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Applications of chemically-modified cyclodextrins: use of hydroxypropyl-β-cyclodextrin as an enabling excipient for brain targeting, redox-based derivatives of estradiol A review of preclinical and clinical findings

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Estrogen depletion associated with the menopause produces a constellation of debilitating symptoms which range the gamut from vasomotor complaints which severely affect over one-third of all climacteric women to cognitive deficits. While many of these complications can be alleviated with traditional hormone replacement therapy (HRT), fear of cancer, cardiovascular problems and other metabolic diseases prompt many women to avoid treatment. Surveys suggest that over 30% of all prescriptions written for HRT are never filled due to these concerns and recent findings from Woman's Health Initiative trials have only added to these trepidations. Selective brain delivery of estrogens, in general, and estradiol, in particular, may address these items. One approach to accomplish CNS deposition is the use of a chemical delivery system for estradiol that selectively targets the central nervous system and may, therefore, reduce the incidence or severity of peripherally manifested side-effects. Owing to the very poor water solubility and limited stability of these derivatives, hydroxypropyl-β-cyclodextrin has proved to be enabling excipients in the development of these drug candidates. Various preclinical evaluations have demonstrated the organ-targeting potential of an estradiol-chemical delivery systems (E2-CDS) and therefore its potential usefulness as a therapeutic adjunct in certain subpopulations of menopausal women including those at risk to breast carcinomas. A very exciting potential use of the E2-CDS is in neurodegenerative diseases. Data suggest that estrogens may improve the mental performance of elderly patents suspected of having Alzheimer's disease.

Key words: Chemical delivery systems (CDS) – Estradiol – Hydroxypropyl-β-cyclodextrin.

I. INTRODUCTION

A variety of approaches have been applied to the delivery of drugs to the brain including physical methods in which the drug is either i) injected into the CSF or brain parenchyma, ii) surgically placed at its site of action using various biodegradable matrices or iii) introduced into the brain through a temporary breakdown of the blood-brain barrier (BBB) which is induced by carotid injections of hypertonic nonelectrolyte solutions [1-10]. Biological approaches have explored the use of macromolecular carriers such as chimeric peptides or antibodies [11-13]. Chemical methods have taken the form of drug analogs and prodrugs [14-17]. A prodrug is a pharmacologically inactive compound which results from transient chemical modification of a biologically active species [18]. The chemical change is designed to improve some deficient physicochemical property of the drug such as membrane permeability or water solubility. When the BBB is considered, increased drug penetration is usually well correlated with the lipophilicity or the octanol: water partition coefficient of a drug [14, 19]. In order, therefore, to improve the entry of drug, esterification or amidation may be performed. This manipulation provides the necessary lipid solubility of the drug and improves BBB penetration. Once in the CNS, hydrolysis of the lipophilicity-modifying group will release the active compound [18].

Chemical delivery systems

Unfortunately simple prodrugs suffer from several important limitations, the most severe of which is nonselectivity in target site delivery. In addition, poor retention and the possibility for reactive catabolism, often act to decrease, not increase, the therapeutic index of drugs when masked as a prodrug. Some of the limitations associated with the prodrug approach are derived from the fact that only one chemical conversion occurs in the activation of the compound. In many circumstances, multiple and facile conversions may not only lead to selectivity in delivery but may also act to decrease the toxicity of a drug as well as sustain its action. A chemical delivery system (CDS) is defined as a biologically inert molecule which requires several steps in its conversion to the active drug which enhances drug delivery to a particular organ or site [20-22]. In designing a CDS for the CNS, the unique architecture of the BBB was exploited. As with a prodrug, a CDS should be sufficiently lipophilic to allow for brain uptake. Subsequent to this step, the molecule should undergo an enzymatic or other conversion to promote retention within the CNS but, at the same time, to accelerate peripheral elimination of the entity [23]. Finally, the intermediate should degrade releasing the active compound in a sustained manner. One system which possesses these attributes is summarized in *Figure 1*. In this CDS, a targetor molecule is used as a lipophilicity modifier. While many moieties may serve such functions, 1,4-dihydronicotinates or trigonellinates have proved to be the most useful. In this approach, a hydroxy-, amino- or carboxylic acid-containing drug is esterified, amidated or otherwise covalently linked to nicotinic acid or a nicotinic acid targetor precursor. This compound is then quaternized to generate the 1-methylnicotinate salt or trigonellinate and chemically reduced

to give the 1,4-dihydrotrigonellinate or CDS. Upon systemic administration, the CDS can partition into several body compartments due to its enhanced lipophilicity; some of which are inaccessible to the unmanipulated compound. At this point in the delivery scheme, the CDS is simply working as a lipoidal prodrug. The carrier molecule is specially designed, however, to undergo an enzymatically mediated oxidation which converts the membrane permeable dihydrotrigonellinate to a hydrophilic, membrane impermeable trigonellinate salt [23].

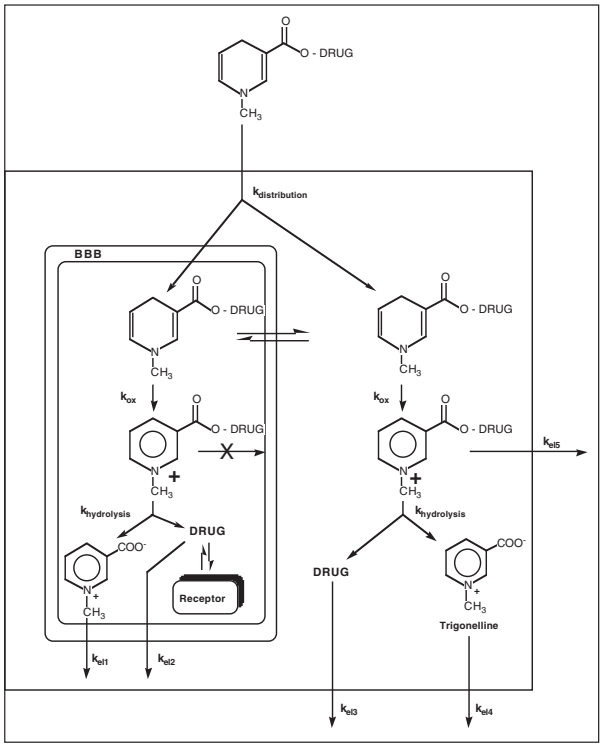

Figure 1.

This conversion occurs ubiquitously. The now polar, oxidized carrier-drug conjugate is trapped behind the lipoidal BBB and is, in essence, depoted in the CNS. Any of the oxidized salt which is present in the periphery will be rapidly lost as it is now polar and easily eliminated by the kidney and liver. The conjugate which is trapped behind the BBB can then slowly hydrolyze to give the active species in a slow and sustained manner. By the system design, concentrations of the active drug are very low in the periphery reducing systemic, dose-related toxicities. In addition, the drug in the CNS is present mostly in the form of an inactive conjugate, thus lowering any central toxicities. This approach should allow for a more potent compound in that a larger portion of the administered dose is shunted to its site of action. In addition, this system should allow for an increased dosing interval. The brain-targeting CDS has been extensively applied to various pharmacologically active agents [24-30]. Several excellent reviews of this technology are available [31-33].

II. AN ESTRADIOL-BASED CHEMICAL DELIVERY SYSTEM (E2-CDS)

The most advanced CDS is a brain-targeting system for estradiol. Estrogens are lipophilic steroids which are not impeded in their entry to the CNS. These compounds readily penetrate the BBB and achieve high central levels after peripheral administration. Unfortunately, estrogens are poorly retained by the brain. This circumstance requires that frequent doses of these steroids be administered to maintain therapeutically significant concentrations. Constant peripheral exposure of estrogen has been related, however, to a number of pathological conditions including cancer, hypertension, and altered metabolism in addition to uterine bleeding and other complications [34-40]. As the CNS is the target site for many of the actions of estrogens, a brain-targeted delivery form of these compounds may provide for safer and more effective estrogens [41, 42].

A broad spectrum of potential clinical applications for estrogen chemical delivery systems is available (*Table I*) and this reflects the wide distribution of estrogen receptors in the brain. Estrogen receptors are found in the hypothalamus and the closely associated preoptic area, where they mediate estradiol effects on LH secretion, sexual behavior in both males and females, appetite and temperature regulation [43, 44]. In addition, estrogen receptors have recently been identified on dopaminergic neurons which innervate the striatum, where estradiol may modulate locomotion and hence may be involved in movement disorders [45]. Also estrogen receptors have been identified in mesocortical dopaminergic neurons where they may mediate estradiol effects on mood [46]. Finally, estrogen receptors are found in the region of the nucleus basalis magnocellularis (NMB), a site of cholinergic cell bodies which innervate the cerebral cortex [47]. These estrogen receptors may mediate the influence of estrogens on cognitive function.

Table I - Potential Indications for an estradiol chemical delivery system.

- Menopausal symptoms
- Prostate cancer
- Endometriosis
- Libido
- Cognition and Alzheimer's disease
- Depression
- Contraception

1. Chemistry

The most potent natural steroid is estradiol (E2), which is chemically a diol containing a phenolic 3-moiety and a 17-alcohol group. The preparation of the 17-based CDS is given in *Figure 2*. This manipulation, in addition to generating a CDS, efficiently destroys the biological potency of the estrogen since 17-substituted estradiol analogs are known not to bind to the estrogen receptor [48]. In the synthesis, E2 is reacted with nicotinoyl chloride hydrochloride to give the 3,17-bis nicotinate [49-51]. This compound is subjected to methanolic potassium bicarbonate which results in selective hydrolysis of the phenolic nicotinate. The resulting secondary ester is quaternized with methyl iodide to give the 17-trigonellinate, or E2Q$^+$, and then reduced using sodium dithionite to give the 17-(1,4-dihydrotrigonellinate), or E2-CDS. This synthesis has been scaled to large batch size high yield and purity. The log P values for the E2-CDS and E2-Q$^+$ were measured using standard procedures and compared to that of E2. The log of the octanol:

water partition coefficient for E2-CDS was found to be 4.50; for E2, 3.76; and for the E2-Q$^+$ salt, 0.144 [49]. This indicates that the quaternary salt form of E2-CDS is 8000 times more hydrophilic than the parent E2 and more than 44,000 times more hydrophilic than the E2-CDS which is approximately five-fold more lipophilic than E2. These values, which are consistent with other derivatives examined, indicate that the compounds synthesized possess the appropriate physicochemical properties for CDS functioning.

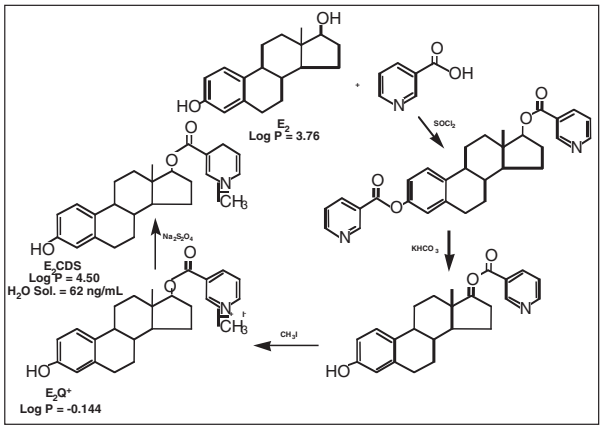

Figure 2 - Preparation of E2-CDS (3-hydroxy-17β-[[(1-methyl-1,4-dihydropyridin-3-yl)-carbonyl]oxy]estra-1,3,5(10)-triene), E2-Q$^+$ (1-methyl-3-[[[3-hydroxyestra-1,3,5(10)trien-17β-yl]oxy]-carbonyl]pyridinium iodide), estradiol nicotinate (3-hydroxy-17β-[(3-pyridinylcarbonyl)oxy]estra-1,3,5(10)-triene and estradiol bis nicotinate (3, 17β-bis[(3-pyridinylcarbonyl)oxy]estra-1,3,5(10)-triene).

2. Formulation

The E2-CDS is designed to penetrate into deep brain compartments and to be biologically labile to generate the requisite *in vivo* conversations. These properties complicate the development of a convenient formulation due to the poor water solubility of the E2-CDS as well as its hydrolytic and oxidative instabilities [52]. This problem was overcome through the use of chemically modified β-cyclodextrins, specifically 2-hydroxypropyl-β-cyclodextrin. These derivatives interact with lipophiles resulting in the formation of a dynamic inclusion complex [53-55]. These complexes act to both increase the water solubility of the E2-CDS (from about 50 ng/ml to over 30 mg/ml) as well as to significantly increase its shelf-life (*Figure 3*) [52]. While a number of the initial studies for E2-CDS were completed using organic vehicles such as DMSO, the toxicological and clinical development of the compound would not have been possible without the use of the functional excipient, HPβCD.

3. *In vitro* studies

The CDS concept requires that the dihydrotrigonellinate-drug conjugate rapidly converts to the pyridinium salt and that this brain lock-in form slowly hydrolyzes to give the parent drug and the carrier molecule (trigonelline). Prior to *in vivo* study, these assumptions were examined *in vitro* using rat organ homogenates as the test matrices. The half-lives of E2-CDS in plasma, liver, and brain homogenates were found to be 156.6, 29.9, and 29.2 min, respectively [49]. Thus, E2-CDS was converted to the corresponding pyridinium salts much faster in the tissue homogenates than in plasma. This is consistent with a membrane-bound enzyme acting as the oxidative catalyst. Candidates for this enzyme include members of the family of NADH transhydrogenases which mediate redox reactions of NADH, a molecule similar in structure to the active portion of the CDS [23]. The second metabolic step required for drug release is hydrolysis of E2 from the E2-Q$^+$ which was demonstrated in plasma as well as in various organ homogenates.

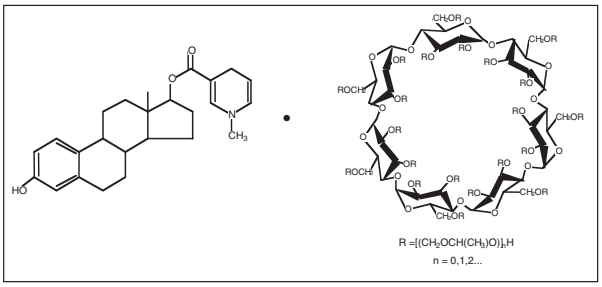

Figure 3 - HPβCD complex with E2-CDS.

4. Tissue distribution and pharmacokinetics

Initial studies on the tissue distribution of E2-CDS involved i.v. administration of large doses of the drug dissolved in DMSO (60 or 15 mg/kg) to conscious restrained rats [49, 65]. The E2-CDS rapidly disappeared from blood after administration reflecting the enzymatic liability and large volume of distribution of this lipophile. The quaternary metabolite of E2-CDS was detected in all tissues soon after drug administration. Analytical determinations were assisted with the development of a pre-column-enriching HPLC system [65]. This method generated limits of accurate detection for plasma samples and organ tissue of 10 ng/ml or ng/g for E2-Q$^+$, 20 ng/ml or ng/g for the E2-CDS and 50 ng/ml or ng/g for E2. As illustrated in *Figure 4*, a significant selectivity was observed for the CNS with tissue half-lives being approximately five-fold greater than those observed in kidney, heart, lung, testis, eye, or peritoneal fat [65].

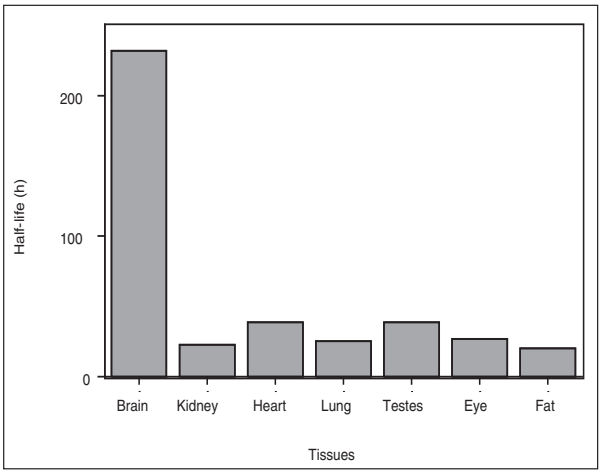

Figure 4 - Elimination half-lives for E2-Q+ from various tissues.

In order to follow E2 released over time from deposited E2-Q$^+$, an RIA technique was developed [57]. The levels of E2 in brain tissue after E2-CDS administration are elevated four to five times longer than after simple E2 treatment (*Figure 5*). In addition, the ratio of the area under the concentration curves (AUC) of E2 in brain and plasma after E2-CDS treatment was approximately 12. This profile confirms that E2 is generated within the CNS and is not sequestered from peripheral sources. In addition, plasma levels of E2 are much more rapidly eliminated than those in brain (*Figure 6*). Pharmacokinetically, serum E2 data derived from E2-CDS dosing could be fitted to a three exponent time-concentration curve in which the initial phase (half-life = 1.0 h) was correlated with a rapid distribution phase, the second phase was associated with elimination of E2 derived from several compartments (both peripheral and central) (half-life = 9.9 h) and the terminal elimination phase (half-life = 123.5 h) was associated primarily with elimination of E2 derived from the brain [57]. The pharmacokinetics of the E2-CDS have also been examined in the dog [58]. The data collected from these evaluations confirm the large volume of distribution for the E2-CDS as well as its dose linearity and slow terminal phase elimination (*Table II*).

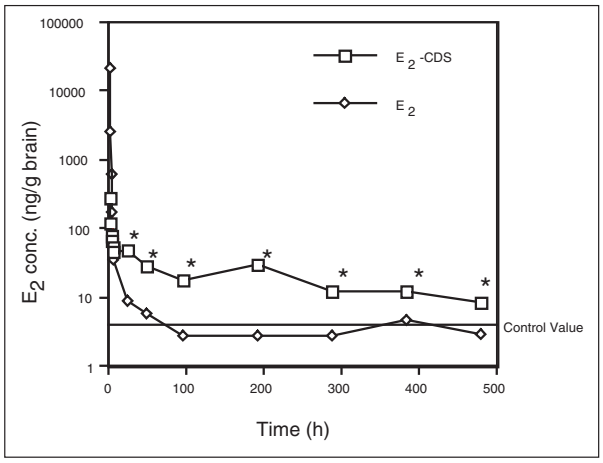

Figure 5 - Brain E2 after E2-CDS (3 mg/kg) or equimolar E2.

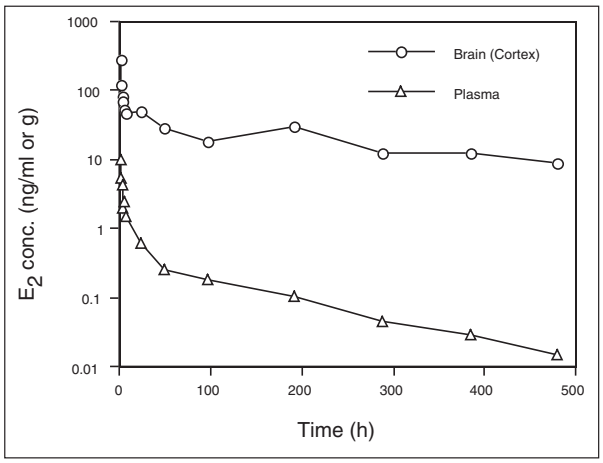

Figure 6 - Brain (cortex) and plasma E2 after a single 3 mg/kg dose of E2-CDS.

Table II - Pharmacokinetics of E2-CDS in the dog.

- E2-CDS (1 mg/kg)
  - Cl_{total} = 11.9 ± 4.7 l/min
  - MRT = 23 ± 11 min
  - Vd_{ss} = 286 l (12 l/kg)
- E2-Q$^+$
  - Protein binding = 87%
  - AUC = 291 ± 23 ng·h/ml (0.5 mg/kg) and 451 ± 20 ng·h/ml (1.0 mg/kg)
  - Cl_{renal} = 37.1 ± 1.8 ml/min
  - k_e = 0.315 d^{-1} ($t_{1/2}$ = 2.2 d)

5. Pharmacology

Given this kinetic advantage, the pharmacology of the estradiol delivery system was examined using a variety of animal models including the effects of the system on i) LH and LHRH secretion, ii) cyclicity and ovulation in the rat, iii) sexual behavior in castrate male rats, iv) testosterone levels and androgen-dependent peripheral tissues, v) body weight and food intake, vi) growth hormone dynamics and other hormonal effects and vii) high affinity choline uptake, neuropeptide Y and cerebral ischemia.

Estradiol, in the ovariectomized female, acts in a negative feedback mode to decrease the secretion of LHRH at the levels of the hypothalamus [59]. The hormonal regulation further acts to reduce LH secretion at the pituitary which then reduces E2 production at the level of the ovaries (*Figure 7*) [60]. It is generally acknowledged that E2 can affect several behaviors at higher brain centers including weight maintenance, cognition, thermoregulation and depression meaning that the estradiol depletion associated with ovarian removal or senescence may alter or complicate these activities [41, 42]. The initial examinations of the E2-CDS were aimed to evaluating its effect on the hypothalamic-pituitary-ovarian axis.

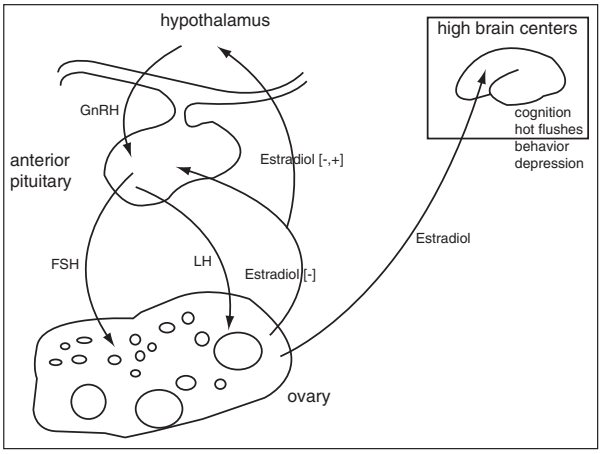

Figure 7 - Estradiol dynamics in the female.

5.1. LH and LHRH

The response of LH secretion to E2-CDS was evaluated in several rat studies. In the first set of experiments, E2-CDS was administered as a single i.v. injection (3 mg/kg) in DMSO to male rats which had been orchidectomized 2 weeks previously [61, 62]. E2 was administered to another

group of rats at an equimolar dose and vehicle-treated rats served as the controls. Both E2 and E2-CDS reduced serum LH equivalently from 4 to 48 h post-drug administration. From 4 to 12 days, LH levels in the E2 treated rats increased progressively to levels equivalent to those in DMSO-treated rats (*Figure 8*). By contrast, LH concentrations in animals treated with E2-CDS remained low and were suppressed by 82%, 88% and 90% when compared to vehicle treated animals at 4, 8 and 12 days after treatment, respectively. In these animals, serum E2 levels remained elevated through 2 days in both rats treated with E2 and E2-CDS. By 4 days after drug treatment, E2 levels in both drug-treated groups returned to that observed in vehicle treated orchidectomized male rats. These data indicated that, despite the clearance of E2 from the plasma, the delivery system achieved chronic suppression of LH secretion for at least 12 days following a single i.v. administration. These data are consistent with the hypothesis that the E2-CDS causes enhanced brain delivery, lock-in of the E2-Q^+, and slow release of E2 to achieve LH suppression. This study was extended to determine the duration of LH suppression following several different doses of the E2-CDS. In this second evaluation, orchidectomized rats were treated with E2-CDS solubilized in DMSO (0.1, 0.3 or 1.0 mg/kg i.v.) or the vehicle and were examined at 12, 18 and 24 days post-treatment [50, 63]. E2-CDS significantly suppressed LH secretion at the lowest dose through 18 days while estradiol or estradiol valerate (E2V, a depot ester of estradiol) were ineffective at doses equimolar to the highest E2-CDS dose at 12 days (*Figure 9*).

The secretion of LH in rats can be controlled or attenuated by numerous factors, including the action of E2 both in a positive and negative feedback mode at the pituitary gland. In addition, hypothalamic release of LHRH stimulates LH secretion. Again, LHRH release can be augmented by the interaction of E2 in the preoptic area. The effect of E2-CDS on LHRH release was, therefore, studied [57]. Animals which had been ovariectomized for at least 2 weeks were given a single i.v. dose of E2-CDS, E2 or vehicle. At either 1 or 16 days post-drug administration, animals were anesthetized and their pituitary stalks exposed for collection of portal blood samples to be used for LHRH RIA. Subsequent to sample collections, hypothalamic tissue was removed for LHRH assay. Portal blood concentrations of LHRH were significantly reduced on day 1 for both E2 and E2-CDS. By day 16, however, only the E2-CDS continued to decrease LHRH secretion, while the E2 treated animals were not different from vehicle controls (*Figure 10*). The decreased LHRH secretion was mirrored by an increase in hypothalamic LHRH levels, suggesting that the inhibition of hormone release resulted in a tissue accumulation of the peptide. Again, hypothalamic LHRH concentrations were significantly elevated at day 1 for both E2-CDS and E2, but by 16 days only the E2-CDS produced a significant accumulation (*Figure 10*). Since previous studies have indicated that long-term exposure of E2 decreases pituitary responsiveness to LHRH, the generated results strongly suggest that the prolonged inhibitory action of E2-CDS on LH release is due primarily to sustained suppression of LHRH secretion from the hypothalamus [64, 65]. The reduced LHRH and LH secretion resulted in an abolition of ovulation as indicated by an

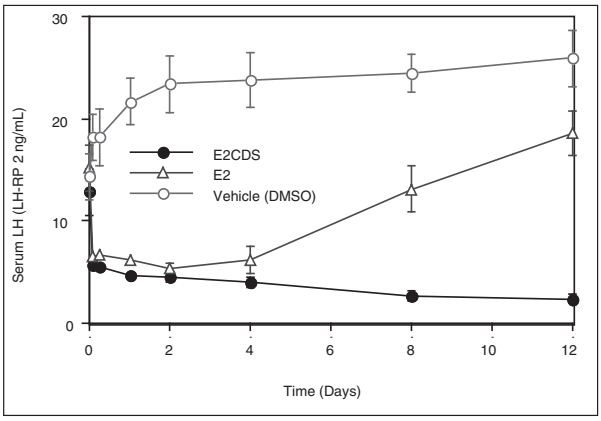

Figure 8 - Serum LH following single doses of E2-CDS (3 mg/kg) or E2 formulated in DMSO to castrate male rats.

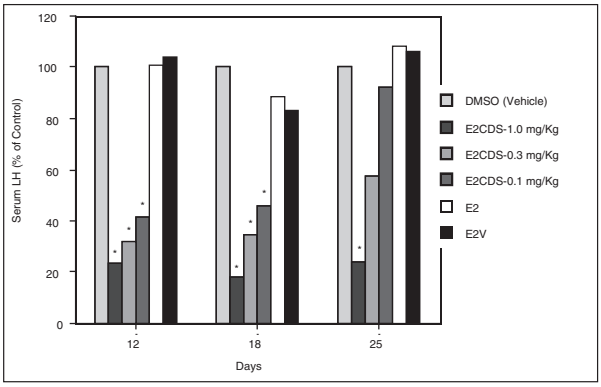

Figure 9 - Dose and duration effects of E2-CDS on LH suppression.

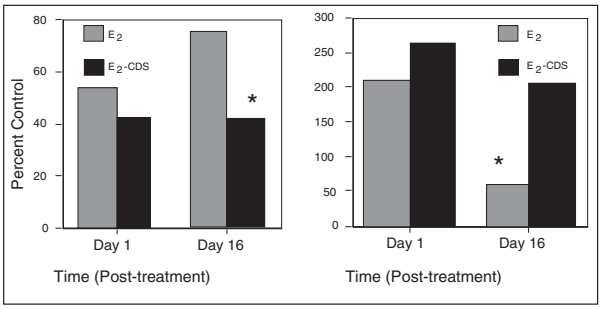

Figure 10 - Effect of E2 or E2-CDS on portal plasma (Left) or hypothalamic (right) LHRH.

elimination of ova found in the oviducts as well as by an increase in the normal ovulation cycle. The increase in the estrus cycle was dose-related and significantly more sensitive to E2-CDS as compared to equimolar E2 (*Figure 11*). While the pharmacokinetic data demonstrated that peripheral estradiol was not elevated compared to E2 treatment, a biological end point was examined to verify this finding. Treatment of intact female rats with E2-CDS resulted in no change in uterine weight consistent with the failure of the E2-CDS to significantly elevate serum E2. On the other hand, equimolar E2 provided for significant uterine ballooning consistent with the expected elevation in serum E2 (*Figure 12*).

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