, passage to the liver and resecretion into bile. During this EPHB cycling, there is deconjugation in the distal small intestine before complete reabsorption occurs from the terminal ileum. In the colon, any conjugates will be deconjugated and fecal UDCA is fully deconjugated. In the distal small intestine and colon, UDCA undergoes the following reactions: a) 7-dehydrogenation (to 7-oxo-UDCA which may be absorbed, pass to the liver and resecreted in the bile as CDCA or remain in the intestine); b) epimerization (to CDCA which can be reconverted to UDCA by intestinal bacteria) and c) 7-dehydroxylation (to LCA). The latter is the most important bacterial biotransformation of UDCA. LCA is not so efficiently absorbed from the large intestine. In the hepatocyte, LCA is not only conjugated to GLY or TAU but also with sulfate (at the 3-position). The Sulfo-LC-Gly and Sulfo-LC-Tau that are thus formed are secreted into bile. These sulfated lithocholyl amidates are poorly conserved in the small intestine and these compounds are rapidly lost from the body. Because of **sulfation, which ensures rapid elimination from the body**, this metabolite of UDCA does not exert the toxic effects (liver toxicity, etc.) amply demonstrated in animals.

UDCA appears to have a number of pharmacodynamic effects. Most of these are of some possible pathogenic and therapeutic relevance. But none of the various PD effects - in isolation - can explain the hepatoprotective actions of the drug in PBC. Understanding of the mechanism of action of UDCA requires an integration of PD data from both *in-vitro* and *in-vivo* animal experiments and data from clinical studies. From data succinctly displayed in Table 3a, it is seen that UDCA has effects on a) cholesterol and bile acid metabolism, b) membranes, c) biliary epithelium, d) the immune system, e) normal hepatocyte and f) on the hepatocyte in cholestatic liver disease. Also included in Table 3a are recently reported anti-inflammatory effects. It is considered that, in spite of the major advances in the understanding of PBC and the sophistication of PD studies, the precise mechanism by which UDCA exerts its benefit in PBC and other cholestatic liver diseases (possibly), remains to be clarified.

TABLE 1a

PD Effects of UDCA With Emphasis on Assumed Mechanisms by Which This Bile Acid Exerts its Beneficial Effects in PBC Patients

PD Effect	Comments
A. Effects on Cholesterol and Bile Acid Metabolism	
1. ↓ Biliary lipid secretion of cholesterol 2. ↓ cholesterol absorption 3. ↑ conversion of CHOL to bile acids - Pool size of primary conjugated BAs becomes smaller; the fractional turnover rate ↑; BA biosynthesis ↑ 4. Desaturates bile and induces CHOL gallstone dissolution	• ↓ input into the exchangeable pool and/or • ↑ conversion to bile acids • Fractional absorption of CHOL does not change or decreases • The actual mass of CHOL absorbed daily from the intestine falls • UDC conjugates have powerful effect on CHOL absorption • ↓ of serum CHOL seen in patients with cholestatic liver disease, hyperlipidemics and healthy subjects
B. Effects on Membranes	
1. When incubated with isolated hepatocytes (or erythrocytes, mast cells or cultured enterocytes), UDCA conjugates are much less toxic than conjugates of CDCA or DCA 2. Protects PL membranes against damage by toxic bile salts	• UDCA is less surface active • In micellar form (above the CMC), there is little solubilization of membrane lipids by the UDCA micelles • Unconjugated UDCA appears to be incorporated into the deeper layers of erythrocyte membranes and rat basolateral hepatocyte membranes, whereas UDCA conjugates bind more superficially
C. Effects on the Biliary Epithelium	
1. ↓ ductule cell injury (improves ductule epithelial cell function) [inferred because when assessed histo-chemically, γ-GT (which ↓ consistently in the plasma of UDCA-treated PBC patients), is located predominantly in the biliary ductular epithelium]	• Mechanism similar to that for the hepatocyte (UDCA lowers the intracellular concentration of toxic conjugated dihydroxy-bile acids • Inhibits the DNA fragmentation process in biliary epithelial cells but not in hepatocytes [H. Koga et al. Gastroenterology 110:A1237 (1996)]
D. Effects on the Immune System	
1. Improves some of the immunological markers (primarily IgM, AMA_o of PBC	• In a recent study [K. Yamazaki et al., Gastroenterology 110:A1364 (1996)] the disappearance of AMA_o upon long-term treatment with UDCA, was significantly more frequent in patients with a low pre-treatment titer.

Table 1a (Con't)

E. Effects on the Normal Hepatocyte	
1. Has potent choleretic effect	• Dilutes toxic bile salts • Does not fully explain the therapeutic effect, since the protective effect is mainly due to UDC conjugates which are not very choleretic [the extremely choleretic taurodehydrocholic acid has not exhibited any hepatoprotective properties [K. Miyai et al., Lab. Invest. 36:249-258 (1977)]
F. Effect on the Hepatocyte in Cholestatic Liver Disease	
1. Improves liver function tests 2. It is hepatoprotective 3. Protects against TPN and AA-induced cholestasis [L. Sichet et al., Gastroenterology 110:A1325 (1996)]	• Prevents the cholestasis and ↓ the hepatic retention of cholestatic bile acids (animals) • In man, UDCA ↓ the concentration of cholestatic BAs within the hepatocytes • It might displace the toxic dihydroxy conjugates from intracellular binding proteins • It might ↑ level of canalicular transporters • Toxic bile salts cause hepatocyte apoptosis, an effect probably mediated through a nuclear serine protease [L.R. Roberts et al., Gastroenterology 110:A1305 (1996)]. Apoptosis might be involved in the cellular injury of biliary epithelial cells and of hepatocytes • The hepatoprotective action may be exerted through regulation of cAMP synthesis [B. Bouscarel et al, Gastroenterology 110:A1156 (1996)]
G. Antiinflammatory Effect	
1. Mediated by its ability to inhibit nitric oxide (NO) production	• This effect may account for the chemoprotective effect of UDCA in animal models of colon cancer, where through its easy conversion to carcinogenic nitrosamines, NO may act as a mutagen [D. Invernizzi et al., Gastroenterology 110:A930 (1996)]

IX. STUDIES SUBMITTED IN SUPPORT OF THE EFFICACY OF UDCA IN PBC

The sponsor is seeking approval for the marketing of 250 mg UDCA (URSO™) film coated tablets for the treatment of patients with all stages of PBC. The medication is to be given at the dose of 13 to 15 mg/Kg/day administered in four divided doses, with the three main meals and a bedtime snack. In support of their request, the firm has submitted NDA 20-675, which includes the clinical results of one adequate and well-controlled U.S. study (K.D. Lindor, Mayo Clinic). Supportive information comes from a foreign well-controlled study (E.J. Heathcote, Toronto Hospital, Canada). The main design features and the usefulness of these two trials are summarized in Table 4.

TABLE 4

Identification, Main Features of Design, Study Population and Initial Appraisal of the Usefulness of the Critical Trial and the Supportive Study for the Approval of the Marketing of UDCA for the Treatment of PBC

Study Identification	Main Design Features	Study Population	Groups Being Compared	Remarks
A. K.D. Lindor Mayo Clinic Published in: Gastroenterology 106:1284-1290 (1994)	Randomized, double-blind, PL-controlled, 2-arm, 2-year, parallel group. Efficacy based on the time to "Tx Fx" (death, need for liver transplantation, development of varices, ascites or encephalopathy, doubling of serum BIL, voluntary withdrawal, marked worsening of fatigue or pruritus).	Liver-biopsy-confirmed PBC with serum AP at least 1.5 ULN and AMA_s+. - Stratified according to histologic stage (early stages I and II vs late stages III and IV); the presence or absence of esophageal varices; and serum BIL levels (≤1.8 mg/dl vs >1.8 mg/dl).	UDCA[a], 250 mg tablets 13-15 mg/Kg/day administered in 4 divided doses [n=89] vs PL[b] (4 times/day) [n=91] [redacted]	• Useful design • Adequate numbers of patients were randomized to either UDCA or PL. • Efficacy is demonstrated by showing statistical superiority over a negative control. • Design does not give dose-response information. • 2-years may be too short a period to demonstrate effects of drug on survival/liver transplant. • After the 2-year D-B Tx period all pts. were offered and accepted participation in the L-T, open-label, active Tx extension. Formulation from two sources were used during the first 2- vs the last 2-y of the 4-year period. For some parameters, efficacy with each and both formulations was assessed.
B. E.J. Heathcote Toronto Hospital, (Canada) Published in: Hepatology 19:1149-1156 (1994)	Randomized, double-blind, PL-controlled, 2-arm, 2-year, parallel group. Efficacy based on the % change in total serum BIL at 2y. Secondary outcome measures: changes in symptoms (pruritus + fatigue) other lab. assessments of cholestasis, serum Ig levels, change in histologic stage, and death and/or liver transplantation.	Liver-biopsy-confirmed PBC with elevated serum AP and AMA_s+ - Stratified according to absence or presence of symptoms at baseline.	UDCA[c], 250 mg capsules 14 mg/Kg/day administered as a single oral dose with evening meals [n=111] vs PL[d] (once-a-day). [n=111] [redacted]	• Useful design. • Adequate numbers of patients were randomized to either UDCA or PL. • Efficacy is demonstrated by showing statistical superiority over a negative control. • Design does not provide dose-response information. • 2-year may be too short a period to demonstrate effects of drug on survival/liver transplant. • L-T open-label extension is on-going (as above).

A. Mayo Clinic Study

"A randomized trial of ursodeoxycholic acid in the treatment of primary biliary cirrhosis"

This U.S. trial was carried out by 4 principal investigators at four institutions, primarily the Mayo Clinic in Rochester, MN. The study began in April 1988 and was designed so that the blind would be broken when the 132nd patient had completed 2y of D-B treatment. This occurred in May 1992, at which time all patients were offered and accepted participation in the L-T, open-label, UDCA treatment extension. Therefore, patients randomized early on in the trial (April 1988) were maintained on double-blind (placebo or UDCA) for 4 years, until May 1992, whereas those randomized late into the trial received double-blind treatment for less than 2 years. The final report to the double-blind portion of this study was submitted to IND [redacted] on February 21, 1995 and the results presented to the Agency during an end-of-phase II meeting held on April 18, 1995. For the purposes of this report, the data were truncated at 2 years for patients maintained on double-blind for more than 2 years.

According to the protocol, this study was based on the hypothesis that liver injury, initiated by possible immune mechanisms, may be potentiated by the retention of potentially toxic BAs. The investigators reasoned that UDCA is a non-toxic BA that experience has shown can be administered with safety to patients. The results of animal studies and at that time (1988) limited prospective but uncontrolled clinical trials in humans [i.e. the initial studies by Poupon et al., reviewed above] showed promise.

1. Specific Aims

These, listed below, were clinically important and clearly stated in the protocol.

a. To compare the effects of UDCA versus PL on AP, AST, BIL, albumin (ALB), IgM and PT.

b. To evaluate the effects of UDCA on symptoms such as fatigue and pruritus.

c. To determine the effects of UDCA on the development or clinical progression of esophageal varices, ascites or edema, and encephalopathy.

d. To determine the effects of UDCA on histologic changes at two years.

e. To determine whether UDCA favorably affects survival or need for transplantation.

f. To assess toxicity and determine the safety of UDCA.

NOTE: In the protocol, the specific aims were listed as shown above. No distinction was made between primary and secondary objectives.

2. Trial Design

The study was conducted in a randomized, stratified, double-blind manner. The plan was to randomize 132 PBC patients into study, with 50% (66 patients) receiving UDCA. Evaluated were clinical, biochemical and histologic responses, in addition to actual and estimated survival.

3. Study Population (Table 5)

The inclusion-exclusion criteria were adequate for this type of study. Entrance criteria required the demonstration of chronic hepatic cholestasis with biopsy-proven PBC. Equally adequate were the exclusion criteria. Among the latter were clinical conditions and medications that could be potentially confounding. Although the patients needed to be sick to show a clinically important improvement, the condition needed not to be advanced so that OLT would be necessary in one year or the patient had hemodynamic complications or signs of liver failure. According to the protocol, the patients had to be on concurrent cholestyramine treatment. Thus, any effect of UDCA or PL on pruritus needed to be demonstrated over and above the baseline effects of cholestyramine.

4. Randomization Procedures, Blinding, Test Medication(s), Dosage Regimen and Concomitant Medications

- In their Appendix C, vol. 35, p. 178, the sponsor provided the randomization schedule.

- The patients were stratified according to histologic stage [early (I or II) vs late (III or IV), presence or absence of esophageal varices (made by upper g.i. endoscopy), and serum BIL (<1.8 mg/dl or greater). It was stated in the protocol that randomization was to be carried out for each of the eight strata separately. But the sponsor did not provide information on the way the randomization/stratification was finally achieved. [This information was requested of and eventually provided by the sponsor (see Results).]

TABLE 5
Mayo Clinic Trial

Characteristics of the Study Population

INCLUSION CRITERIA	REASON FOR EXCLUSION
• M or F patients, who were 18y of age or older, who had given voluntary consent as evidenced by signing the ICForm and who had PBC, defined as follows: - Chronic cholestatic liver Dz of at least 6 months duration. - Serum AP level at least 1.5 times the ULN. - Positive AMA_o. - U/S, CT or cholangiography of the biliary tree which excluded biliary obstruction. - Liver biopsy within the previous 3 months (available for review) that was compatible with the diagnosis of PBC.[a]	• <18y of age. • Pregnant, or F patients presently not using birth control. • Previous treatment with UDCA or CDCA during the 3 months prior to randomization into the study. • Previous treatment with colchicine, corticosteroids, azathioprine, cyclosporin, chlorambucil or d-penicillamine during the 3 months prior to randomization into the study. [These medications were also proscribed during the actual trial.] • Anticipated need for OLT in one year or recurrent variceal bleeds, spontaneous encephalopathy, or refractory ascites. • No concurrent cholestyramine treatment. • Findings that were highly suggestive of liver Dz other than PBC such as chronic alcoholic liver Dz, chronic hepatitis B infection, CAH, hemochromatosis, PSC, Wilson's disease, or alpha-1-antitrypsin deficiency.

a) See text of Review.

ABBREVIATIONS USED
M=male F=female ICForm=Informed Consent Form PBC=Primary Biliary Cirrhosis Dz=disease AP=alkaline phosphatase ULN=upper limit of normal AMA_o=antimitochondrial antibody U/S=ultrasound CT=computed tomography OLT=orthotopic liver transplantation CAH=chronic active hepatitis PSC=primary sclerosing cholangitis

- The patients were randomized on a 1:1 basis; 89 patients were assigned to UDCA and 91 to PL.
- This study was, by design, double-blind. Neither the patient nor the investigator knew the identity of the medication the patient was receiving.[31]
- Test medications (UDCA and matching PL tablets) were formulated by [redacted].
- Tablets were shipped in bulk to the Mayo Clinic Rochester, Investigational Pharmacy in Minnesota. Clinical Trial Medication (CTM) was further distributed to all satellite centers from the Mayo Clinic

[31] If, in order to make therapeutic decisions, the Investigator needed to know the identity of the treatment a patient was receiving, a provision was made to permit breaking the blinding scheme. Both the clinical pharmacist and Mayo Clinic Rochester statistician were unblinded in this study. If clinically necessary, the pharmacist could provide the identity of treatment for a patient.

Rochester pharmacy and further packaged into final patient dispensing containers (white opaque bottles, 360 tablets per bottle).[32]

- Drug accountability was maintained using the NIH and NCI Investigational Drug Accountability Record sheet (sponsor's Appendix F). This sheet was used to record all incoming lots of CTM, CTM dispensed to satellite centers from the Mayo Clinic Rochester Pharmacy, as well as all CTM dispensed to individual patients from the Mayo Clinic Rochester center.

- CTM was dispensed to patients at ca. 3-month intervals, and patients would call in to the pharmacy for resupply. All resupplies of CTM were cleared through the Study Coordinator by the Pharmacist.

- The Pharmacy maintained a copy of the randomization code and randomized patients using this code based on the patient's stratification.

- Similar procedures as above were followed at all satellite centers.[33]

- Patients were prescribed sufficient UDCA 250 mg tablets to provide for a total daily dose of 13-15 mg/Kg. The tablets were taken in divided doses, with meals and a bedtime snack. PL-treated patients received an identical number of matching PL tablets administered in an identical fashion.

- Regarding concomitant medications, it was operationally defined that patients were not permitted to receive any associated concomitant medications for the treatment of PBC (listed in Table 5). Initially, patients requiring concomitant therapy with cholestyramine resin were excluded from the study. However, because of the appearance or the exacerbation of pruritus (particularly in patients who had discontinued cholestyramine upon entry into the study), the concomitant use of QUESTRAN was subsequently allowed. Patients were instructed to take cholestyramine and UDCA or PL 2h apart in order to avoid impeding the absorption of the BA.

5. Clinical Assessments

The study consisted of two phases: Baseline and Treatment

[32] The containers were provided with labels indicating the date, prescription number, number of tablets dispensed, dosage, storage directions, the FDA new drug cautionary statement, the name, location and telephone number of the Principal Investigator and dispensing pharmacy. Further two-part dispensing labels were produced with one part kept for state records and the other attached to the copy of the patient's entry form that was kept by the pharmacy.

[33] A sufficient number of tablets, together with quality control certifications, were retained by the Investigator. CTM was distributed from the Mayo Clinic, Rochester, MN to the satellite centers.

a. **Baseline Phase**

During this phase patients presenting with well-defined PBC and who were AMA_s positive were enrolled.[34]

- A complete Hx and P.E. were performed, and serum biochemistries, Ig levels and PT were measured.
- A serum sample from each patient was stored for BA analysis.
- Abdominal U/S, upper endoscopy, and liver biopsy were performed.
  - At the time of upper endoscopy, bile samples were obtained after CCK stimulation to measure biliary acid composition.
- Patients were stratified according to histologic stage (I and II vs III and IV), serum BIL level (≤1.8 mg/dl vs. >1.8 mg/dl) and varices (present or absent).

b. **Treatment Phase**

During this phase the patients were treated double-blind with UDCA or placebo, and treatment effects were assessed.

- A complete blood count and serum chemistries were determined every three months.
- Annual examinations included a complete Hx, P.E. and measurement of CBC, serum biochemistries, Ig levels and PTs.
- Plasma samples were obtained for BA analysis.
- U/S, upper g.i. endoscopy, measurement of biliary BA composition, and liver biopsies were repeated at the two year endpoint, or sooner if clinically indicated.

c. **Schedule of time and events (Table 6)/Monitoring**

- This trial was approved by the Mayo Clinic Rochester IRB for the three Mayo Clinic Centers. IC was obtained from each patient prior to enrollment in the trial. The trial centers selected by the Lead Principal Investigator were Mayo Clinic (Rochester, MN), Mayo Clinic (Scottsdale, AZ), Mayo Clinic (Jacksonville, FL) and Scott and White Clinic (Temple, TX).
- Patients were seen by the Investigator at the initial Baseline visit and at yearly intervals for the duration of the trial. During each visit, patients were re-evaluated with complete Hx and P.E. as well as measurements of hepatic biochemical and serologic values (Table 6).

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[34] A Patient Entry Form was completed on each patient and included the name of the investigator entering the patient into the study, a Patient Eligibility Checklist, and patient stratification. Blinded copies were retained by the Investigator and Coordinator Center at Center 01, and unblinded copies were maintained by the Pharmacy and Statistical Department at Mayo Clinic Rochester.

Abdominal U/S was performed to assess liver parenchyma, the biliary tree, vascular patency, the presence of ascites and to look for evidence of portal hypertension. Determination of the presence of esophageal varices was made by upper g.i. endoscopy, at which time bile was obtained after CCK stimulation (40 ng/Kg i.v.). Additional details are given in the Footnote to Table 6.

- The sponsoring monitor for the study was Dr. David Jacobus. In addition, the study conduct was internally monitored by Drs. A. Czaja and D. McGill from the Mayo Clinic Rochester clinic.

TABLE 6
Mayo Clinic Trial

Schedule of Time and Events

	MONTHS								
Assessments	**Entry**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**
History[a]	x				x				x
P.E.[b]	x				x				x
Chemistry	x	x	x	x	x	x	x	x	x
CBC	x	x	x	x	x	x	x	x	x
Immunoglobulins	x				x				x
PT	x				x				x
Liver Bx[c]	x								x
U/S[d]	x								x
Endoscopy[e]	x								x
Biliary BAs[f]	x								x
Stored Serum for BAs	x				x				x
Bone Mineral Densitometry	x				x				x

a,b) The clinical assessment was done checking for signs and symptoms of PBC including ascites, encephalopathy, esophageal varices, fatigue and pruritus.

c,d,e) Liver biopsies, abdominal U/S and upper g.i. endoscopy were repeated at the two-year visit.

f) BA levels in duodenal contents were determined at entry and at 2y. Samples were stored at -70°C and analyzed for UDCA and other BAs by HPLC in the laboratory of Dr. Alan F. Hofmann of California, San Diego using previously validated methods. Serum BA composition was measured using HPLC described by P.S. Tietz et al. [J. Chromatogr. 336:249-257 (1984)].

- All observed toxicities that were reported in this trial were followed-up by the investigators until resolved.

d. Efficacy parameters

1) Primary efficacy parameter

The primary indicator of efficacy was evaluated by the proportion of patients in each group with treatment failure during the first 2y of D-B treatment. The protocol-stipulated original definition of Tx failure consisted of the following parameters.

a) death
b) need for liver transplantation (see below)
c) histologic progression by 2 stages or to cirrhosis (see below)
d) doubling of total serum BIL [second observed value ≥1.5 mg/dl] (eventually omitted from FDA revised definition of Tx Fx)
e) development of varices, ascites or encephalopathy (see below) in patients without these findings at entry
f) inability to tolerate the drug regimen
g) voluntary D/C of drug Tx for any reason (eventually omitted from FDA revised definition of Tx Fx)
h) marked worsening of pruritus or fatigue [as defined by progression of two stages or development of disabling fatigue or pruritus] (see below).

In reply to our request of May 09, 1996 for clarification and additional information, the sponsor enclosed the following in their letter of May 14, 1996.

NEED FOR LIVER TRANSPLANTATION

Guidelines for referral for liver transplantation were primarily clinical. Patients considered for transplantation would undergo review by a Transplant Selection Committee where the final decision was made. Typically the reasons for referral and selection for transplantation included decompensated liver disease such as diuretic-resistant ascites or recurrent variceal bleeding, incapacitating symptoms such as pruritus (a rare indication) or evidence of advanced liver disease, usually as manifested by an estimated one-year survival of less than 70-80 percent using the Mayo Risk Score which typically was reflected by **serum bilirubins greater than 8 mg/dl**.

HISTOLOGIC STAGING

The definitions used were those published by J. Ludwig et al. [Virchows Arch. A. Path. and Histol. 379:103-112 (1978)]. These authors call PBC a chronic nonsuppurative destructive cholangitis (CNDC) and use the following criteria to characterize the condition.

<table>
<tr><th colspan="4">S T A G E</th></tr>
<tr><th>I</th><th>II</th><th>III</th><th>IV</th></tr>
<tr><td>Portal Hepatitis
With little or no periportal inflammation or piecemeal necrosis (PMN)

<u>Comment</u>
Granulomas and inflammatory destruction of bile ducts may be identifiable, but their presence or absence does not affect the staging.</td><td>Periportal Hepatitis
Absence of bridging necrosis and of septal fibrosis.</td><td>Septal Fibrosis
or
Bridging Necrosis
or
both</td><td>Cirrhosis
"True" PBC:
fibrous septa and nodular regeneration

<u>Comment</u>
In a few instances, the Bx evidence at this stage may be difficult to distinguish from that of other types of cirrhosis.</td></tr>
<tr><td colspan="3"><u>Comments</u>
Usually, PMN is present.

The biopsy evidence at this stage may be indistinguishable from chronic active or various other types of hepatitis (Ludwig, 1977).

Granulomas, inflammatory destruction of bile ducts, and ductular proliferation, in various combinations, are often identifiable; but presence or absence of these features does not affect the staging.</td></tr>
</table>

ENCEPHALOPATHY

Encephalopathy was recognized clinically, usually corroborated by the presence of an elevated blood ammonia level in a patient with advanced liver disease having fetor hepaticus and confusional status.

PRURITUS AND FATIGUE

To score these symptoms the following 4-grade scale was used.

Grade	Pruritus	Fatigue
0	None	None
1	Mild	Mild, does not interfere with activity
2	Some interference with sleep	Requires extra rest, limits activity, able to work
3	Excoriations, substantial sleep disturbance	Unable to work a full day

2) Other Efficacy Parameters

Other outcome measures included assessment of:

- albumin
- IgM
- AP
- ASAT
- PT

e. Criteria for patient withdrawal

These were adequate and included:

1) Development of AE (such as intractable diarrhea or other AE such as severe nausea or pruritus) requiring treatment or that was unresponsive to dosage reduction.

2) Experiencing the occurrence of an illness or intercurrent Tx that required the termination of test medication.

3) Non-compliance.

4) Request of their discontinuation from the study by the patient or their referring physician.

5) Pronounced worsening of liver biochemistries over a 3-month period (tripling of AP, SGOT or BIL).

6) Need for liver transplantation.

f. Additional details of efficacy variables

The following rules were used for life table analyses:

- If the patient died, the endpoint was considered to be the date of death.
- If the patient had a liver transplant, the endpoint date was the date the patient was severed for potential transplant (not date of transplant)
- If the patient failed, the endpoint date was the date of the visit on which failure was declared; or if the patient failed and was severed from study, it was date of severance.
- If patient did not fail, the endpoint was the date of severance, or if the patient did not sever from study, it was the date of the last visit.

g. Safety Parameters

Safety assessments were based primarily on yearly histories and physicals, the collection of toxicity data, and clinical laboratory parameters which were assessed every 3 mo. during the Tx period. Other evaluations included urinalysis, chest radiographs, mammograms when clinically appropriate, and bone mineral densitometry performed on a yearly basis.

6. Data Collection/Validation

- The Mayo Clinic used its inhouse-developed [redacted] database system for entry of the data on the CRFs into its computer system. It was determined that data entry was a single entry system. For the most part, the study coordinator, [redacted], did the majority of the data entry into the [redacted] database. Once in the Clinfo database, the data were then converted to a SAS database by a Mayo Clinic Rochester statistician.

- Once in a SAS® dataset format, the data were downloaded into diskette and transferred to [redacted] a statistician at [redacted], for further analysis. It should be noted that the Mayo Clinic CRFs were designed around eleven modular "Panels" each of which were used at one or multiple study visits. Thus, the SAS data listings were formatted by panel, by patient number sequentially, and by study visit date chronologically.

- Under the direction of [redacted], four [redacted] clinical research staff members were assigned to obtain a complete copy of all available CRFs on the 180 patients enrolled in the study, along with all available source documents including signed consent forms and medical charts.[35]

- Since the Mayo data entry was a single entry procedure and the [redacted] database had not been verified against the CRF data, it was determined that [redacted] would conduct a manual 100% verification of the SAS data listings against the data on the CRF copied previously at the Mayo. Additionally, [redacted] identified any obviously missing items on the CRFs or on the SAS data listing or both. In all cases, [redacted] returned the edited datasets or CRF copies to Coordinating Section for reconciliation and/or correction. All changes in the original CRFs or the [redacted] database or the derived SAS dataset were made by Mayo study personnel only. Furthermore, cross-validation of data for inconsistencies were done at Rutgers and inconsistencies were resolved by [redacted] and Coordinating Center and data was updated and locked.

7. Statistical Methodology

a. Power and Justification of Sample Size

- The sample size required to detect a 25% change in proportion of patients failing (e.g., from 50 percent to 25 percent) with 80 percent power, testing $\alpha = 0.05$, was determined to be 114 patients randomized (57 PL, 57 UDCA).

- Controlling for an estimated dropout rate of 7%, a minimum of 132 patients[36] was to be enrolled. In this trial, a total of 180 patients was randomized.

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[35] This copying process was carried out over a period from October, 1992 to July, 1993. All copies made during the site visits were hand carried to [redacted] offices. A total of over 8500 pages were photocopied. Each page was then reviewed manually to blackout any personal identifiers (i.e., names, addresses, phone numbers) to assure patient confidentiality.

[36]

$$\frac{1}{(1-0.07)^2} \times 114 = 132$$

b. Mid-point evaluation

- The primary purpose of the mid-term evaluation (2 years from initiation of the study) was to assure justification for continuing the trial. The analyses were performed blinded and results were reviewed by an independent group of physicians. The criteria for continuing the trial was that drug toxicity be acceptable, and that no clear-cut improvement in survival or need for transplantation be found. The independent group, after review of the results from the interim analyses, recommended that the trial be continued.

c. Baseline comparability

- A baseline comparison of demographic and other background information that was collected pre-drug was described for each Tx group.

d. Efficacy analysis

- The primary analysis compared the percentage of UDCA and placebo patients who were classified as treatment failures during the first two years of double-blind treatment by

  1) Fisher's exact test and

  2) logistic regression, controlling for strata by entering them as covariates.

- Time to treatment failure data was also analyzed using life-table procedures.

  - Difference in time to treatment failure was compared using the Log rank test corroborated by the Wilcoxon Test.

  - Cox's analyses were performed to control for covariates such as histological stage and BIL at baseline.

- Individual variables that make up the failure index were compared using a t-test for continuous variables and Fisher's exact test or a Chi-square test for categorical variables.

- Other efficacy parameters with continuous data were compared for change from baseline using paired t-test or Wilcoxon signed rank test.

  - Comparison in change from baseline between the groups was done using two sample t-test and corroborated using the Wilcoxon rank sum test.

  - For categorical parameters, the Chi-square test was used for comparing the distributions between the groups.

e. Safety analysis

Toxicity data were summarized and compared between the two treatment groups. Clinical non-hepatic lab data, physical examination data, urinalysis, and blood pressure data were also compared between the two treatment groups. Treatment emergent changes in selected labs from baseline to the end of the study were also examined.

f. Sensitivity analysis

In the main text of the report, endpoint analyses were presented. Sensitivity analysis using last observation carry forward (LOCF), and observed case analysis (OCA) [see definitions below] were also performed for the majority of parameters and were presented in the sponsor's Appendices.

OCA Analyses: Only data actually recorded for a given patient was used in this analysis.

LOCF Analyses: If an observation for a given visit was missing for a patient, the data from the previous visit were carried forward into the visit in question. If necessary, the BL visit was carried forward into the post-BL visits. If the BL observation was missing, the observation was left as missing.

Endpoint Analyses: The endpoint was the last post-BL observation available for the patient.

8. Results

a. Patient accountability

In this trial, the data were collected over a 4-y period (1 April 1988 through 31 May 1992) during which the blind was maintained for all patients. The blinding was broken when the 132nd patient randomized into the trial completed 2y of D-B treatment. For those patients randomized early on in the trial and maintained on D-B for more than 2y, the data have been truncated at 2-y for efficacy analysis. The data was truncated at the last visit for those patients maintained on D-B for <2y.

Mid-term analyses were carried out in 153 patients (UDCA, n=77; PL, n=76). Of the 77 UDCA patients, 54 had at least one year of follow-up. Of the 76 PL patients, 49 had at least one year of follow-up. Results revealed no evidence of toxicity from the drug and there were no statistically significant differences between the Tx groups in the rate of Tx failure or in the need for transplantation.

b. Number of patients per center

Overall, a total of 180 patients were recruited into the trial. The number of patients per site per Tx group was:

Center[a] #	UDCA	PL	TOTAL
01 Mayo Clinic Rochester, MN [K. Lindor, M.D. and R. Dixon, M.D.]	79[b]	81[c]	160[d]
03 Mayo Clinic Scottsdale, AZ [M. Anderson, M.D.]	5	4	9
04 Mayo Clinic Jacksonville, FL [S. Lange, M.D.]	3	5	8
05 Scott and White Clinic, TX [G. LeSage, M.D.]	2	1	3
TOTAL	89	91	180[e]

a) Center 02 was a local medical facility where some patients were allowed to receive their annual evaluations after being randomized into the trial at an established site.
b) F=91%; M=9%.
c) F=85%; M=15%.
d) 160 represents 90% of the 180 totally enrolled.
e) This total of 180 patients had been enrolled into the D-B trial when the study was unblinded in May 1992. At that time, 112 of the 180 randomized patients had completed at least 24 months of exposure to test medication.

c. Number and percentage of patients per each annual visit (Table 7)

The data in this table (the percentages are rounded figures) show that the proportion of patients being treated at 24 months in the UDCA group was ca. 31% higher than the proportion being treated at 24 months in the PL group. This difference was largely due to more treatment failures in the PL group compared to the UDCA group

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TABLE 7
Mayo Clinic Trial

Number/Proportion (%) of Patients Being Treated
at Each Annual Visit

	Visit Month		
	0	12	24[a]
UDCA	89 (100%)	84 (94%)	63 (71%)
PL	91 (100%)	78 (86%)	49 (54%)

a) The ca. 31% difference between UDCA and PL at this time was mainly due to more Tx Failures in the PL group, compared to the UDCA group.

d. Center 02 patient accountability

A subset of patients was allowed to undergo their annual evaluations at their local medical facilities, with the necessary documentation of required evaluations being mailed in to the Principle study center (Mayo Clinic, Rochester, MN). These patients were considered Center 02 patients. The following patients had Center 02 visits after entering the trial at the Mayo Clinic Rochester:

#706 #709 #711 #721 #731 #732 #751 #753 #759 #781
#783 #816 #826 #830 and #832

- Pt. #791 originally entered the trial at Mayo Clinic Rochester 25 May 1989 and later severed/DC. This pt. re-entered the trial at Mayo Clinic Scottsdale.

- Pts. #785 and #786 entered the trial at the Scott and White Clinic. They had follow-up visits coded as Center 02. However, due to a change in the data sheet these should have been Center 05 (Scott and White) visits.

e. Patient severance/discontinuation (Table 8)

As shown in this Table, a total of 12 (14%) of the UDCA patients and 23 (25%) of the PL patients D/C the study for a variety of reasons. These reasons included deaths, transplant (these patients were either evaluated for transplant or referred for transplant). Overall, reasons for severance/DC were not statistically significantly different between UDCA and PL.

TABLE 8
Mayo Clinic Trial

Patient Severance/Discontinuation

Reason	UDCA (n=89)	PL (n=91)	p-value (all reasons)
Death [n=9]	3 (3%)	6 (7%)	N.S.
Voluntary W/D [n=16]	5 (6%)	11 (13%)	
Transplantation [n=8]	3 (3%)	5 (5%)	
Other [n=2]	1 (1%)	1 (1%)	
TOTAL	12 (12%)	23 (25%)	

In this Table, patients are counted only once. For instance, pt. #804 who voluntarily W/D on 06/04/90 and died on 11/16/90 was counted only once under the most serious of the two outcomes, i.e. death.

f. Patients for whom the blind was broken

- The blind was broken for patient #734 during the study.

This was a 41-year old woman who had been diagnosed with PBC in 1985. She entered the trial on 20 September 1988. In July, 1990 she began to notice generalized muscle weakness. A muscle Bx in November, 1990 demonstrated denervation and mild myopathic changes. The patient was maintained on D-B. She was followed clinically. In January, 1992 another neuromuscular examination was performed due to continued weakness. Electrophysiological studies and muscle Bx demonstrated changes consistent with an active inflammatory myopathy.

The blind was broken for this patient because of literature reports of bile sequestering agents causing myopathy with muscle necrosis that would mimic polymyositis. The patient was on PL .

The patient was D/C from the D-B study.

- The blind was broken for patient #770 at the request of her physician.

She entered the study on 5 January 1989. In November 1989 she was evaluated by her physician who felt her clinical condition and liver Dz was worsening. In addition, her BIL levels were rising.

At the physician's request the CTM was stopped and the patient was decoded and found to be on UDCA. The test medication was stopped and the patient was D/C on 10 November 1989.

This pt. was included in the voluntary withdrawals group.