Fenretinide-polyvinylalcohol Conjugates: New Systems Allowing Fenretinide Intravenous Administration

I. Orienti,[†] G. Zuccari,[†,*] V. Bergamante,[†] R. Carosio,[‡] R. Gotti,[†] M. Cilli,[§] and P.G. Montaldo[‡]

Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy, Laboratory of Oncology, G. Gaslini Children's Hospital, Genoa, Italy, and Animal Facilities, Istituto Nazionale Ricerca sul Cancro, Genoa, Italy

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N-(4-hydroxyphenyl)retinamide (fenretinide, 4-HPR) has been shown to be active toward many tumors without appreciable side effects. However its *in vitro* activity does not match a correspondent efficacy *in vivo*. The main reason is that the drug's hydrophobicity hinders its bioavailability in the body fluids. Even if the drug is previously dissolved in organic solvents, such as ethanol or DMSO, the subsequent dilution in body fluids trigger its precipitation in fine aggregates characterized by very low dissolution efficiency, never reaching amounts suitable for therapeutic response. To date no intravenous formulation of 4-HPR exists on the market. The 4-HPR linkage to a hydrophilic polymer by a covalent bond easily hydrolyzable in aqueous environment is expected to increase the drug's aqueous solubility, providing the free drug after hydrolysis of the covalent bond. This may be a useful tool for the preparation of aqueous intravenous formulations of 4-HPR. For this purpose, we linked 4-HPR to polyvinylalcohol (PVA) by a carbonate bond at different drug/hydroxy vinyl monomer molar ratios. We demonstrated that conjugation increased 4-HPR aqueous solubility and strongly inhibited neuroblastoma cell proliferation. In addition, in an *in vivo* neuroblastoma metastatic model, we obtained a significant antitumor effect as a consequence of the improved drug bioavailability.

Introduction

N-(4-hydroxyphenil)retinamide (fenretinide, 4-HPR), a synthetic retinoid, has been recognized as modulator of cell growth, differentiation, proliferation, and apoptosis.[1] In addition, 4-HPR was shown to suppress carcinogenesis in a variety of tissue types including oral mucosa, skin, bladder, lung, prostate, and breast cancer tissues in experimental animals.[2] These findings further confirmed that 4-HPR might be useful in both chemotherapy and chemoprevention of human cancers.[3] 4-HPR has been evaluated in Phase I−II trials in neuroblastoma (NB) patients.[4] However, its plasma levels in patients treated with oral dosage forms have been far lower than the effective plasma concentration required to induce apoptosis (usally 10 μM). Therefore, the pharmacological activity *in vitro* does not correspond to an equivalent efficacy *in vivo* due mainly to the poor bioavailability of 4-HPR. Moreover, neuroblastoma xenografts from rats, treated daily with 75 mg of oral fenretinide for 10 days, showed no statistically significant reduction in tumor volume and weight compared to that of corresponding control tumors.[5] In fact, the bioavailability of a drug from oral dosage forms is influenced by many factors among which aqueous solubility, variable adsorption in the gastrointestinal tract, and the epatic first pass effect play, in this case, an important role.[6,7] The possibility to obtain parenteral formulations mantaining high plasma concentrations of 4-HPR would be particularly interesting in NB, where the elimination of the nonproliferating residual cells from chemotherapy remains a very important and unsolved challenge, which is often the cause of a relapse of the disease.[8]

In an attempt to improve 4-HPR bioavailability, we have conjugated the hydrophobic drug with a hydrophilic polymer by a covalent bond easily hydrolyzable in biological environment. The main achievement obtained by this formulation technology is that several factors act together to affect the pharmacokinetic and, therefore, the bioavailability of 4-HPR such as the reduction of renal excretion, tumor localization by EPR (enhanced permeability and retention) effect, drug protection from enzymatic degradation, and increase in the drug's solubility.[9,10] Therefore, this approach allows one to enhance drug solubility simultaneously, providing long circulating systems suitable for parenteral administration.[11] In previous work, we prepared amphiphilic polymers based on PVA partially substituted with lipophilic acyl chains by either ester or carbamate bonds between the hydroxyls of PVA and the carbonyl of the acyl moieties. These polymers formed micelles in an aqueous environment because of the aggregation of the grafted acyl chains.[12] Entrapment of hydrophobic drugs in the micelles was observed, with a subsequent increase in their solubility.[13−15]

This study focuses on the 4-HPR conjugation to PVA by a carbonate bond between the phenol hydroxyl of 4-HPR and the vinyl hydroxyl of PVA (Figure 1) to further increase the 4-HPR aqueous solubilization. We covalently linked 4-HPR to PVA at different 4-HPR/hydroxyvinyl monomer molar ratios, choosing the carbonate linkage between the drug and the polymer because it is easily hydrolyzable in water. The physicochemical characteristics of 4-HPR-PVA conjugates were analyzed and correlated with their ability to release the free drug in aqueous environments. Subsequently, the best formulation, providing

* Corresponding author: Guendalina Zuccari, Department of Pharmaceutical Sciences, University of Bologna, Via San Donato 19/2, 40127 Bologna, Italy. Tel/Fax: +39 051 253664. E-mail: guendalina.zuccari@unibo.it.

[†] University of Bologna.

[‡] G. Gaslini Children's Hospital.

[§] Istituto Nazionale Ricerca sul Cancro.

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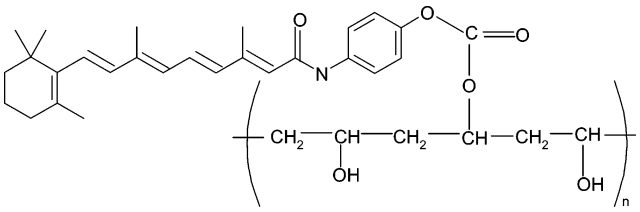

Figure 1. Polyvinylalcohol conjugated to 4-HPR.

higher 4-HPR solubilization, was evaluated *in vitro* and *in vivo* to test its antitumoral effect.

Materials and Methods

Materials. 4-HPR was a gift from Dompè (Milan, Italy), polyvinylalcohol (MW = 10,000 Da, 80% hydrolyzed) was a commercial sample from Aldrich Chemical Co. (Steinheim, Germany), 1,1′-Carbonyldiimidazole was purchased from Sigma Chemical Co. (St. Louis, MO). All the other reagents and solvents employed were from Fluka Chemie GmbH (Buchs, Suisse).

Preparation of the 4-HPR-PVA Conjugates. The 4-HPR-PVA conjugates were prepared following a procedure previously described for the synthesis of carbonates from alcohols.[16] Briefly, 4-HPR (195.77 mg; 0.5 mmol) was dissolved in 10 mL of *N*-methylpyrrolidone (NMP) in the presence of carbonyldiimidazole (0.5 mmol). The solutions were stirred at room temperature in the dark for 24 h and subsequently supplemented with different PVA amounts to obtain 0.5%, 1%, 2%, 4% 4-HPR/PVA weight ratios. Coupling reactions were incubated overnight at room temperature and finally supplemented with diethyl ether to induce the precipitation of the 4-HPR-PVA conjugates. The solid obtained was reprecipitated twice from NMP and dried under vacuum to constant weight.

The solid products obtained were stored in a desiccator. The conjugation degree (4-HPR/PVA weight ratio in the final product) was determined by a capillary electophoretic (CE) method and ^{1}H-NMR.

The CE experiments were performed on a BIORad Biofocus 2000 instrument from BioRad (Hercules, CA). The data were collected on a personal computer equipped with the Biofocus System Integration software (BioRad). The separations were carried out on a fused-silica capillary of 50 μm internal diameter (ID) with a total length of 23 cm (effective length 19.5 cm). The electrophoretic runs were performed at a constant voltage of 5.0 kV with controlled temperature (25 °C). The samples were injected hydrodynamically using a pressure of 2 psi × s (1 psi = 6894 Pa); the detection wavelength was 365 nm. The running buffer constituted of an aqueous solution of sodium tetraborate (20 mM; pH 9.2) supplemented with 100 mM sodium dodecylsulfate (SDS). In order to obtain reproducible migration times, the capillary was rinsed between the runs with water (2 min) and running buffer (2 min). The samples were prepared in methanol, and they were filtered through a 0.45 μm membrane before the analysis.

The developed method was validated for linearity of the response in the concentration range of 0.020−0.250 mg/mL of 4-HPR; a five point calibration graph was obtained by triplicate injections for each standard 4-HPR solutions (methanol), and the ratios of the peak area to migration time (corrected peak area, *Y*) were plotted against drug concentration (*C*; mg/mL). The linear regression analysis provided the following equation: $Y = 3812 \times 10^3 (\pm 79867)\, C - 41 \times 10^3 (\pm 9698)$ with a correlation coefficient $r = 0.999$. The sensitivity of the method expressed as the limit of detection (LOD) corresponding to a signal-to-noise ratio (S/N) of approximately 3, was found to be 5 μg/mL; the limit of quantification can be considered as the lowest calibration point. The repeatability of migration time and corrected peak area was estimated by the relative standard deviation (RSD %) of five consecutive analysis ($n = 5$) on a standard solution of 4-HPR (0.04 mg/mL); RSD % values corresponding to 1.2% and 2.3% were obtained for the migration time and corrected peak area, respectively.

The quantitation of 4-HPR in the actual samples was performed by the external standard method, on the basis of the comparison of the response (corrected peak area) of a standard solution of 4-HPR with that of the considered samples.

The ^{1}H-NMR analysis was performed by using an Inova 600 spectrometer and recording the spectrum in $(CD_3)_2SO$.

Solubilization Studies. The solubility of conjugated 4-HPR was evaluated by dispersing excess of 4-HPR-PVA in water at 37 °C and, subsequently, ultracentrifuging at 12,000 rpm to eliminate the undissolved material. The clear solutions obtained were spectrophotometrically analyzed at 350 nm for total drug content. PVA solutions, at concentrations corresponding to those present in the solubilization studies of conjugated 4-HPR, were used as blanks to avoid any interference on the analytical determination of the drug.

Dynamic Light Scattering (DLS) Measurements. The DLS measurements were performed in aqueous solutions of the conjugates at different concentrations to evaluate their mean size and gain information on their suitability in pharmaceutical parenteral formulations. The solutions were prepared by dissolving the conjugates in water at concentrations ranging from 1 to 10 mg/mL. The measurements were performed by a Brookhaven 90-PLUS instrument equipped with a 50 mW He-Ne laser (532 nm) and thermoregulated at 37 °C. The scattering angle was fixed at 90°. Results were the combination of three 10-min runs for a total accumulation correlation function (ACF) time of 30 min. The mean size of the polymer aggregates in solution was provided by the average hydrodynamic radius. The results were volume-weighted.

Drug Release Studies from the Conjugates. In order to evaluate the ability of the conjugates to release the free 4-HPR responsible for the therapeutic effect, drug release was analyzed in phosphate buffered saline at pH 7.4 (PBS). The release studies were carried out by placing 1 mL of 4-HPR-PVA solution (5 mg/mL) in a releasing cell separated by a dialysis membrane (10,000 Da MW cut off; 3.80 cm^2 area) from a receiving compartment containing 3 mL of PBS and 1 mL of chloroform. The membrane allowed permeation only to the free drug, released from the conjugate, and not to the drug−polymer conjugate whose mean molecular weight exceeds the membrane cut off. The drug release from the polymer, due to the easy hydrolysis of the carbonate linkage in aqueous environment,[17] was followed by drug diffusion through the membrane and partition toward chloroform. The organic solvent behaved as an extractive phase for the free 4-HPR, assuring sink conditions throughout the experiment. The system was thermostated at 37 °C, and every hour, the organic phase was removed, evaporated, and the residue dissolved in methanol. The methanolic phase was analyzed for the determination of the free drug content by RP-HPLC. The HPLC assays were carried out by a Nova-Pak C18 (150 × 3.9 mm, 4 mm, Waters) column with a UV detector at 350 nm. The mobile phase was a mixture of water (20%) and acetonitrile (80%); the injecting volume was 20 μL; and the flow rate was 1.0 mL/min. The system was thermostated at 37 °C. In these chromatographic conditions, the retention time of 4-HPR was 11.00 min. These studies were performed in triplicate for each sample, and the S.D. was less than 5%.

Biological Studies. The adrenergic MYCN amplified neuroblastoma cell line HTLA-230 was used. Cells were maintained in DMEM growth medium containing 10% fetal bovine serum, 2 mM L-glutammine, and 100 ng/mL each penicillin and streptomycin (all from Sigma) at 37 °C in a humidified 95% air/5% CO_2 atmosphere. Experiments were performed during the logarithmic phase of cell growth. Cells were seeded in 12-well plates (Corning Incorporated, NY) (8×10^4 cells/well) as triplicates. After 72 h, the cells were untreated or treated with growth medium containing 2, 3, 4, or 5 μM free 4-HPR (previously dissolved in ethanol), 2, 3, 4, or 5 μM conjugated 4-HPR (dissolved in PBS), or an excess (25 μM) of the pure polymer. The effects of 4-HPR and 4-HPR-PVA on cell growth and death were determined by cell count and the trypan blue dye exclusion method. Cells maintained in medium alone and treated with pure polymer were used as controls.

The results were statistically evaluated and analyzed using the two-tailed unpaired *t* test with Welch's correction, with confidence intervals set at 99%.

In Vivo Therapeutic Experiments. In order to mimic the clinical features of metastatic NB, we injected CD1 *nude/nude* mice with human HTLA-230 (4×10^6 cells/mouse, 10 mice per group) as described.[18] The mice were then randomly assigned to the control or treatment groups. Twenty-fout hours after the tumor cell inoculation, the animals were treated with conjugated 4-HPR (13.5 mg/Kg), pure polymer (1928 mg/Kg), or vehicle alone (PBS), given slowly through the tail vein in a volume of 200 μL. We could not intravenously inject a corresponding amount of free 4-HPR because of the excess of cosolvent needed for its solibilization. The treatment was repeated four times after 3 days. To determine treatment efficacy, the animals were monitored routinely for weight loss and general behavior, and survival time was used as the main criterion. The statistical significance of differential survival between experimental groups of mice was determined by Kaplan−Meier curves and log-rank (Peto) test by the use of StatDirect statistical software (CamCode, Ashwell, UK).

Results and Discussion

Characterization of 4-HPR-PVA Conjugates. Capillary electrophoretic analysis on the studied polymers revealed that the conjugation degree in the final products was 0.21%, 0.52%, 0.71%, 0.70% (4-HPR/PVA weight ratio) starting from 0.5%, 1%, 2%, and 4% (4-HPR/PVA weight ratio), respectively, in the preparative mixture.

By ^{1}H NMR analysis, the substitution degree was obtained comparing the integral of the peak at 7.40 δ assigned to the aromatic protons of 4-HPR with the integral of the peak at 1.95 δ assigned to the methyl protons ($COCH_3$) of the acetyl moiety present at 20% in the PVA backbone. The substitution degree calculated from the ^{1}H NMR spectra was found to be 0.23%, 0.48%, 0.69%, and 0.70% starting from 0.5%, 1%, 2%, and 4% (4-HPR/PVA weight ratio), respectively, in the preparative mixture.

These data indicated that the conjugation degree of 4-HPR to PVA reaches a maximum value of about 0.70% when the 4-HPR/PVA weight ratio is 2% in the preparative mixture. The additional increase in the weight ratio does not further raises the 4-HPR conjugation to PVA in the present experimental conditions. This may be due to the progressive steric hindrance provided by the increased presence of 4-HPR molecules in the PVA backbone that hinder, beyond 0.70%, any further conjugation of the drug to the polymer. On the basis of these data, the substitution degree of the conjugates may be considered a mean of the values obtained by both CE and ^{1}H NMR analysis: 0.22%, 0.50%, and 0.70%.

The conjugates will therefore be referred to throughout the text as 4-HPR-PVA $_{0.22}$, 4-HPR-PVA $_{0.50}$, and 4-HPR-PVA $_{0.70.}$

4-HPR Aqueous Solubility. Conjugation strongly increased 4-HPR aqueous solubility to 271.98 ± 2.36 μg/mL (4-HPR-PVA$_{0.22}$), 294.13 ± 2.14 μg/mL (4-HPR-PVA$_{0.50}$), and 343.16 ± 3.96 μg/mL (4-HPR-PVA$_{0.70}$) with respect to the pure drug (1.71 ± 0.08 μg/mL).[15] This is due to the presence of PVA whose hydration holds 4-HPR solubilization in aqueous medium. The solubility of 4-HPR did not greatly change in the different 4-HPR-PVA conjugates. The increase in conjugation degree generates two opposite trends: it decreases the solubility of 4-HPR-PVA, thus decreasing the number of macromolecules in solution but at the same time allowing each solubilized macromolecule to hold more 4-HPR molecules in solution. As a result, the aqueous solubility of 4-HPR is almost the same in the different conjugates. In the case of conjugates characterized by the highest conjugation degree, 4-HPR solubilization may be considered more efficient as supported by less solubilizing PVA molecules. On the basis of these considerations, 4-HPR-PVA$_{0.70}$ may be regarded as the conjugate characterized by the highest solubilizing efficiency toward 4-HPR among the analyzed systems.

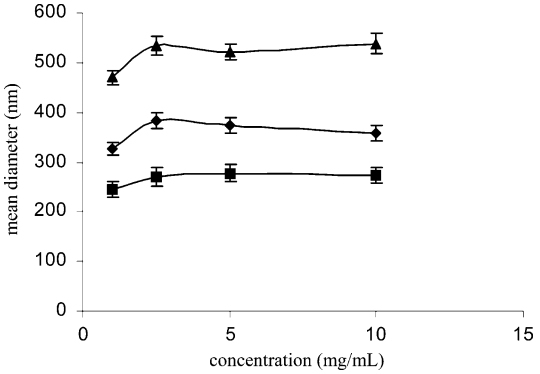

Figure 2. Mean diameter (nm) of the conjugates formed at 37 °C in PBS by dissolution at different concentrations of 4-HPR-PVA$_{0.22}$ (■), 4-HPR-PVA$_{0.50}$ (♦), and 4-HPR-PVA$_{0.70}$ (▲). The results are presented with standard error of the mean.

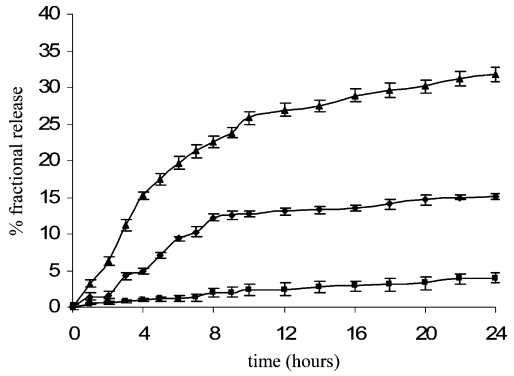

Figure 3. Fractional release (%) of 4-HPR from 4-HPR-PVA$_{0.22}$ (■), 4-HPR-PVA$_{0.50}$ (♦), and 4-HPR-PVA$_{0.70}$ (▲) (±SEM).

DLS Studies. The DLS examination of the aqueous solutions of the conjugates revealed the presence of nanosized structures whose size was almost unaffected by concentration (Figure 2). The increase in conjugation degree slightly raised the size of the aggregates because of the presence of increasing 4-HPR hydrophobic molecules in the self-assembled structures. In each case, low polydispersion (min 0.350 ± 0.013, max 0.580 ± 0.032) was observed for the different systems, and the mean size of the aggregates never exceeded 538 nm, suggesting their suitability for parenteral administration and the possibility of their accumulation in the solid tumors following extravasation.[11]

Release of the Free Drug from the Conjugates. The release of the free drug from the conjugates has been evaluated by the fractional release obtained by comparing the free drug concentration in the receiving phase to the total drug concentration (free and conjugated) solubilized in the releasing phase. The release rate of 4-HPR from the 4-HPR-PVA conjugates increases with increasing the conjugation degree (Figure 3). This may be explained according to the different steps characterizing drug release from the conjugates: the hydrolysis of the drug−polymer bond and drug diffusion through the hydrated polymer toward the aqueous bulk. While the facile hydrolysis of the 4-HPR-PVA carbonate bond is not expected to be appreciably influenced by the degree of conjugation, the subsequent drug diffusion through the hydrated polymeric structure is favored in the presence of the lowest amount of polymer per drug

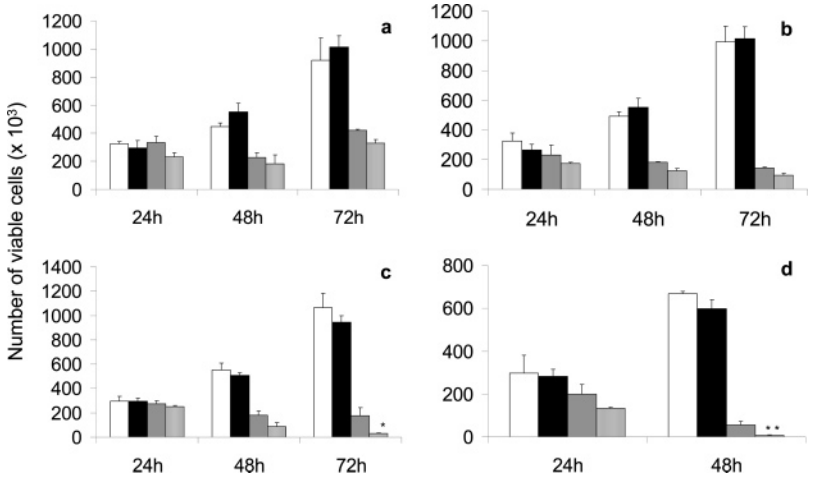

Figure 4. Time- and dose-dependent inhibitory effects of free and conjugated 4-HPR at 2 (panel a), 3 (panel b), 4 (panel c), and 5 μM (panel d) and pure polymer (an excess, 25 μM) on human neuroblastoma HTLA-230 cells. White columns, control; black columns, pure polymer; gray columns, free 4-HPR; hatched columns, conjugated 4-HPR. *$p < 0.001$ and **$p < 0.01$ vs free 4-HPR.

diffusing molecule, as in the presence of the highest degree of conjugation. In addition to the ability to provide the free drug in anaqueous environment, an ideal drug−polymer conjugate, suitable for parenteral administration of antitumor drugs, should also delay drug release in the average time period (6 h) of circulation before accumulating in the solid tumor or its metastases. In this period, the stability of the drug carrier toward release is very important in order to prevent the uncontrolled distribution of the drug in the body and thus cause unforeseen toxicity. The analyzed 4-HPR-PVA conjugates match these features, providing releases not exceeding 20% in the first 6 h and almost constant release rates in the subsequent period (Figure 3).

Inhibition of Neuroblastoma Cell Proliferation. Cell proliferation was inhibited in a time- and dose-dependent manner by free and 4-HPR-$PVA_{0.70}$. The growth trend, obtained by counting viable cells, is depicted in Figure 4. 4-HPR-$PVA_{0.70}$ enhanced the monolayer growth inhibition of free 4-HPR in HTLA-230 cells. The higher activity of 4-HPR-$PVA_{0.70}$ compared to that of 4-HPR may be attributed to the improved aqueous solubilization of the conjugated and the consequent improved availability of the drug hydrolyzed from the polymer thus providing a continuous supply of molecularly dispersed (solubilized) drug to the cells. The free 4-HPR, in contrast, is present in solution in molecular aggregates generated after the dilution of the ethanolic solution in the aqueous medium.[19] The low dissolution rate of the molecular aggregates decreases the aqueous concentration of the solubilized drug and thus its bioavailability and also decreasing its antitumor activity.

***In Vivo* Anti-Tumor Activity of 4-HPR-$PVA_{0.70}$.** Three times a week, mice were monitored for their body weight, general physical, and performance status, as well as for externally visible tumor mass or ascite formation. Animals were sacrificed whenever excessive (>25%) weight loss, huge tumor growth, massive ascites or impairment of motor functions due to spinal cord compression took place. Figure 5 shows the survival profile of treated versus control mice engrafted with HTLA-230. A highly significant ($p = 0.0017$, log-rank test Peto) increase in mean survival time could be observed in mice that received 4-HPR-$PVA_{0.70}$. Moreover, while control mice underwent rapid and extensive metastatic tumor growth, mainly involving the adrenal gland, kidney, ovary, liver, spleen, and bone marrow, in treated mice these events took place more slowly and in a less extensive amount. These results may be explained by the high peak and possibly steady-state level of conjugated 4-HPR injected (13.5 mg/kg per shot).

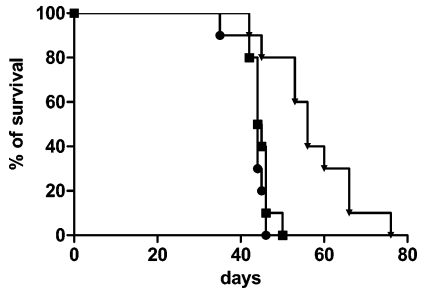

Figure 5. Survival of human and murine neuroblastoma-bearing CD1 *nude/nude* mice after 4-HPR-$PVA_{0.70}$ treatment. CD1 *nude/nude* mice were injected i.v. with 4×10^6 human NB HTLA-230 cells/mouse (10 mice per group). After tumor cell inoculation, the animals received five injections of conjugated 4-HPR (13.5 mg/Kg, i.v. in sterile saline solution (▼)) and pure polymer (1928 mg/Kg, i.v. in sterile saline solution (■)), while control mice received the same volume (200 μL) of vehicle alone (PBS (●)). The experiments have been repeated three times with similar results. The value of p is <0.002.

We are presently devising different treatment dosage and schedules in order to find out whether different regimens could further enhance the therapeutic potential of the conjugate HPR-$PVA_{0.70}$.

Conclusions

4-HPR covalently linked to polyvinylalcohol through a carbonate bond provides conjugates, increasing the drug aqueous solubility up to 200 times with respect to the free drug. This allows preparation of 4-HPR parenteral formulations not yet available on the market because of the strong hydrophobicity of 4-HPR. The improved 4-HPR aqueous solubilization has been proven to enhance drug cytotoxicity toward neuroblastoma cell lines with respect to the pure drug. The *in vivo* studies on nude mice injected with human neuroblastoma cells supported the enhanced activity observed *in vitro*, confirming that the achievement of adequate doses of 4-HPR for anti-cancer therapy relies on the ability of these conjugates to improve drug solubilization, providing the free drug in adequate time periods. The enhanced activity of 4-HPR by means of conjugation with PVA could provide new insights into the matter of the minimal residual desease treatment of neuroblastoma as well as other tumors responding to 4-HPR, where the persistence of hidden prolif-

erating or quiescent cells is often the cause of the relapsation of the disease.

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References and Notes

(1) Lotan, R. Retinoids and apoptosis: implications for cancer chemoprevention and therapy (editorial). *J. Natl. Cancer Inst.* **1995**, *87*, 1655−1657.
(2) Moon, R. C.; Metha, R. G.; Rao, K. J. Retinoids and Cancer in Experimental Animals. In *The Retinoids*; Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds.; Raven Press: New York, **1994**; pp 573−595.
(3) Niles, R. M. Recent advances in the use of vitamin A (retinoids) in the prevention and treatment of cancer. *Nutrition* **2000**, *16*, 1100−1101.
(4) Lo Piccolo, M. S.; Formelli, F.; Lulsch, R.; Ponzoni, M.; Montaldo, P. G.; De Bernardi, B.; Bellini, F. F.; Garaventa, A. Phase I study of fenretinide in neuroblastoma. *Am. Assoc. Cancer Res.* **2000**, PC22: 61.
(5) Ponthan, F.; Lindskog, M.; Karnehed, N.; Castro, J.; Kogner, P. Evaluation of anti-tumor effects of oral fenretinide (4-HPR) in rats with human neuroblastoma xenografts. *Oncol. Rep.* **2003**, *10*, 1587−1592.
(6) Ragazzi, M. B.; Icona, I.; Gervasutti, C.; Lazzarino, M.; Toma, S. Clinical pharmacokinetics of tretinoin. *Clin. Pharmacokinet.* **1997**, *32*, 382−402.
(7) Adamson, P. C.; Pilot, H. C.; Balis, F. M.; Rubin, J.; Murphy, R. F.; Poplack, D. G. Variability in the oral bioavailability of all-trans-retinoic acid. *J. Natl. Cancer Inst.* **1993**, *85*, 993−996.
(8) Reynolds, C. K.; Matthay, K. K.; Villablanca, J. G.; Maurer, B. J. Retinoid therapy of high-risk neuroblastoma. *Cancer Lett.* **2003**, *197*, 185−192.
(9) Duncan, R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discovery* **2004**, *2*, 347−360.
(10) Veronese, F. M.; Morpurgo, M. Bioconjugation in pharmaceutical chemistry. *Il Farmaco* **1999**, *54*, 497−516.
(11) Maeda, H.; Wu, J.; Sawa, T.; Matsamura, Y.; Hori, K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Controlled Release* **2000**, *65*, 271−284.
(12) Orienti, I. et al. Self-assembling poly(vinyl alcohol) derivatives, interactions with drugs and control of release. *J. Pharm. Sci.* **2001**, *90*, 1435−44.
(13) Orienti, I.; Zuccari, G.; Carosio, R.; Fini, A.; Rabesco, A. M.; Montaldo, P. G. Modified doxorubicin for improved encapsulation in PVA polymeric micelles. *Drug Delivery* **2005**, *12*, 15−20.
(14) Zuccari, G.; Carosio, R.; Fini, A.; Montaldo, P. G.; Orienti, I. Modified polyvinylalcohol for encapsulation of all-trans-retinoic acid in polymeric micelles. *J. Controlled Release* **2005**, *103*, 369−380.
(15) Orienti, I.; Zuccari, G.; Bergamante, V.; Mileo, E.; Lucarini, M.; Carosio, R.; Montaldo, P. G. Amphiphilic poly(vinyl alcohol) derivatives as complexing agents for fenretinide. *Biomacromolecules* **2006**, 7, 3157−3163.
(16) Aresta, M.; Dibenedetto, A.; Fracchiolla, E.; Giannoccaro, P.; Pastore, C.; Papai, I.; Schubert, G. Mechanism of formation of organic carbonates from aliphatic alcohols and carbon dioxide under mild conditions promoted by carbodiimides. DFT calculation and experimental study. *J. Org. Chem.* **2005**, *70*, 6177−6186.
(17) Binderup, E.; Bjorkling, F.; Hjarnaa, P. V.; Latini, S.; Baltzer, B.; Carlsen, M.; Binderup, L. EB1627: a soluble prodrug of the potent anticancer cyanoguanidine CHS828. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2491−2494.
(18) Raffaghello, L.; Pagnan, G.; Pastorino, F et al. In vitro and in vivo antitumor activity of liposomal fenretinide targeted to human neuroblastoma. *Int. J. Cancer* **2003**, *104*, 559−567.
(19) Orienti, I.; Zuccari, G.; Bergamante, V.; Carosio, R.; Montaldo, P. G. Enhancement of oleyl alcohol anti-tumour activity through complexation in polyvinylalcohol amphiphilic derivatives. *Drug Delivery* **2007**, *14*, 209−217.

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