In Vitro and in Vivo Study of Water-Soluble Prodrugs of Dexanabinol

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Abstract □ Trialkylammonium acetoxymethyl esters of dexanabinol were synthesized and evaluated as water-soluble prodrugs. Syntheses were performed by conventional methods; solubility in water and stability in buffers and human plasma were determined by HPLC, and in vivo tissue distribution studies were performed in a rat model. Most of the new derivatives were soluble in water (~50 mg/mL). They were relatively stable in water, while rapidly hydrolyzed in human plasma. Distribution studies indicated that peak concentrations of drug both in blood (30 μg/mL) and brain (2 μg/mL) were rapidly (5 min) achieved after iv administration of a selected prodrug to rats. The blood concentration decreased faster than brain levels which were detectable even after 24 h. Some of the examined esters could be further developed as water soluble prodrugs of dexanabinol.

Introduction

Dexanabinol (HU-211), [(6a*S-trans*)-6,6-dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran-9-methanol] (**1**), a synthetic, nonpsychotropic cannabinoid,[1] is a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist[2] and an effective radical scavenger.[3] The compound is currently in clinical trials as a neuroprotective agent with potential use in the treatment of brain damage associated with stroke, head trauma, and cardiac arrest.[4−6]

An obstacle in the development of dexanabinol as a single-dose neuroprotective agent, considering the intravenous route as the best way of administration, is its very poor solubility in water, which makes formulation in aqueous compositions extremely difficult. The large dimethylheptyl side-chain and potential formation of stable, lipophilic molecular aggregates, such as dimers, mediated by strong hydrogen bonding[7] account for this behavior.

Cosolvent systems containing cremophor EL used in vivo and in phase I and II clinical trials are associated with allergic-type side effects and, accordingly, not unanimously accepted. Other technologies for solubilization of dexanabinol in aqueous compositions, including water-soluble prodrug approaches, have been investigated.

Various polar combinations or combinations bearing permanent charges were synthesized for dexanabinol as esters at either the allylic hydroxyl or phenolic functionalities. They included glycinate and N-substituted glycinates,[8,9] esters of amino acids containing tertiary or quaternary heterocyclic nitrogen,[10] and hemiesters of dicarboxylic acids[8] and phosphates;[11] more than 30 combinations were used in a preliminary screening process in which solubility in water and stability in water and plasma (rat and human) were determined. The results demonstrated that only trialkylammonium glycinate salts possessed properties required by prodrugs: solubility and stability in water and rapid hydrolysis in human plasma.

The in-depth investigation of trialkylammonium moiety containing glycinates, targeting identification of a prodrug of practical use for dexanabinol, has been considered in this work. Syntheses, solubility, and stability determinations, in vivo tissue distribution in a rat model and preliminary formulation experiments of a selected prodrug, are presented herein.[12]

Materials and Methods

Synthesis—Conventional procedures were used for the synthesis of the novel derivatives. Melting points are uncorrected and were determined on an Electro-thermal melting point apparatus (Fisher Scientific). Elemental microcombustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectra (NMR) were recorded on a Varian XL-300 spectrometer. Samples were dissolved in an appropriated deuterated solvent, and chemical shifts were reported as parts per million (δ) relative to tetramethylsilane (0.00) which served as an internal standard. Coupling constants (*J*) are reported in hertz. The progress of various reactions were followed by thin-layer chromatography (TLC). TLC was performed on EM Reagents DC aluminum foil plates coated to a thickness of 0.2 mm with silica gel (60 mesh). All solvents and chemicals were of reagent grade. Dexanabinol was synthesized in-house.

(6a*S-trans*)-9-(Chloroacetoxymethyl)-6,6-dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran—A solution of dexanabinol (4.93 g, 12.7 mmol) and chloroacetic anhydride (2.05 g, 12.0 mmol) in chloroform (20 mL) was stored under argon in a dark room at 22 °C for 4 days. The reaction mixture was poured onto crushed ice (100 g), neutralized with 5% aqueous $NaHCO_3$, and extracted with chloroform (200 mL). The extract was washed with 5% aqueous $NaHCO_3$ (100 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the residue was purified by column chromatography (silica gel, hexanes:diethyl ether, 9:11) to give 4.73 g (80%) of the product as a sticky oil of 98% purity (HPLC). Anal. Calcd for $C_{27}H_{39}ClO_4$: C, 70.03; H, 8.49; Cl, 7.66. Found: C, 70.28, H, 8.56; Cl, 7.46.

[(6a*S-trans*)-6,6-Dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-9-(trimethylammonioacetoxymethyl)-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran] Chloride (**2**)—Anhydrous trimethylamine (0.65 g, 11.0 mmol) was added via a needle to a solution of (chloroacetyl)dexanabinol (1.90 g, 4.1 mmol) in anhydrous toluene (30 mL) and stirred under argon in a flask with a septum at 40 °C for 3 days. The precipitate was filtered, washed with toluene (20 mL), and dried in a vacuum oven (0.5 Torr, 60 °C, 4 h) to give

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2.05 g (96%) of product of 98% purity (HPLC); mp 212 °C. Anal. Calcd for $C_{30}H_{48}ClNO_4$: C, 69.01; H, 9.27, Cl, 6.79; N, 2.68. Found: C, 68.93; H, 9.26; Cl, 6.82; N, 2.69.

(6a*S-trans*)-9-(Bromoacetoxymethyl)-6,6-dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran—A modified previously described procedure[9] was used, starting from dexanabinol (3.40 g, 8.8 mmol) and bromoacetic anhydride (3.43 g, 13.2 mmol) in anhydrous toluene (20 mL). After stirring 20 h at 22 °C, ethyl ether (20 mL) and 5% aqueous $NaHCO_3$ were added, and the mixture was stirred vigorously for 30 min. The organic phase was dried on $MgSO_4$, the solvent evaporated, and the resulting oil dried in a vacuum oven (0.5 Torr, 80 °C, 6 h). No column chromatography was required, the purity of compound (4.08 g, 91%) being 98% (HPLC peak area). Anal. Calcd for $C_{27}H_{39}BrO_4$: C, 63.90; H, 7.75; Br, 15.74. Found: C, 63.79; H, 7.80; Br, 15.68.

[(6a*S-trans*)-6,6-Dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-9-(trimethylammonioacetoxymethyl)-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran] Bromide (**3**) (reported earlier[9])—A solution of (bromoacetyl)dexanabinol (1.10 g, 2.2 mmol) and anhydrous trimethylamine (0.25 g, 4.3 mmol) in hexanes (20 mL) was kept at − 5 °C for 3 days. The resulting precipitate was filtered, rinsed with hexanes, and dried in a vacuum oven (0.5 Torr, 70 °C, 4 h) to give 1.10 g (90%) of product with 98.6% purity (HPLC); mp 215 °C. Anal. Calcd for $C_{30}H_{48}BrNO_4$: C, 63.59; H, 8.54; N,2.47; Br, 14.01. Found: C, 63.75; H, 8.62; N, 2.45; Br, 14.08.

[(6a*S-trans*)-6,6-Dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-9-(triethylammonioacetoxymethyl)-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran] Bromide (**4**) (reported earlier[9])—A solution of (bromoacetyl)dexanabinol (1.00 g, 1.97 mmol) and triethylamine (0.35 mL, 2.50 mmol) in hexanes (10 mL) was stored under argon in a dark room at 22 °C for 3 days. The deposited crystalline material was separated by decantation, washed with hexanes, and dissolved in hot tetrahydrofuran (1.7 mL). The solution was diluted with diethyl ether (10 mL), filtered through a sintered glass funnel, and stirred at 22 °C for 2 days. The obtained precipitate was filtered, washed with diethyl ether, and dried in a vacuum oven (0.5 Torr, 50 °C, 24 h) to give 404 mg (34%) of product of 98% purity (HLPC); mp 127 °C. Anal. Calcd for $C_{33}H_{54}BrNO_4$: C, 65.11; H, 8.94; Br, 13.13; N, 2.30. Found: C, 65.23; H, 8.80; Br, 13.42, N, 2.42.

[(6a*S-trans*)-6,6-Dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-9-(*N,N*-dimethyl-*N*-ethylammonioacetoxymethyl)-6a,7,10,-10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran] Bromide (**5**)—A solution of (bromoacetyl)dexanabinol (622 mg, 1.23 mmol) and *N,N*-dimethylethylamine (0.162 mL, 1.50 mmol) in anhydrous diethyl ether (10 mL) was stored under argon at − 5 °C for 4 days. The obtained precipitate was separated by filtration, rinsed with diethyl ether, and dried in a vacuum oven (0.5 Torr, 60 °C, 4 h) to give 0.69 g (97%) of product of 98.6 purity (HPLC); mp 134 °C. Anal. Calcd for $C_{31}H_{50}BrNO_4$: C, 64.12; H, 8.68; Br, 13.76; N, 2.41. Found: C, 63.87; H, 8.69; Br, 13.99; N, 2.37.

[(6a*S-trans*)-6,6-Dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-9-(*N*-methyl-*N,N*-diethylammonioacetoxymethyl)-6a,7,10,-10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran] Bromide (**6**)—A solution of (bromoacetyl)dexanabinol (622 mg, 1.23 mmol) and *N,N*-diethylmethylamine (0.182 mL, 1.50 mmol) in anhydrous ether (10 mL) was stored at 22 °C for 1 week and then at − 15 °C for an additional week. The resulting precipitate was filtered, rinsed with diethyl ether, and dried in a vacuum oven (0.5 Torr, 50 °C, 2 h) to give 424 mg (59%) product of 91.9% (HPLC) purity; mp 134 °C. Anal. Calcd for $C_{32}H_{52}BrNO_4$: C, 64.63; H, 8.81; Br, 13.44; N, 2.36. Found: C, 64.35; H, 8.81; Br, 13.58; N, 2.42.

[(6a*S-trans*)-6,6-Dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-9-(tripropylammonioacetoxymethyl)-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran] Bromide (**7**)—A solution of bromoacetyl dexanabinol (622 mg, 1.23 mmol) and tripropylamine (0.285 mL, 1.50 mmol) in diethyl ether (5 mL) was stored under argon at 22 °C for 10 days. The resulting mixture was triturated with an additional portion of diethyl ether (5 mL). The crystals were filtered, washed with diethyl ether (2 × 5 mL), and dried in a vacuum oven (0.5 Torr, 60 °C, 1 h) to give 720 mg (90%) product of 97.5% purity (HPLC); mp 158 °C. Anal. Calcd for $C_{36}H_{60}BrNO_4$: C, 66.44; H, 9.29; Br, 12.28; N, 2.15. Found: C, 66.52; H, 9.35; Br, 12.41; N, 2.30.

Solubility—The solubility of the novel derivatives in deionized water (pH 5.6−5.7; determined with a Ionalyzer7 model 501, Orion Research pH meter) was determined by preparation of saturated solutions at 21 °C (sonication for 60 min), filtration of the undissolved material, and determination of the concentration of the resulting solutions by HPLC. Calibration solutions of 0.1, 0.25, 0.50, and 1.00 mg/g concentrations were prepared. The four-point calibration had correlation of 0.99991. Average results of three determinations are reported.

Stability—The stability in aqueous buffers and human plasma of selected prodrugs was determined.

Sample Preparation and Incubation Conditions for Stability in Buffers—Stock solutions were prepared by dissolving prodrugs in water (pH 6.1) at concentrations of 50 mg/mL. Work solutions were prepared by diluting stock solutions with buffers of various pHs to final concentrations of 5 mg/mL. Samples were then incubated at 21 °C. Aqueous samples were analyzed by HPLC at various time points (pHs were determined each time both at the beginning and at the end point of determinations and proved to be unchanged). For HPLC determinations 100 μL of work solutions were diluted with 900 μL water to concentration of 0.5 mg/mL. Buffers used were: pH 7.4, Dulbeko's phosphate saline buffer (2.00 g/L KCl; 2.00 g/L KH_2PO_4; 8.00 g/L NaCl; 1.78 g/L Na_2HPO_4); pH 9, 0.05 M solution of tris(hydroxymethyl)aminomethane 5.47 g/L; pH 1.2 (simulated gastric fluid): 1 g of NaCl, 1.6 g of pepsin, 3.5 mL of HCl dissolved in 500 mL solution (according to USP XXII); pH 5.5: 0.1 N citric acid (6.4 g/L) (pH, 2.2) adjusted with 1 N NaOH; pH: 3.0 was prepared from 0.05 M K_2HPO_4 (pH 8.95) by adjusting pH with 0.1 N HCl. Aliquots were analyzed by HPLC, and concentrations of dexanabinol resulting from hydrolysis were determined. Hydrolysis followed first- order kinetics, and half-lives of compounds were calculated by plotting time versus the natural logarithm of peak area. Average values for three experiments were reported.

Sample Preparation and Incubation Conditions for Stability in Blood—Stock solutions were prepared at concentrations of 30 mg prodrug/mL in pH 7.4 buffer (0.05 M, $\mu = 0.15$). Work solutions were obtained by diluting stock solution with freshly collected blood to give 3 mg/mL or 200 μg/mL concentrations. Samples were incubated at 37 °C, and HPLC determinations were performed at various time points by extracting 100 μL samples with acetonitrile (900 μL for higher concentration and 400 μL for the lower concentration), followed by vortexing and immediate centrifugation and analysis.

Assay Method—High performance liquid chromatography (HPLC) (reversed-phase) was used for quantitative determinations. The HPLC consisted of a Spectra-Physics SP 8810 precision isocratic pump, Spectra-Physics SP 4290 system 2 integrator, Spectra-Physics SP 8880 autos ampler, Kratos Spectroflow 757 UV absorbance detector, and a Hewlett-Packard-HP3365, series II, version 3.33 Chemstation. The chromatographic conditions: HPLC columns, Alltima C8, 5 μm, 250 × 4.6 cm; mobile phase: acetonitrile: buffer (5 mL acetic acid and 5 mL triethylamine/L), 75:25 (v/v); flow rate: 1.0 mL/min; UV detection: 230 nm; volume of injection: 10 μL.

In Vivo Tissue Distribution Studies—The tissue distribution of a selected prodrug (**2**) was determined in a rat model. Male Sprague−Dawley rats (250−300 g body weight) were injected via tail vein with an aqueous (deionized water) solution of prodrug at 6.75 mg/kg body weight dose (250 g rats received ~200 μL of solution obtained by dissolving 25 mg prodrug in 3 mL water, the final pH being ~5). Animals were sacrificed by decapitation at 5, 15, 30, 60, 120, 240, 480, and 1440 min, and blood and brain tissues were collected. Three rats were used per time point. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised in 1985).

Sample Preparation—Plasma was obtained from trunk blood by centrifugation (1500 rpm). Brain was removed and rinsed with ice-cold saline. Samples were kept frozen until extraction. Acetonitrile (4 mL) was added to plasma (1 mL) followed by addition of the internal standard, a deuterated dexanabinol, synthesized in-house[13] (0.1 μg dexanabinol-d_5 in DMSO). Brain was weighed and homogenized in 2 mL of deionized water, and then acetonitrile (4 mL) was added, followed by the addition of the internal standard (0.5 μg/g brain). Multistep liquid−liquid extraction was then applied (both for plasma and brain homogenate). After the protein precipitation by acetonitrile, the supernatants were removed and concentrated to ~1 mL. A solution of NaOH (2 N, 2 mL) was added, followed by extraction with hexane/ethyl acetate (9/1, v/v) (4 mL). The organic phase was separated and washed with 0.1 N aqueous HCl solution (4 mL), and the solvent was evaporated under a

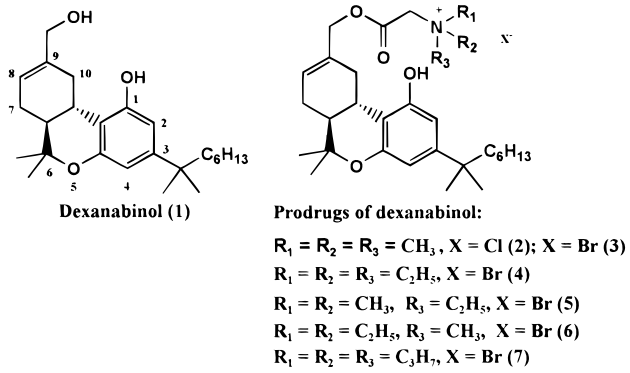

Figure 1—Structures of dexanabinol (**1**) and its prodrugs.

stream of nitrogen gas. Sample reconstitution was done in the HPLC mobile phase (100 μL). Average recovery for **1** was 55–60%.

Analytical Method—LC/MS methods were developed for determination of concentrations of **1** in plasma and brain. HPLC separation was done on a Supelco (Bellefonte, PA) LC–CN (cyanopropylsilica) 3.3 cm × 4.6 mm i.d. analytical column, protected with a Supelguard LC–CN (2 cm × 4.0 mm i.d.) cartridge. The mobile phase was 45% methanol and 55% aqueous acetic acid (1% v/v) at 1.0 mL/min flow rate. The solvent delivery system consisted of a Kratos (Manchester, UK) Spectroflow 400 isocratic pump. A Finnigan MAT (San Jose, CA) LCQ[7] ion-trap mass spectrometer with the manufacturer's APCI source was used. Injection volume was 5 μL. Retention time for analyte and internal standard was 2.70 min. Selected-ion monitoring (SIM) of the protonated molecules *m/z* 387.40 and 392.33 were used for the analyte and internal standard, respectively.

Quantitation was based on calibration by tissue spiked with known amount of analyte (0.1, 10, and 150 μg/mL plasma and 0.1, 1.0, and 5.0 μg/g brain) and the ratio of the area under the peak for the analyte ion (*m/z* 387.40, 1-amu window) to the area under the curve for the ion of the internal standard (*m/z* 392.33, 1-amu window) was used for regression to concentration (linear fitting, with quadratic weighing for concentration).

Results and Discussion

The investigated prodrugs are summarized in Figure 1. The synthetic procedures included acylation of dexanabinol (**1**) with chloroacetic or bromoacetic anhydride, followed by reaction of the resulting haloacetyl dexanabinol with appropriate tertiary amines. The allylic hydroxyl group is chemically more reactive than the sterically hindered and more rigid phenolic group. Accordingly, the acylation was quite specific, resulting in allylic esters without formation of any side products. The bromoacetate of dexanabinol reacted rapidly with trimethylamine, even at low temperature (−5 °C), affording high purity product with good yield. Consecutive substitution of methyl groups with ethyl groups led to slower reactions, lower yields, and products with lower melting points; the isolation of triethylammonium derivative, which proved to be quite lipophilic and soluble in common organic solvents, was difficult. However, this trend was surprisingly reverted to some degree in the case of derivatives containing higher alkyl groups, such as the tripropylammonium derivative.

To determine the influence of the counterion on the solubility of the quaternary derivatives, the chloride of the trimethylammonium acetate was also synthesized.

Attempts to use a similar procedure for the synthesis of trialkylammonium propionate derivatives of dexanabinol failed, because the reaction of bromopropionyl ester with tertiary amines gave predominantly dehydrobromination (elimination of hydrogen bromide) with formation of the acrylic ester, rather than the quaternization. While the dimethylamino derivative could be obtained from acrylate, by reaction with dimethylamine, its quaternization with methyl iodide was not possible since the resulting trimethylammonium derivative easily eliminated hydrogen iodide with formation of the acrylate.[14]

Table 1—Stability (half-lives) of Prodrugs in Buffers[a]

	half-lives ($t_{1/2}$) (days, mean ± SEM) at various pH			
prodrug	1.2	3.0	5.5	7.4
2	114.0 ± 4	52.00 ± 5	26.11 ± 1	14.64 ± 0.5
3	NA	NA	11.96 ± 0.8	3.64 ± 0.4
4	140.3 ± 5	51.30 ± 0.6	27.68 ± 0.9	11.94 ± 0.6
5	NA	NA	14.80 ± 1	10.30 ± 0.8
6	NA	NA	19.90 ± 0.7	10.45 ± 0.9

[a] SEM = standard error means. NA = data not available.

Prodrugs of practical use should have adequate solubility and sufficient stability in water to allow for formulation and storage. On the other hand, they should rapidly convert to the active parent drug within the body, particularly in blood since the administration is performed intravenously.

Solubility studies indicated that all of the investigated prodrugs were soluble in water. The determined solubilities were around 50 mg/mL (53.4, 52.2, 47.5, 47.0 mg/mL for **3**, **2**, **6**, **4**, respectively) except for compound **5** which proved to be less soluble (5 mg/mL) (no data are available for **7**). This spectacular increase in the solubility of the esters, as compared to dexanabinol which is insoluble in water, is a result of two factors: (1) the ionic character of the molecule induced by the permanently charged ammonium moiety, and (2) the prohibition of formation of strong dimers by double hydrogen bonding. It is difficult to explain some anomalies in solubilities in the examined series. While derivatives **2** and **3** were expected to have the best solubility due to smaller, less lipophilic alkyl groups linked to the quaternary nitrogen, the much lower solubility of **5** compared to **4** and **6** is unusual. The counterion, Cl^- or Br^- in **2** and **3**, respectively, does not have much influence, the two otherwise similar esters having almost the same solubility in water.

The esters were relatively stable in aqueous buffers. In each case, the stability was higher at the pH of distilled water (5.5), as compared to physiological pH (7.4) (Table 1).

Stability of selected prodrugs was determined at other pHs as well. At basic pH (9.0), the tested prodrugs were rapidly hydrolyzed (half-lives could not be calculated due to the fast rate of hydrolysis), while at strongly acidic pH (such as at 1.2, the pH of the stomach fluid) their stability increased considerably.

The results indicate that esters of **1** can be easily dissolved in water (buffered or unbuffered), but since the resulting solutions are not stable enough to allow for long time storage, they should be stored in the form of lyophilized powders and reconstituted a short time before their use.

Stability of **2** and **4** in human whole blood and plasma was determined at body temperature (37 °C). At concentration of 200 μg/mL, half-lives were 102 and 114 min (whole blood) and 47 and 90 min (plasma) for **2** and **4**, respectively. At lower concentration, which better reflects the real situation (doses used in clinical trials are in the range of ~100–300 mg dexanabinol/person), **2** hydrolyzed even faster, $t_{1/2}$ in plasma being 26.3 min at 50 μg/mL and very short at 20 μg/mL (70% of prodrug hydrolyzed at time point zero). This difference indicates saturation at higher concentration. As demonstrated during preliminary studies,[8,9] glycinate esters containing quaternary ammonium moieties are substrates for blood esterases and as a result are readily hydrolyzed. There are numerous esters containing

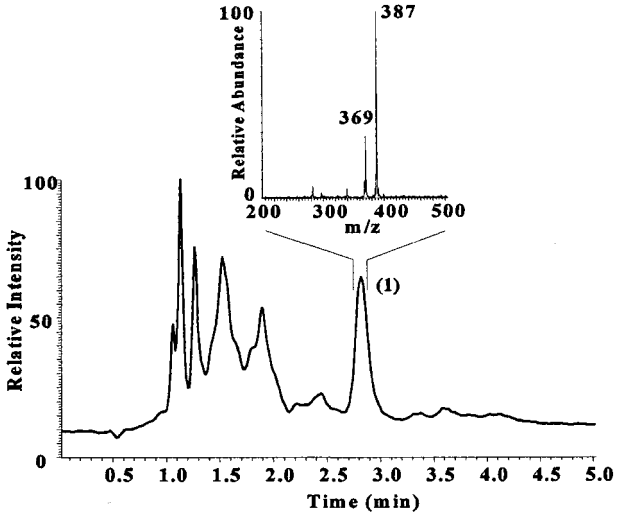

Figure 2—Representative LC-MS total-ion current (TIC) chromatogram of an extract of plasma spiked with 1.5 μg/mL dexanabinol (**1**). Inset: atmospheric pressure chemical ionization (APCI) mass spectrum of the peak indicated.

quaternary ammonium groups which are good substrates for enzymes which possess an anionic site. That includes obviously acetylcholine which is hydrolyzed by acetylcholinesterase present in postsynaptic membranes and also in plasma. Cholinesterases from plasma (pseudocholinesterases) are less specific than those from brain and they, or related esterases, might have a role in the hydrolysis of dexanabinol esters. These results indicate that the quaternary ammonium moiety containing esters can be used as prodrugs since they rapidly release the active **1** after their intravenous administration.

In vivo tissue distribution studies were performed in a rodent model. A selected prodrug (**2**) was administered intravenously to groups of rats, animals were sacrificed at various time points, and blood and brain concentrations of **1** were determined. These studies also addressed analytical method development for LC-MS, which offered an improvement over the gas chromatographic separation developed for compounds with similar structures, where sample derivatization was needed.[15] The HPLC assay developed during this study has benefits over similar methods using alkyl silica bonded phases, because the endogenous lipids extracted from the tissue elute before the analyte upon using cyanopropylsilica bonded phase. The short (3.3-cm), 3-μm particle-size column afforded reduced analysis time (5 min), compared to the 15-cm or 25-cm (5 μm) columns employed previously. A representative total-ion current chromatogram and the APCI mass spectrum of **1** are shown in Figure 2.

The in vivo distribution results are presented in Table 2 and in Figure 3. In agreement with the in vitro experiments performed in rodent blood,[9] a rapid hydrolysis of the prodrug was noticed. At 5 min following the administration, plasma levels of ~30 μg/mL were obtained. At the same time point, brain concentrations of dexanabinol reached a peak concentration of 2 μg/g as well. As the blood concentration of dexanabinol decreased following a typical two-compartment pharmacokinetic model, brain concentrations also became lower. However, the brain/blood concentration ratio increased from 0.07% at 5 and 15 min to 0.13, 0.35, 0.65, 2.48, 2.68, and 3.90 at 30, 60, 120, 240, 480, and 1440 min, respectively. Apparently, the lipophilic compound was retained by the brain tissues, while eliminated from blood. Even after 24 h, detectable levels of dexanabinol were identified in brain. It is important that peak concentrations of drug can be obtained rapidly (5 min) after administration, since the faster the neuroprotectant agent reaches the

Table 2—Plasma and Brain Concentrations of Dexanabinol (1) after iv Administration of 6.75 mg/kg Prodrug (2, equivalent to 5 mg/kg of 1) to Male Sprague–Dawley Rats

	concentrations (average ± SEM)	
time (min)	plasma (μg/mL)[a]	brain (μg/g)
5	29.36 ± 5.86	2.06 ± 0.31
15	10.73 ± 1.19	0.75 ± 0.11
30	4.34 ± 2.54	0.57 ± 0.16
60	1.26 ± 0.47	0.44 ± 0.05
120	0.40 ± 0.12	0.26 ± 0.05
240	0.25 ± 0.07	0.37 ± 0.08
480	0.19 ± 0.09	0.51 ± 0.08
1440	0.12 ± 0.03	0.47 ± 0.16

[a] Area under the curve = 14.51 μg mL^{-1} h, clearance = 0.345 L kg^{-1} h^{-1}, mean residence time = 8.0 h. Two-compartment pharmacokinetic model[16] for plasma concentration of 1, $c = Ae^{-\alpha t} + Be^{-\beta t}$: A = 33.7 μg/mL, B = 0.392 μg/mL, α = 0.066 min^{-1}, β = 0.0011 min^{-1} (nonlinear curve fitting by Scientist for Windows, Version 2.0, MicroMath, Inc., Salt Lake City, UT). k_{el} = 0.0393 min^{-1}, k_{12} = 0.0259 min^{-1}, k_{21} = 0.0019 min^{-1}, volume of the central compartment = 0.147 L kg^{-1}; apparent volume, steady state = 2.201 L kg^{-1}.

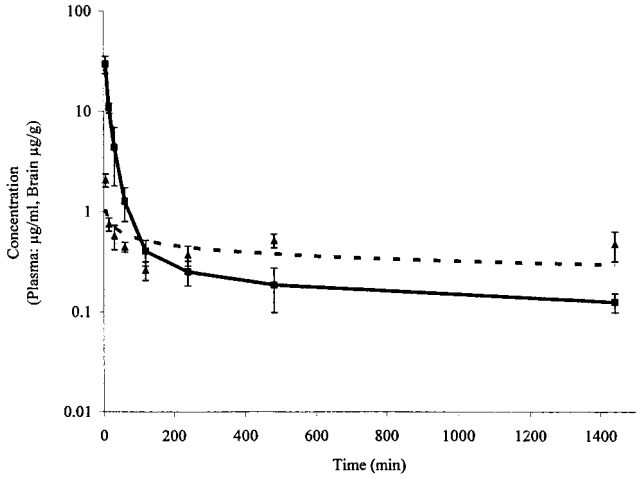

Figure 3—Concentration versus time profile of dexanabinol (**1**) in rat plasma (■, solid line) and brain (▲, dashed line) after iv administration of 6.75 mg/kg of prodrug **2**.

target, the more effective it is. It has to be mentioned that distribution results generated in rodent model might be different in humans, since esterases in mice and rats are much more active than in more evolved mammals. However, the in vitro experiments indicated that the selected combinations are good substrates for human blood esterases as well. It is then safe to affirm that the presented distribution study can be extrapolated to humans. Table 2 also contains some important pharmacokinetic parameters, calculated from the concentration−time profiles, including area under the curve, clearance, mean residence time, etc.

No control experiments, using **1** dissolved in a cosolvent system were performed. Such a study, in which **1** (5 mg/kg) was administered to rats in a cremophor/ethanol vehicle,[17] has been available for comparison and indicated a different tissue distribution profile. For example, the blood levels of dexanabinol were lower (maximum 4.5 μg/mL) and brain concentrations higher (maximum 10 μg/g) as compared to the results of the present study. The comparison of data might be misleading due to a variety of differences induced by solvents, including effects on the permeability of the blood−brain barrier.

Preliminary formulation studies were performed for selected prodrugs. Compounds **2** and **3** were dissolved in pure water (concentrations of ~50 mg/mL), and the resulting solutions were filtered through S&S Nylon-66 mem-

brane (45 μm pore size) and then freeze-dried in a Labconco freeze-dryer, model 77500, equipped with a Sargent-Welch vacuum pump, at a vacuum of 10 mmHg and temperature of − 50 °C for 6 h. The resulting powder was stored in dry conditions in dark, closed containers. The compound was analyzed by HPLC following the lyophilization and at a 1 month interval for 3 months; no degradation was registered, the purity being constantly 98%.

Conclusions

Several quaternary nitrogen-containing glycinate esters of dexanabinol were synthesized and investigated. Most of these combinations possess the required properties to be used as water-soluble prodrugs; they are soluble and fairly stable in water, but rapidly hydrolyze in human blood. In vivo distribution studies performed in a rat model indicated the brain uptake of **1**, when administered intravenously in the form of a prodrug, as a result of the rapid hydrolysis of the water-soluble ester. The prodrugs should be formulated as freeze-dried powders and reconstituted in water prior to their use. Prodrugs might present an advantageous alternative to the currently used formulations of dexanabinol.

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Supporting Information Available— ^{1}H and ^{13}C NMR data for novel derivatives 9-(chloroacetoxymethyl)dexanabinol, **2**, and **5**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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