Improved Oral Delivery of *N*-(4-Hydroxyphenyl)Retinamide with a Novel LYM-X-SORB Organized Lipid Complex

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Abstract

Purpose: Fenretinide [*N*-(4-hydroxyphenyl)retinamide (4-HPR)] is a cytotoxic retinoid that suffers from a wide interpatient variation in bioavailability when delivered orally in a corn oil capsule. The poor bioavailability of the capsule formulation may have limited responses in clinical trials, and the large capsules are not suitable for young children. To support the hypothesis that a novel organized lipid matrix, LYM-X-SORB, can increase the oral bioavailability of fenretinide, fenretinide in LYM-X-SORB matrix and in a powderized LYM-X-SORB formulation was delivered to mice.

Experimental Design: Fenretinide was delivered orally to mice as the contents of the corn oil capsule, in LYM-X-SORB matrix (4-HPR/LYM-X-SORB matrix) or in a LYM-X-SORB matrix powderized with sugar and flour (4-HPR/LYM-X-SORB oral powder). Levels of 4-HPR, and its principal metabolite, *N*-(4-methoxyphenyl)retinamide, were assayed in plasma and tissues.

Results: In a dose-responsive manner, from 120 to 360 mg/kg/d, delivery to mice of 4-HPR in LYM-X-SORB matrix, or as 4-HPR/LYM-X-SORB oral powder, increased 4-HPR plasma levels up to 4-fold ($P < 0.01$) and increased tissue levels up to 7-fold ($P < 0.01$) compared with similar doses of 4-HPR delivered using capsule contents. Metabolite [*N*-(4-methoxyphenyl)retinamide] levels mirrored 4-HPR levels. Two human neuroblastoma murine xenograft models showed increased survival ($P < 0.03$), when treated with 4-HPR/LYM-X-SORB oral powder, confirming the bioactivity of the formulation.

Conclusions: 4-HPR/LYM-X-SORB oral powder is a novel, oral drug delivery formulation, suitable for pediatric use, which warrants further development for the delivery of fenretinide in the treatment of cancer. A phase I clinical trial in pediatric neuroblastoma is in progress.

A synthetic retinoid made in the late 1960s, *N*-(4-hydroxyphenyl)retinamide (4-HPR; fenretinide), has been reported to be cytotoxic to, or inhibit the growth of, primary tumor cells, cell lines, and/or xenografts of various cancers, including those of neuroblastoma (1–3), colorectal (4), prostate (5, 6), breast (7, 8), ovarian (9–11), small-cell lung cancer (12), and both acute lymphoid and myeloid leukemias (13–15). Fenretinide cytotoxicity *in vitro* may be mediated through retinoic acid receptor–dependent and retinoic acid receptor–independent mechanisms (16), is p53 independent (12, 17–19), and can be caspase independent (19). Mechanisms of fenretinide cytotoxicity may involve reactive oxygen species generation (19–22) and/or an increase in ceramide species (saturated and desaturated *N*-acyl-sphingolipids; refs. 3, 15, 22–24).

Clinically, fenretinide has been studied in phase I, II, and III chemoprevention and chemotherapeutic trials using both low- and high-dose schedules using an oral gelatin capsule containing fenretinide (100 mg) in corn oil and polysorbate 80 [currently available through the National Cancer Institute (NCI)]. The systemic toxicity of fenretinide using chronic, low-dose schedules (generally 100–400 mg/d, obtaining ≤ 3 μmol/L plasma levels) has been minimal with the major clinical toxicity being reversible nyctalopia (decreased night vision) due to reduced plasma retinol levels (25). High-dose schedules (1,800 mg/m^2/d, divided into two or three daily doses, for 7 days, every 21 days) have also been well tolerated in adults (26). In pediatric cancer patients, occasional cases of hepatic toxicity and non-dose–related pseudotumor cerebri were observed in one pediatric phase I study, which determined a maximum tolerated dose at 2,450 mg/m^2/d (divided thrice daily, for 7 days, every 21 days; ref. 27); however, no dose-limiting toxicities were

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Note: Certain intellectual property rights to fenretinide/LYM-X-SORB organized lipid matrix formulations may be reserved by Childrens Hospital Los Angeles (Los Angeles, CA) and/or BioMolecular Products, Inc. (Byfield, MA).

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observed in another pediatric study using the capsule formulation at up to 4,000 mg/m^2/d (single daily dosing, for 4 weeks, every 5 weeks; ref. 28). Dose escalation in this later study was terminated without reaching a maximum tolerated dose due to patient noncompliance with the number of capsules that needed to be consumed. However, even high-dose schedules of the capsule formulation have obtained relatively low micromolar plasma levels with a wide interpatient variation that has complicated interpretation of response data (26–28). Thus, developing improved formulations of fenretinide that obtain higher and/or more consistent plasma levels in a more patient-friendly dosing formulation could enhance 4-HPR antitumor activity.

LYM-X-SORB is an organized lipid matrix of lysophosphatidylcholine, monoglyceride, and free fatty acids, specifically designed to improve the solubility and oral bioavailability of drugs by forming chylomicron-like particles in the stomach and enhancing drug absorption via the lymphatics in the proximal intestine (29, 30). The LYM-X-SORB matrix is composed of U.S. Food and Drug Administration Generally Regarded As Safe list components and has a safety and tolerance showed in a 1-year double blind oral feeding study in cystic fibrosis patients (31). We have incorporated fenretinide into a LYM-X-SORB matrix (4-HPR/LYM-X-SORB matrix) and formulated it as a free-flowing powder, with roughly the flavor of raw cookie dough, for direct oral administration or for mixing in foods, juices, or nonmilk fat–containing liquid oral nutritional supplements. We report here that this new formulation, fenretinide/LYM-X-SORB oral powder (4-HPR/LYM-X-SORB oral powder), obtained significantly higher plasma and tissue levels in mice than did an equivalent dose of fenretinide delivered using the contents of the corn oil capsule. We also report that 4-HPR/LYM-X-SORB oral powder prolonged survival in two of three human neuroblastoma murine xenograft models. Additionally, it is anticipated that the powdered format of the formulation will be more acceptable to patients than the several dozen large fenretinide corn oil capsules currently required daily for an adult to obtain 4-HPR plasma levels >10 μmol/L. Thus, 4-HPR/LYM-X-SORB has the potential to improve the clinical anticancer activity of fenretinide by increasing drug systemic exposure, decreasing interpatient variability in absorption, and increasing patient compliance.

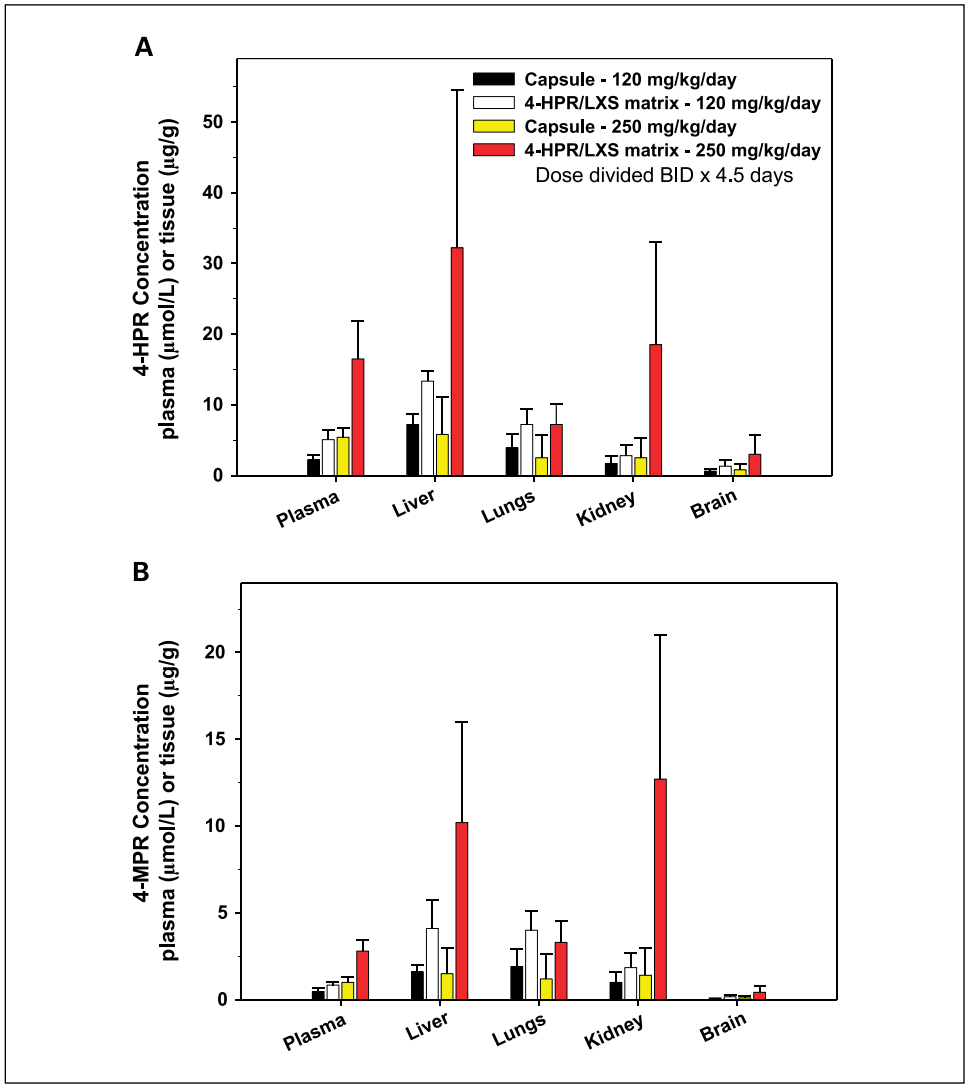

Fig. 1. Fenretinide (4-HPR) and metabolite (4-MPR) levels obtained in mouse plasma and tissues using fenretinide were administered in 4-HPR/LYM-X-SORB (*LXS*) organized lipid matrix or as the contents of NCI corn oil capsules. BALB/c mice were orally administered the extracted contents of NCI fenretinide capsules mixed in crushed mouse chow (4-HPR, 120 mg/kg/d, $n = 5$, *black columns*) or in a nutritional shake (4-HPR, 250 mg/kg/d, $n = 5$, *yellow columns*) or administered fenretinide inserted into LYM-X-SORB matrix (4-HPR/LYM-X-SORB molar ratio: 0.8:1.0; LYM-X-SORB composition, 1:3:3, molar ratio of lysophosphatidylcholine, monoglyceride, and free fatty acid). 4-HPR/LYM-X-SORB matrix was softened for delivery in water ($n = 5$), or in nutritional shake ($n = 5$), at two different doses (4-HPR, 120 mg/kg/d, *white columns*; 4-HPR, 250 mg/kg/d, *red columns*). Animals were administered drug in two equal daily doses (twice daily), for nine total doses, and sacrificed for analysis 3 h after the last dose. *A,* fenretinide (4-HPR) levels in plasma (μmol/L) and tissues (μg/g) were increased by delivery in LYM-X-SORB matrix compared with capsule contents: in plasma, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.01$); in liver, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.01$); in lungs, 120 mg/kg/d ($P < 0.02$) and 250 mg/kg/d ($P = 0.02$); in kidney, 120 mg/kg/d ($P = 0.14$) and 250 mg/kg/d ($P < 0.01$); and in brain, 120 mg/kg/d ($P = 0.05$) and 250 mg/kg/d ($P = 0.03$). *B,* metabolite (4-MPR) levels in plasma (μmol/L) and tissues (μg/g) were increased by fenretinide delivery in LYM-X-SORB matrix compared with NCI capsule contents: in plasma, 120 mg/kg/d ($P = 0.05$) and 250 mg/kg/d ($P < 0.01$); in liver, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.01$); in lungs, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.03$); in kidney, 120 mg/kg/d ($P < 0.04$) and 250 mg/kg/d ($P < 0.01$); and in brain, 120 mg/kg/d ($P = 0.04$) and 250 mg/kg/d ($P = 0.02$). Columns, 4-HPR concentration (*A*) and 4-MPR concentration (*B*); bars, SD.

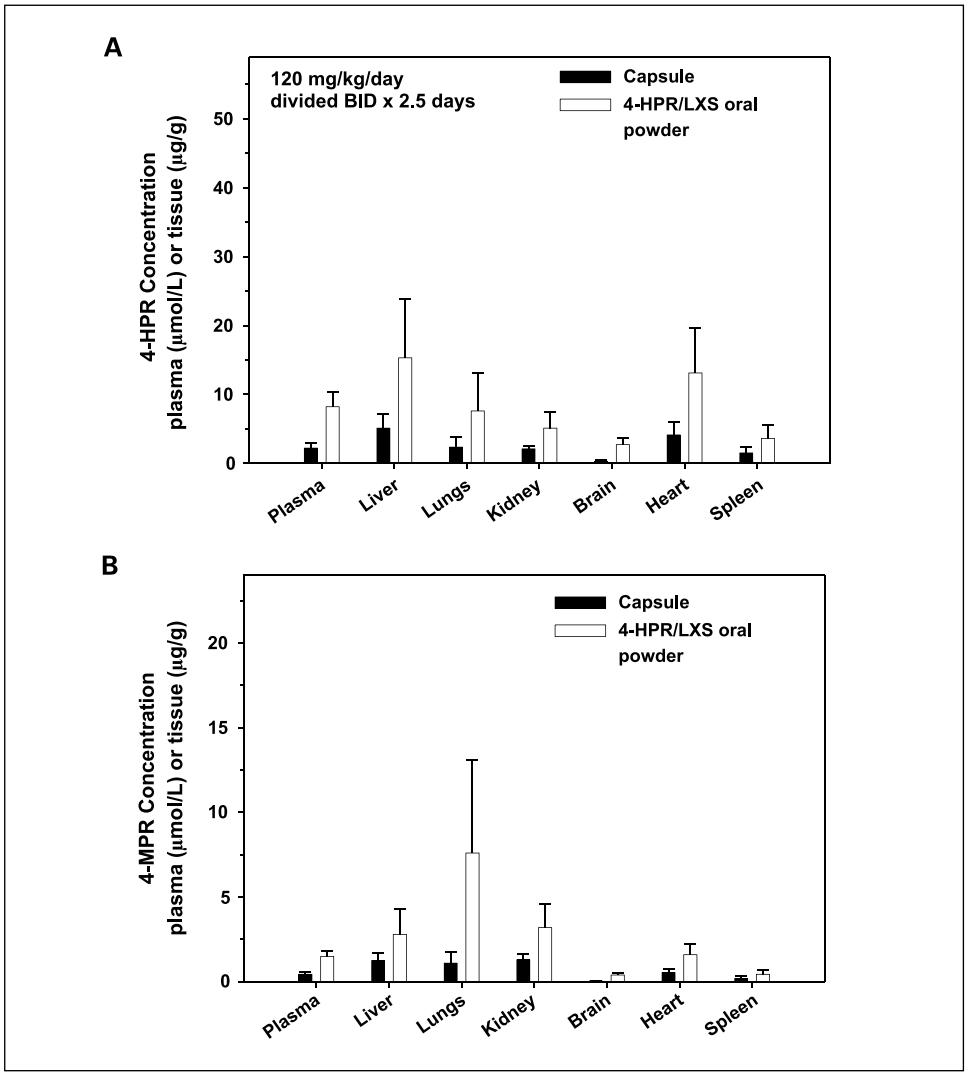

Fig. 2. Fenretinide (4-HPR) and metabolite (4-MPR) levels obtained in mouse plasma and tissues using 4-HPR administered in powderized 4-HPR/LYM-X-SORB matrix or as the contents of NCI corn oil capsules. Nude mice were orally administered fenretinide (4-HPR, 120 mg/kg/d) as the extracted contents of NCI fenretinide capsules mixed in a nutritional shake ($n = 9$, *black columns*) or in 4-HPR/LYM-X-SORB matrix (4-HPR/LYM-X-SORB molar ratio: 0.8:1.0; LYM-X-SORB composition, 1:4:2, molar ratio of lysophosphatidylcholine, monoglyceride, and free fatty acids) that was powderized with flour and sugar. 4-HPR/LYM-X-SORB oral powder (*white columns*) was slurried for delivery in water ($n = 5$) or in a nutritional shake ($n = 5$). Animals were administered drug in two equal daily doses (twice daily), for five total doses, and then sacrificed for analysis 4 h after the last dose. All animals were assayed for plasma levels. For tissue levels, animals administered 4-HPR/LYM-X-SORB oral powder in nutritional shake ($n = 5$) were analyzed for comparison against animals administered NCI capsule contents in nutritional shake ($n = 4$). *A,* fenretinide (4-HPR) levels in plasma and most tissues were increased by delivery in 4-HPR/LYM-X-SORB oral powder compared with capsule contents: in plasma, liver, kidney, brain, and heart, all $P < 0.05$; in lungs, $P = 0.09$; and in spleen, $P = 0.08$. *B,* metabolite (4-MPR) levels in plasma and most tissues were increased by 4-HPR delivery in powderized LYM-X-SORB matrix compared with NCI capsule contents: in plasma, kidney, brain, heart, and spleen, $P < 0.05$; in liver, $P < 0.08$; and in lungs, $P < 0.06$. Columns, 4-HPR concentration (*A*) and 4-MPR concentration (*B*); bars, SD.

Materials and Methods

Chemicals. 4-HPR (fenretinide), *N*-(4-methoxyphenyl)retinamide (4-MPR), and *N*-(4-ethoxyphenyl)retinamide were obtained from the NIH NCI. All chemicals were high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich Co. Matrigel Matrix HC was from BD Biosciences.

Cell lines. The human neuroblastoma cell lines, SMS-KCNR, CHLA-140, and CHLA-90, have been described previously (32–35). SMS-KCNR is a p53-functional cell line established at progressive disease after dual-agent induction chemotherapy; CHLA-140 is a multidrug-resistant, p53 functional cell line established at progressive disease after intensive multiagent chemotherapy that overexpresses MDR1; and CHLA-90 is a multidrug-resistant, p53 mutant cell line derived at relapse after myeloablative therapy and autologous bone marrow transplant that overexpresses MDR1.[6] Cell lines were maintained at 37°C in a humidified incubator containing 95% room air + 5% CO_2 atmosphere as described (3).

Drugs and formulation. Clinical grade, bulk fenretinide was obtained from the NCI and formulated into various compositions of LYM-X-SORB organized lipid matrix (4-HPR/LYM-X-SORB matrix) at a 4-HPR/LYM-X-SORB molar ratio of 0.8:1.0 under conditions of Good Manufacturing Practice by Avanti Polar Lipids, Inc. (36), operating under license from LYM-DRUG Products, LLC, and BioMolecular Products, Inc. (30). Fenretinide/LYM-X-SORB oral powder (4-HPR/LYM-X-SORB powder) was formulated by blending the 4-HPR/LYM-X-SORB matrix with sugar and wheat flour to final products that were either 2.2% or 3% by weight fenretinide, 16% or 22% LYM-X-SORB (lysophosphatidylcholine, monoglycerides, and free fatty acids, 1:4:2), ~20% sucrose, and the remainder wheat flour (37). 4-HPR capsules (100 mg in corn oil and polysorbate 80; NSC #374551, IND# 40294) were obtained from the NCI.

Animals. Five- to six-week-old BALB/c and Harlan athymic nude-Foxn1^{nu} mice were obtained from commercial vendors. For delivery of fenretinide from the (corn oil) capsules currently available from the NCI, the contents of the capsules were expressed after puncturing the capsule, or extracted with a needle and syringe, into an Eppendorf tube, and the amount of fenretinide obtained was quantified by HPLC assay. The capsule contents were then vortexed and mixed with crushed mouse chow or SlimFast liquid nutritional supplement ("nutritional shake") for oral or gavage delivery. For delivery of fenretinide in 4-HPR/LYM-X-SORB matrix (a hard wax at room temperature), 4-HPR/LYM-X-SORB matrix was compounded in crushed chow, or slurried in nutritional shake for oral syringe or gavage feeding, depending on the dose and volume required. For delivery of fenretinide in 4-HPR/LYM-X-SORB

[6] N. Keshelava, personal communication.

oral powder, the powder was mixed with water or nutritional shake for gavage feeding.

Cohorts of mice were administered the indicated dose of 4-HPR, on a divided daily schedule, for the number of doses indicated. In general, cohorts of five mice were used for each experimental condition or associated controls; the exact number of animals used for each experiment is listed in the figure legends. 4-HPR was well tolerated in all dose forms. The ability of mice to tolerate drug administration was assessed by daily examination of general activity and overall appearance and body weights at least twice weekly. Animals were sacrificed at 3 or 4 h after the last dose by carbon dioxide narcosis, and blood and organs were harvested and stored at -80°C for HPLC analysis. Fenretinide-containing materials were wrapped in foil, or kept in tinted tubes, to reduce exposure to light. For xenograft models, human neuroblastoma cell lines were established s.c. in nude mice. For passage and expansion, 16 to 20 million cells of established tumors were mixed in Matrigel Matrix HC per manufacturer's directions and injected s.c. (total volume of 0.2 mL) above the shoulder blade. All experiments were done using xenografts of ≤ passage 3 in mice. Drug treatment was begun when tumors measured 100 to 200 mm^3. Animals received 4-HPR/LYM-X-SORB oral powder, or powderized LYM-X-SORB matrix-alone (controls), mixed in 0.2 cc nutritional shake by gavage, in divided daily doses, Mondays to Fridays, for up to 18 weeks. Dosing by gavage was used to insure consistent dosing of the animals. Tumors were measured using calipers twice weekly and tumor volumes were calculated as 0.5 × height × width × length (38). Animals were sacrificed by carbon dioxide narcosis when tumor volumes exceeded 1,500 mm^3 per Institutional Animal Care and Use Committee guidelines. Treatment was well tolerated. All animals were housed and treated according to protocols approved by the Institutional Animal Care and Use Committee.

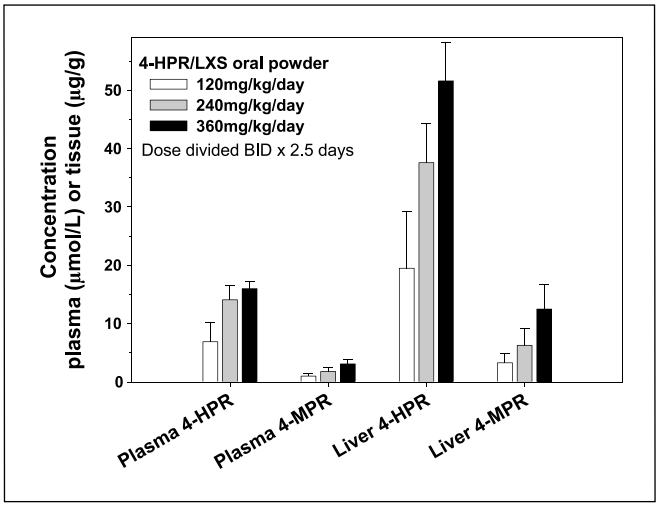

Fig. 3. Fenretinide (4-HPR) and metabolite (4-MPR) levels obtained in mouse plasma and liver using 2.2% and 3% clinical grade 4-HPR/LYM-X-SORB oral powder. Nude mice were gavaged with 4-HPR/LYM-X-SORB oral powder formulated under Good Manufacturing Practice conditions. 4-HPR/LYM-X-SORB matrix (4-HPR/LYM-X-SORB molar ratio: 0.8:1.0; LYM-X-SORB composition, 1:4:2, molar ratio of lysophosphatidylcholine, monoglyceride, and free fatty acid) was powderized with flour and sugar to final 4-HPR concentrations of 2.2% or 3%. Mice received the following: 4-HPR/LYM-X-SORB oral powder (4-HPR, 120 mg/kg/d, *white columns*) slurried for delivery in water (2.2% powder, $n = 5$; 3% powder, $n = 5$) or in a nutritional shake (3% powder, $n = 5$); 4-HPR/LYM-X-SORB oral powder (4-HPR, 240 mg/kg/d, *gray columns*) slurried for delivery in water (2.2% powder, $n = 5$; 3% powder, $n = 3$) or nutritional shake (3% powder, $n = 5$); or 4-HPR/LYM-X-SORB oral powder (4-HPR, 360 mg/kg/d, *black columns*) slurried for delivery in nutritional shake (3% powder, $n = 5$). Animals were administered drug in two equal daily doses (twice daily), for five total doses, and then sacrificed for analysis 4 h after the last dose. Both 4-HPR and 4-MPR levels in plasma and liver significantly increased with drug dosage: 120 versus 240 mg/kg/d, $P < 0.01$; 240 versus 360 mg/kg/d, $P < 0.05$. For a given fenretinide dosage level, there was no statistical differences in 4-HPR or 4-MPR plasma or liver levels obtained between animals receiving the 2.2% versus 3% powder or in plasma levels obtained using powder slurried in water versus nutritional shake. 4-HPR and 4-MPR liver levels trended lower in animals receiving oral powder in nutritional shake compared with water at 120 mg/kg/d ($P < 0.04$ and $P < 0.01$, respectively) but not at 240 mg/kg/d ($P = 0.20$ and $P = 0.14$, respectively). Columns, concentration; bars, SD.

Fenretinide assay. Fenretinide (4-HPR), and its principal metabolite, 4-MPR, were quantified in plasma and tissues using a HPLC method as described previously (39). Briefly, the plasma samples were added to internal standard, and ice-cold acetonitrile was added before homogenate was mixed by vortex and placed in an ultrasonic bath for 10 min. The mixture was then centrifuged at 10,000 × *g* at 4°C for 5 min. The supernatant was transferred to an autosampler and injected directly into the HPLC system. Tissues were homogenized using a Tissue Tearor (Biospec, Inc.) with a 1:3 ratio of physiologic saline (0.9% NaCl, w/v).

The HPLC used was a Waters Alliance 2690 Separation Module with a Waters 717 autosampler. Waters 2487 UV-Visible absorbance detector was set at a wavelength of 340 nm and autosampler temperature was set at 4°C. The column used for separation was a chemically bonded reversed-phase Symmetry C_{18} column (3.5 μm, 150 × 4.6 mm) with a Symmetry C_{18} guard column (5 μm, 3.9 × 20 mm). Isocratic elution with acetonitrile/water/glacial acetic acid (88:10:2, v/v/v) was used at a flow rate of 1.0 mL/min. Drug levels were calculated using the Empower Pro chromatographic data system. Calibration curves were prepared in methanol/acetonitrile (1:1, v/v) with concentrations ranging from 0.16 to 20 μg/mL depending on expected levels of analyte in the unknown samples. 4-HPR and 4-MPR were used as standards, and *N*-(4-ethoxyphenyl)retinamide was used as an internal standard. Blanks and low, middle, and high quality control samples were incorporated in each run. The same extraction procedure used for plasma and tissue samples was used for the calibration curves. The plasma concentration of 4-HPR and 4-MPR was presented in micrograms of drug per milliliter of plasma (μg/mL) or in micromolar per liter (μmol/L). The final tissue concentrations of drugs were presented in micrograms of drug per gram of wet tissue weight (μg/g).

Statistical analysis. The statistical significance of differences in means was evaluated by the Student's *t* test using Microsoft Excel 2000 software. *P* values were two sided. Analysis of human neuroblastoma murine xenograft models was by Kaplan-Meier log-rank analysis of survival. All tests were considered significant at $P < 0.05$.

Results

LYM-X-SORB matrix increased fenretinide levels. LYM-X-SORB organized lipid matrix can be formulated at various ratios of lysophosphatidylcholine, monoglycerides, and free fatty acids, to optimize incorporation of the target drug. Fenretinide (4-HPR) was incorporated into several hard, wax-like LYM-X-SORB lipid matrix (4-HPR/LYM-X-SORB matrix) preparations with the LYM-X-SORB matrix variously formulated with lysophosphatidylcholine/monoglycerides/free fatty acids ratios of 1:2:4; 1:4:2; or 1:3:3. Mice were orally dosed, either with these 4-HPR/LYM-X-SORB matrix formulations or with the expressed (expelled) contents of the currently available NCI fenretinide capsule (essentially a microcrystal slurry in corn oil). Levels of fenretinide, and its principal metabolite, 4-MPR, were then measured in plasma and tissues.

Figure 1 shows the fenretinide plasma and tissue levels obtained when 4-HPR incorporated into LYM-X-SORB matrix (1:3:3) was administered in various vehicles at doses of 120 and 250 mg 4-HPR/kg/d for 4.5 days compared with 4-HPR levels obtained by the contents of NCI 4-HPR capsules.

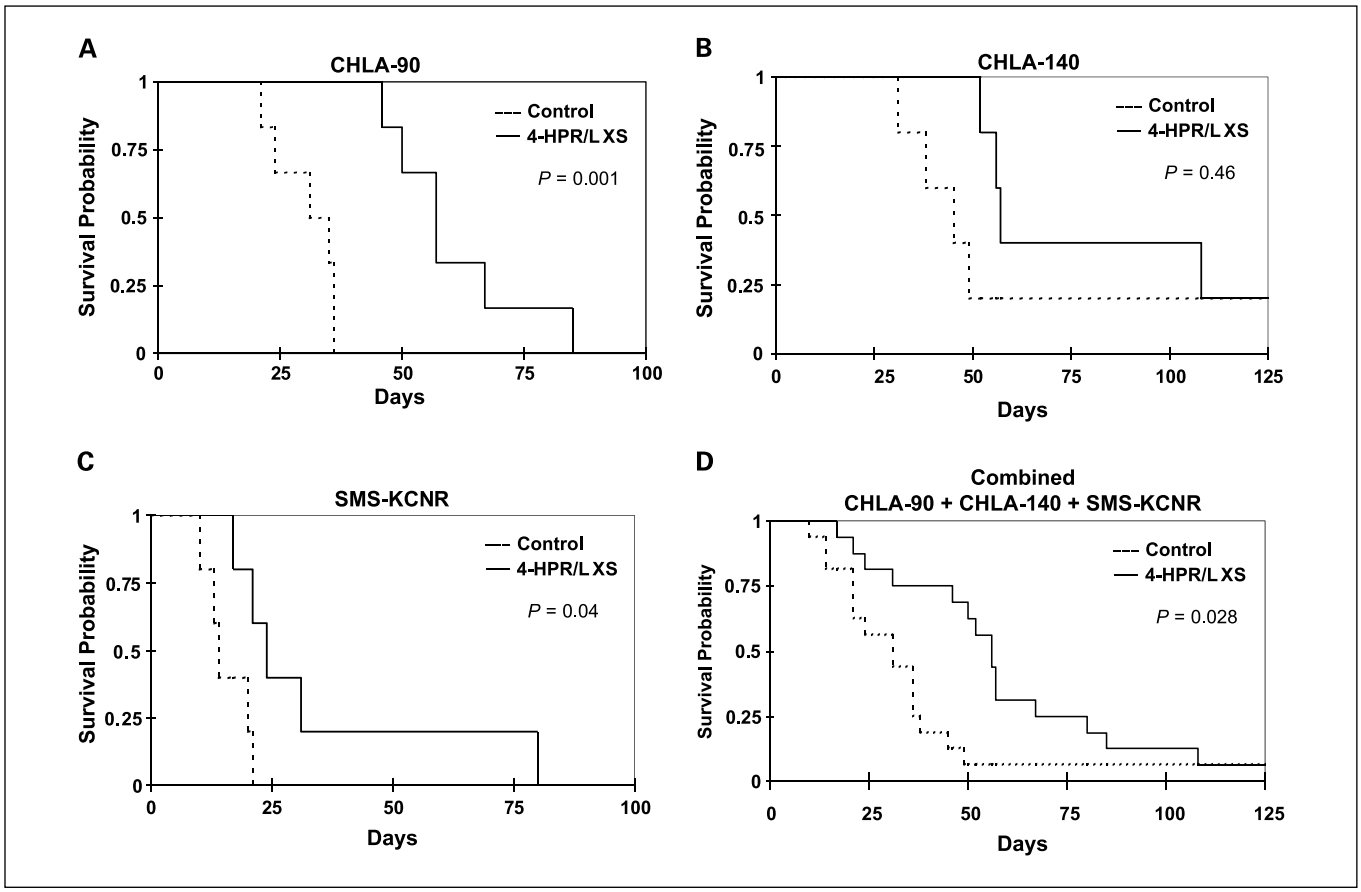

Fig. 4. 4-HPR/LYM-X-SORB oral powder prolonged survival in human neuroblastoma murine xenografts. Human neuroblastoma cell lines SMS-KCNR, CHLA-90, and CHLA-140 were established as s.c. xenografts in *nu/nu* mice. Animals received 120 mg/kg/d, by gavage, in daily divided doses, 5 d a week (CHLA-140, $n = 5$ per cohort), or 240 mg/kg/d, by gavage, in daily divided doses, 5 d a week (SMS-KCNR, $n = 5$ per cohort; CHLA-90, $n = 6$ per cohort). Control animals received powderized LYM-X-SORB matrix-alone. Animals were sacrificed when tumors reached 1,500 mm^3. *A,* CHLA-90. *B,* CHLA-140. *C,* SMS-KCNR. *D,* combined survival for SMS-KCNR, CHLA-90, and CHLA-140 ($n = 16$ per cohort). 4-HPR/LYM-X-SORB oral powder significantly prolonged survival in CHLA-90 and SMS-KCNR and in the combined survival data of the three models. Survival analysis by Kaplan-Meier log-rank test.

Compared with NCI capsule contents, at doses of 120 mg/kg/d, use of the wax-like 4-HPR/LYM-X-SORB matrix approximately doubled plasma and tissue levels of 4-HPR; at doses of 250 mg kg/d, 4-HPR plasma levels increased over 3-fold and tissue levels up to 6-fold (Fig. 1A). In accordance with results reported for rats (40), 4-HPR levels in the intact brain were the lowest of the tissues assayed, but even in brain, the 4-HPR/LYM-X-SORB matrix increased 4-HPR levels ~4-fold at doses of 250 mg/kg/d. 4-MPR levels in plasma and tissues generally mirrored 4-HPR levels, albeit at lower levels (Fig. 1B). Results obtained with 4-HPR/LYM-X-SORB matrix formulated at lysophosphatidylcholine/monoglycerides/free fatty acids ratios of 1:2:4 and 1:4:2 at doses of 120 mg/kg/d, for 4.5 days, were comparable with those obtained with the 1:3:3 matrix (data not shown).

As the three different LYM-X-SORB matrix lysophosphatidylcholine/monoglycerides/free fatty acids ratios tested seemed to be approximately equivalent in increasing 4-HPR bioavailability, formulation development proceeded with a LYM-X-SORB matrix ratio of 1:4:2, as LYM-X-SORB matrix-alone at this ratio had previously received phase I clinical testing as a nutritional supplement in cystic fibrosis patients (31).

Powderized LYM-X-SORB matrix increased fenretinide levels. LYM-X-SORB matrix, a hard wax at room temperature, can be consumed neat, or delivered in capsules. However, to maximize patient acceptance of the 4-HPR/LYM-X-SORB matrix, especially to children, and to facilitate delivery through nasogastric feeding tubes, the 4-HPR/LYM-X-SORB matrix (1:4:2) was "powderized" by blending the matrix oil with table sugar and wheat flour to a final 4-HPR concentration of 3% by weight. This formulation, 4-HPR/LYM-X-SORB oral powder, could be delivered neat, mixed with applesauce, or slurried in liquid carriers for delivery. 4-HPR/LYM-X-SORB oral powder delivered at 120 mg 4-HPR/kg/d, for 2.5 days, increased plasma levels ~3.5-fold and tissue levels up to 3-fold compared with capsule contents (Fig. 2A). 4-MPR levels generally mirrored 4-HPR levels, albeit at lower levels (Fig. 2B). 4-HPR/LYM-X-SORB oral powder at this dose increased 4-HPR plasma levels ($P < 0.01$) compared with the wax-like 4-HPR/LYM-X-SORB matrix delivered by itself (Fig. 1A), perhaps as a result of powderization facilitating absorption by reducing the particle size of the ingested LYM-X-SORB matrix.

Supported by the above data, a grant was secured from the NCI Developmental Therapeutics Program's Rapid Access to

Intervention Development program for the Good Manufacturing Practice (i.e., clinical grade) production of 4-HPR/LYM-X-SORB oral powder to support phase I trials in pediatrics and adults. 4-HPR/LYM-X-SORB oral powder was prepared at final 4-HPR concentrations of 2.2% and 3%. Both preparations were free-flowing powders; whereas the 2.2% powder had superior dissolution properties in liquids, the 3% powder could be adequately slurried in liquids and had a consistency more amenable to direct ingestion or mixing with solids for ingestion. Clinical grade 4-HPR/LYM-X-SORB oral powders (2.2% and 3%) were administered to mice at doses from 120 to 360 mg/kg/d, for 2.5 days, and plasma and liver 4-HPR and 4-MPR levels were assayed (Fig. 3). 4-HPR levels in plasma and liver were increased 3- to 7-fold compared with 4-HPR delivered at similar doses using capsule contents (Figs. 1 and 2) and the levels obtained were dose responsive in this range.

Fenretinide/LYM-X-SORB oral powder prolonged survival in neuroblastoma xenograft models. Three human neuroblastoma cell lines were established as xenografts in nude mice. SMS-KCNR is drug-sensitive *in vitro*, whereas CHLA-140 and CHLA-90 are multidrug-resistant *in vitro* (32–35). CHLA-90 has loss of p53 function via a *TP53* mutation; both CHLA-140 and CHLA-90 overexpress the *MDR1* gene.[6] Xenografts received 4-HPR/LYM-X-SORB oral powder, or powderized LYM-X-SORB matrix-alone (controls), by gavage for up to 18 weeks. Treatment with 4-HPR/LYM-X-SORB oral powder prolonged survival in two of the three xenograft models and for the combined data from all three models, as shown by Kaplan-Meier analysis (Fig. 4). This showed that fenretinide delivered in 4-HPR/LYM-X-SORB oral powder was both bioavailable and bioactive. Chronic dosing of 4-HPR/LYM-X-SORB oral powder seemed well tolerated by mice; mouse body weights were not different from the controls (Fig. 5).

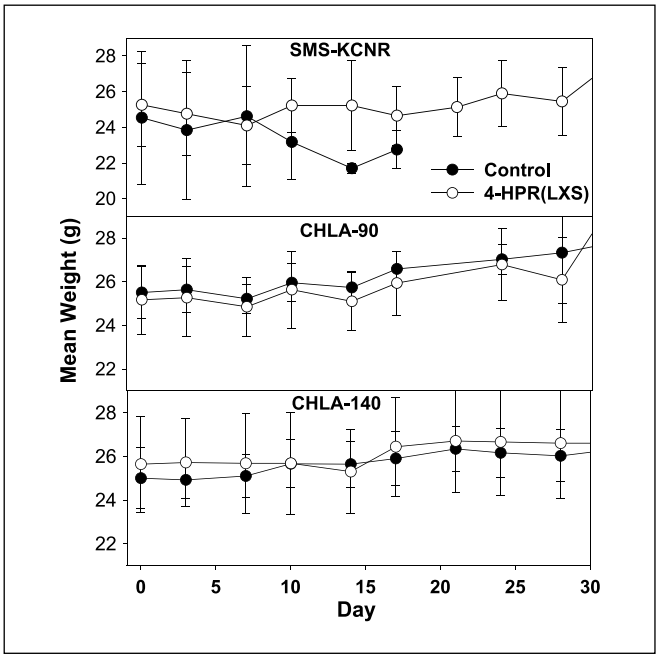

Fig. 5. Body weights of mice during the initial 30 d of the experiment presented in Fig. 4. Weight comparisons could only be carried out for the initial 30 d (a total of four 5-d courses of 4-HPR/LYM-X-SORB oral powder) as mice were culled due to tumor burdens. Statistical differences were assessed at day 14; there was no significant difference ($P > 0.05$) in body weights for those mice treated with 4-HPR/LYM-X-SORB compared with controls for mice carrying CHLA-90 and CHLA-140 xenografts. The SMS-KCNR control mice had significantly ($P = 0.035$) lower weights than 4-HPR-treated mice due to morbidity from tumor xenograft growth.

Discussion

To date, clinical investigations of 4-HPR (fenretinide) have principally focused on its oncologic use as a chemoprevention or chemotherapeutic agent, although recent preclinical data suggest that fenretinide may have clinical potential in the prevention or treatment of obesity-related type II diabetes (41, 42) and/or retinal degenerative diseases, such as age-related macular degeneration and Stargardt's macular degeneration (43–45). Direct clinical activity observed with fenretinide in cancers to date has been modest, possibly due to the low drug levels obtained, although tumor responses and/or disease stability have been noted in neuroblastoma (27, 46), platinum-refractory ovarian cancer (47), and oral leukoplakia (48). Fenretinide has been generally well tolerated on both low-dose and high-dose schedules with a reversible decrease in night vision being the most common side effect (25). Decreased night vision (nyctalopia), however, has not prevented daily fenretinide administration for up to 5 years (49).

One impediment to the clinical development of fenretinide has been its historical delivery vehicle, a large, corn oil-based capsule of limited bioavailability that delivers plasma levels with a wide interpatient variability. In one trial, the daily consumption of up to 4,000 mg/m^2 fenretinide (40 capsules/m^2), for 4 weeks of every 5 weeks, resulted in mean plasma levels of only 12.9 μmol/L (28). Patient dissatisfaction with the capsule formulation on high-dose schedules has been noted (28, 50).

In an attempt to improve drug delivery and maximize tumor response, we have placed fenretinide in a novel, organized lipid matrix, called LYM-X-SORB, and powderized the 4-HPR/LYM-X-SORB matrix for delivery. As the data presented show, this formulation increased plasma and tissue levels in mice from 3- to 7-fold compared with identical doses delivered as the contents of fenretinide in corn oil capsules and prolonged survival in human neuroblastoma xenograft models. We observed that 4-HPR delivered in any form of LYM-X-SORB matrix increased 4-HPR bioavailability. However, working with the hard, wax-like LYM-X-SORB matrix produced intermouse variability in drug levels obtained, likely due to the technical difficulties associated with its consistency. Powderizing the 4-HPR/LYM-X-SORB matrix produced a product that was easier to deliver mechanically and that obtained higher drug levels with a lower interanimal variance.

In the formulation ultimately developed for clinical trials, fenretinide/LYM-X-SORB oral powder has roughly the consistency of brown sugar. It is intended to be dissolved or slurried in nonmilk fat–containing liquids, mixed with foods, or consumed neat for patient delivery. It is hoped that this powderized format will be user-friendly to patients and facilitate compliance on high-dose fenretinide schedules.

Indeed, fenretinide/LYM-X-SORB oral powder slurried into a liquid nutritional supplement has been delivered via nasogastric tube to a cancer patient with restricted oral intake.[7] Powderized LYM-X-SORB organized lipid matrix (as described in this report, but lacking fenretinide) will also be tested as a dietary supplement in cystic fibrosis patients in an upcoming randomized, double-blinded, nutritional intervention study funded by the NIH National Institutes of Diabetes, Digestive and Kidney Diseases (grant 2 R44 DK060302-02A1).

Based on the preclinical data reported here, a phase I trial of fenretinide/LYM-X-SORB oral powder (NL2004-04, B. Maurer, Chair) in pediatric neuroblastoma patients is in progress in the NCI-funded, New Approaches to Neuroblastoma Therapy consortium[8] with the drug supplied via by a Rapid Access to Intervention Development grant from the NCI Developmental Therapeutics Program. It is anticipated that this novel formulation will deliver higher and/or more uniform fenretinide levels for use in future single- and combination-agent clinical trials in cancer and other disease states.

[7] B.J. Maurer, unpublished data.

[8] http://www.nant.org

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