Effect of a Chemical Delivery System for Dexamethasone (Dex-CDS) on Peritumoral Edema in an Experimental Brain Tumor Model

Tali Siegal,[1] Ferenc Soti,[2] Anat Biegon,[3] Emil Pop,[2] and Marcus E. Brewster[2,3,4]

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INTRODUCTION

Primary malignancies of the central nervous system (CNS) affects upwards of 24,000 individuals annually in the United States, accounting for approximately 2% of all cancer-related deaths in the adult population (1,2). Furthermore, primary brain tumors are second only to trauma as causes of death in children and is the second most common cancer type in the adolescent (3). The incidence of metastatic brain tumors is higher than that of primary CNS neoplasms and about 24% of patients dying of cancer will develop metastatic CNS lesions (2). The prognosis of individuals with malignant brain tumors is poor with untreated patients dying within 3–6 months of diagnosis and treated patients having a five year survival rate of less than 5% (3).

In addition to the tumor mass itself, a factor that significantly contributes to morbidity and mortality in brain neoplasms is the associated vasogenic edema (3,4). The etiology of the edema is related to increases in microvascular permeability resulting in extravasation of plasma proteins, salt and the accompanying plasma water. This increase in extracellular osmotic pressure leads to increases in the intracranial pressure (ICP) which may induce headaches, nausea and vomiting as well as seizures and focal neurologic deficits. If ICP is not controlled, lethargy, stupor, cerebral herniation and brain stem compression, may follow (1,3).

The vasogenic edema of brain tumors is attenuated by treatment with antiinflammatory steroids such as dexamethasone (5,6). High dose dexamethasone may improve or resolve signs and symptoms related to increased ICP within 12 to 48 h of drug administration. Unfortunately, several factors reduce the utility of dexamethasone therapy including limited CNS uptake but particularly the systemic adverse effects (7). These untoward effects include adrenal atrophy, hepatomegaly, hepatocellular necrosis, myopathy, cushingoid features, duodenal ulcers and immunosupression. Both the pharmacokinetic limitations and toxicological adverse effects may be addressed by the targeting and sequestration of the steroid into the brain. One approach to achieve this goal is the use of a redox-based chemical drug delivery system (CDS) (8,9). A CDS for dexamethasone (Dex-CDS) has been prepared (i.e., the 21-(1-methyl-1,4-dihydronicotinate) ester) and evaluated previously (7,10). These studies suggested that the Dex-CDS provoked more potent and sustained glucocorticoid-related action than did the parent compound using inhibition of stress-induced ACTH release as an end point. The Dex-CDS was also found to have a more beneficial pharmacokinetic profile (7). In the current communication, we evaluate the utility of the Dex-CDS in the treatment of experimental peritumoral edema using a rat brain tumor model.

MATERIALS AND METHODS

Chemistry

Dex-CDS(dexamethasone 21-dihydrotrigonellinate or 9α-fluoro-11,17-dihydroxy-21-[{(1-methyl-1,4-dihydropyridin-3-yl)carbonyl}oxy]-16-methylpregn-1,4-dien-3,20-dione) was prepared based on a modification of a previously reported procedure (7). Dexamethasone was treated with nicotinic anhydride in pyridine to give dexamethasone 21-nicotinate which was then alkylated with methyl iodide giving rise to the 21-(1-methylnicotinate) salt (Dex-Q+). The reduction of the Dex-Q+ to the Dex-CDS was accomplished using a basic aqueous solution of sodium dithionite containing a co-solvent. Optimal yields and purity were obtained using a 50% aqueous N-methylpyrrolidinone solution for the reduction, five equivalents of $NaHCO_3$ and three equivalents of $Na_2S_2O_4$. The obtained crude material was recrystallized from methanol yielding pure (97.5%) 1,4-dihydronicotinate as indicated by HPLC. The main contaminant was the 1,6-isomer (1.9%) meaning the total dihydronicotinate content was 99.4%.

An aqueous vehicle suitable for in vivo use was prepared for Dex-CDS. One g of Dex-CDS was suspended in 66 mL of a borate buffer (0.01 M) containing 5% w/v glucose and 45% w/v 2-hydroxypropyl-β-cyclodextrin (HPβCD) (11). The system was cooled in an ice bath, sparged with nitrogen and stirred for 3 h. The suspension was then filtered and the filtrate frozen in liquid nitrogen and lyophilized. The resulting powder was milled through a 60 mesh sieve generating 25 g of the Dex-CDS·HPβCD complex. Analysis of the powder by UV spectrophotometry indicated an incorporation of 25.8 mg Dex-CDS/g total powder. The analysis was completed using a HP 8451A diode array spectrophotometer. Plots of Dex-CDS concentration versus UV absorbance were linear ($r > 0.9999$) over the concentration range of interest. For administration to animals, 388 mg of the lyophilized Dex-CDS·HPβCD powder were reconstituted to 1.0 mL with water to generate a 10 mg/mL Dex-CDS solution which was further diluted as necessary for administration to animals. An equimolar system containing Dex in HPβCD was also prepared.

Animal Studies

Groups (n = 4–14) of female Fischer rats (BW = 180–200 g) were anesthetized (30 mg/kg pentobarbital i.p.) and inoculated with an unselected rat tumor line of a methylcholantrene-

[1] Neuro-Oncology Clinic, Hadassah Hebrew University Hospital, P.O. Box 12000, Jerusalem 91120, Israel.
[2] Pharmos Corp, Two Innovation Dr., Alachua, Florida 32615.
[3] Pharmos, Ltd., Rehovot 76326, Israel.
[4] To whom correspondence should be addressed.

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induced malignant fibrous histiocytoma. Tumor cell suspensions (10^5 in 2 μL RPMI medium) were stereotactically inoculated into the right cerebral hemisphere (P = 3.5, L = 2, H = −4 mm, relative to the bregma as a zero point) over a 10 min time course (12). These coordinates target the tumor cells to sub-cortical white matter. Sham-treated animals received an intracranial injection of the same volume of medium. Ten days after tumor induction, the animals manifested weight loss, apathy and scissoring of the hindlimbs in response to tilting. At this point, treatment with either dexamethasone (Dex), Dex-CDS or vehicle was initiated. Three paradigms were followed. In the first, vehicle (n = 10), Dex (n = 14) or Dex-CDS (n = 10) were administered i.v. at time 0, 8 h and 24 h at doses equimolar to 2.0 mg/kg Dex. At 30 h (i.e., 6 h after the last injection), a solution of Evans Blue dye (2%) in saline was administered (13). Animals were sacrificed by pentobarbital overdose and the rats were subjected to transcardiac perfusion (150 mL 0.9% NaCl at 100 mm Hg) to clear the dye from the circulation. The brains were then removed and the hemispheres sliced into 3 mm thick coronal sections. The tumor mass (which was easily detected by the naked eye and well delineated from adjacent tissue) and one to 2 mm regions adjacent to the tumor were separated with the aid of a dissecting microscope, weighed and treated with two volumes of dimethylformamide (DMF). The tissue was homogenized by sonication, incubated for 24 hours at 50°C and centrifuged at 12,000 g for 20 min. The extracted dye was quantitated by UV spectrophotometry at the λ_{max} for the dye (635 nm). The amount of dye per gram brain weight was determined using a Beer's Law plot of Evans Blue concentration versus absorbance. In the second study, vehicle (n = 4) or a single i.v. dose of either Dex (n = 4) or Dex-CDS (n = 4) (equimolar to 2.0 mg/kg Dex) was administered to tumor-bearing rats and 30 h after drug administration animals were prepared as above and Evans Blue extravasation determined. In the third set of studies, vehicle (n = 4), a single i.v. dose of Dex-CDS (n = 4) (equimolar to 2.0 mg/kg Dex) or three i.v. injections of Dex (n = 4) (all at 2.0 mg/kg) at time 0, 8 h and 24 h were administered. At 30 h after initiation of treatment, animals were sacrificed and extravasation of Evans Blue into the tumor and the areas around the tumor was assayed. Differences among groups and brain regions were assessed by analysis of variance (ANOVA) and a post-hoc Student-Neuman-Keul's test. A $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Dex-CDS was prepared by sequential conversion of Dex to its 21-nicotinate, 21-(1-methylnicotinate) iodide (Dex-Q+) and finally the Dex-CDS (21-(1-methyl-1,4-dihydronicotinate)) (Figure 1) (7). The synthetic yields and purity of the Dex-CDS were significantly improved over previous methods by manipulation of the solvent system. While both methanol-water and ethanol-water gave rise to considerable hydrolysis of the Dex-Q+ ester, use of a 50% aqueous N-methylpyrrolidinone solution generated high yield of the dihydronicotinate with regioseletivity for the 1,4-dihydropyridine (i.e., the ratio of 1,4-dihydronicotinate:1,6-dihydronicotinate was 98:2).

The Dex-CDS was prepared to increase its membrane permeability and, therefore, it was not unexpected that the compound manifested poor water solubility. In order to generate aqueous vehicles for the Dex-CDS, 2-hydroxypropyl-β-cyclo-

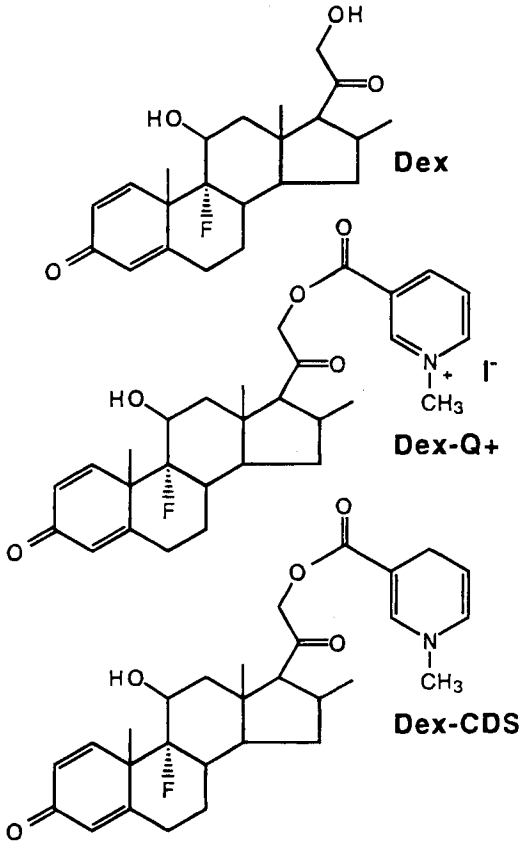

Fig. 1. Structure of Dexamethasone (Dex), Dexamethasone 21-(1-methynicotinate) Iodide (Dex-Q+) and Dexamethasone 21-(1-methyl-1,4-dihydronictonate) (Dex-CDS).

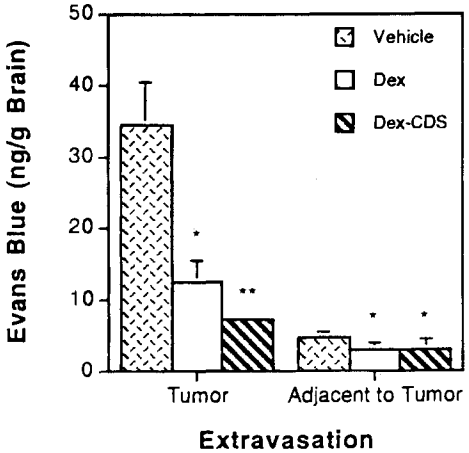

Fig. 2. Blood-Brain Barrier Integrity as Measured by Evans Blue Extravasation at an Implanted Tumor or in Areas Surrounding the Tumor after Treatment with either Vehicle, Dex or Dex-CDS. The drugs dose was equimolar to 2.0 mg/kg Dex × 3 injections at 0, 8 h and 24 h. Extravasation measurements were made at 30 h. * Indicates a significant difference ($p < 0.05$) relative to vehicle while ** indicates a significant difference relative to both vehicle and Dex treatment.

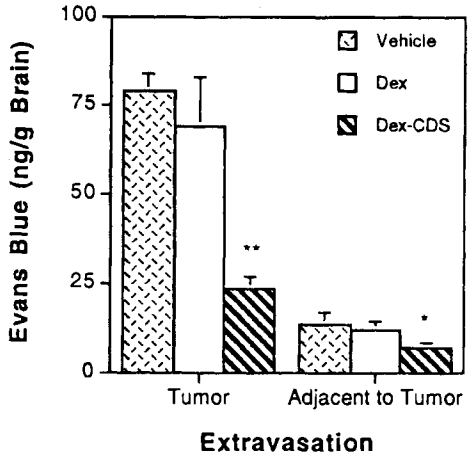

Fig. 3. Blood-Brain Barrier Integrity as Measured by Evans Blue Extravasation at an Implanted Tumor or in Areas Surrounding the Tumor after Treatment with either Vehicle, Dex or Dex-CDS. The drugs dose was equimolar to 2.0 mg/kg Dex × 1 injections at time 0. Extravasation measurements were made at 30 h. * Indicates a significant difference ($p < 0.05$) relative to vehicle while ** indicates a significant difference relative to both vehicle and Dex treatment.

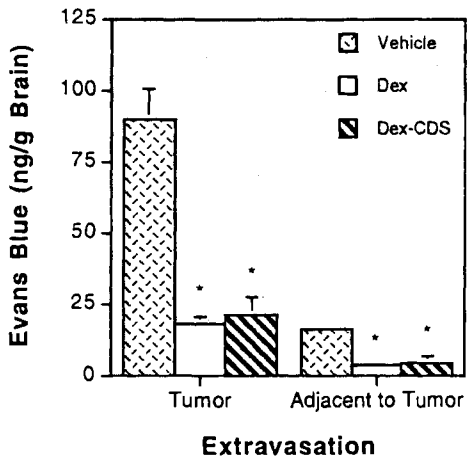

Fig. 4. Blood-Brain Barrier Integrity as Measured by Evans Blue Extravasation at an Implanted Tumor or in Areas Surrounding the Tumor after Treatment with either Vehicle, Dex or Dex-CDS. Dex was given at a dose of 2.0 mg/kg × 3 injections at 0, 8h and 24 h while Dex-CDS was given once at time 0 at a dose of 2.6 mg/kg (equimolar to 2.0 mg/kg Dex). Extravasation measurements were made at 30 h. * Indicates a significant difference ($p < 0.05$) relative to vehicle.

dextrin (HPβCD) was considered (11,14). This derivative effects solubilization of lipophiles through dynamic complex formation and, unlike the parent β-cyclodextrin, is highly water soluble. HPβCD also has a benign parenteral toxicological profile and has been extensively evaluated in humans (11). HPβCD increased the aqueous solubility of Dex-CDS as a linear function of cyclodextrin concentration generating a phase-solubility profile qualitatively similar to that seen with Dex and HPβCD (11). A 38.8% HPβCD solution of Dex-CDS was found to contain 10 mg/mL of the steroid.

Various pharmacological effects of Dex-CDS in the aqueous HPβCD formulation were analyzed using a rat brain tumor model which induces a breakdown of the blood-brain barrier (BBB). The BBB disruption was quantitated by Evans Blue dye extravasation (12,13). The animal studies were designed to assay both the potency and pharmacokinetic behavior of Dex-CDS relative to Dex. In the first study, vehicle, Dex or Dex-CDS (at doses equimolar to 2.0 mg/kg Dex) were administered using a dosing schedule found to be optimal for Dex (i.e., three doses given at 0, 8 and 24 h). As illustrated in Figure 2, Dex-CDS was highly effective in reducing vascular permeability both at the tumor and in areas around the tumor and was more potent than Dex in reducing the extravasation at the level of the tumor. Treatment with Dex reduced Evans Blue extravasation by 67% while Dex-CDS gave rise to an attenuated extravasation of 80% within the tumor mass. In the area around the tumor, equimolar doses of Dex-CDS or Dex decreased extravasation by 37.2 and 37.9%, respectively. To assay pharmacokinetic performance, Dex-CDS was compared to Dex after a single dose at time 0, with dye extravasation assayed after 30 h. As shown in Figure 3, such a single injection of Dex (2.0 mg/kg) had a weak but not significant effect on the increased permeability measured either in the tumor mass or in the regions around the tumor (12.3 and 12.5% reduced permeability, respectively). On the other hand, a single injection of an equimolar dose of Dex-CDS (2.6 mg/kg) significantly attenuated Evans Blue extravasation in both the tumor as well as in the area surrounding the tumor (with decreases in extravasation of 70 and 50%, respectively). Finally, a single i.v. dose of Dex-CDS (2.6 mg/kg) was compared to the three-injection schedule of Dex (2.0 mg/g × 3 at 0, 8 and 24 h) with BBB permeability determined at 30 h after the first dose. As illustrated in Figure 4, Dex-CDS under these conditions was as effective as three doses of Dex administered over the indicated time course.

Collectively, these data suggest that Dex-CDS exerts a more potent anti-edema action at the level of the implanted brain tumor than does Dex. The mechanism for the increased potency is probably pharmacokinetic in origin, consistent with improved and/or sustained brain delivery. These data also support the contention that the Dex-CDS is converted to the active compound in a timely way after central penetration since 21-esters of glucocorticoids have been shown to be inactive (15). The current results are in keeping with previous studies that evaluated the effects of Dex-CDS on suppression of stress-induced ACTH release. Thus, Dex-CDS was found to be more effective and exerted more prolonged action than did dexamethasone (7). In addition, the pharmacokinetic profile of Dex-CDS reported previously indicated that Dex-CDS delivered higher total Dex concentrations to the brain while blood and peripheral organs levels were lower when compared to free Dex treatment. The higher brain to blood and brain to liver ratios for Dex obtained after Dex-CDS treatment are consistent with attenuated peripheral exposure and with a potentially improved safety profile. Therefore, treatment of tumor-related edema, which often necessitates the use of high drug doses for extended periods, could benefit from this improved delivery approach.

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